PETITION

Terpene Polymers
(Pinene Polymers)
For Addition to National List
Under the Provisions of Organic Foods Production Act

Requirement:

7 CFR § 205.601
Synthetic Substances Allowed for Use in Organic Crop Production

Prepared By:

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Petitioner:

Miller Chemical & Fertilizer Corporation
P.O. Box 333, 120 Radio Road
Hanover, PA 17331

July 26, 2005

Statement of Commercial Confidential Information Claims

The following information was claimed Confidential Business Information (CBI):

1) Manufacturing process for Terpene Polymers
2) Statement of Formula for SUSTAIN

1) Manufacturing Process for Terpene Polymers

Miller Chemical and Fertilizer Corporation ("Petitioner") believes that the manufacturing process for terpene polymers is proprietary information and does not want to disclose it for competitive reasons. Therefore, the Petitioner requests the USDA to treat this information confidential.

2) Statement of Formula for SUSTAIN

The Petitioner further claims that the composition of the formulation for SUSTAIN CBI because the compositional information is considered "Trade Secret" This information is used in Petitioner's business and maintained in secrecy. Therefore, the Petitioner requests the USDA to treat this information confidential.

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July 26, 2005

Program Manager
National Organic Program/TMP
Agricultural Marketing Service
United States Department of Agriculture
1400 Independence Avenue, SW
Room 4008-So., Ag Stop 0268
Washington, D.C. 20250

SUBJECT: Petition to Include Terpene Polymers (Pinene Polymers) on the National List
Petitioner: Miller Chemical & Fertilizer Corporation

Dear Sir/Madam:

On behalf of Miller Chemical & Fertilizer Corporation ("**Miller Chemical**"), we are submitting this petition to you for the addition of Terpene Polymers (also referred to as Pinene Polymers) to National List under the provisions of Organic Foods Production Act of 1990, as amended (**OFPA or Act**).

Terpene polymers, which are also called pinene polymers, are the polymers derived from natural constituents of the pine tree. They are the synthetic substances which are exempt from the requirement of a tolerance under 40 CFR 180.910 by the Environmental Protection Agency for use in pesticide formulation applied to growing crops and crops after harvest (pre- and post-harvest uses). The EPA classified Terpene Polymers (Pinene Polymers) as List 4 Inerts of Minimal Concern.

Miller Chemical intends to use Terpene Polymers for organic crop production because the use of these substances is not expected to contribute to contamination of crops, soil or water. We have prepared the petition in accordance with the regulations at 7 CFR 205.601 and 205.607 and also with the procedures outlined for the National List Petition Process (FR 65 43259; July 13, 2000).

Enclosed please find the subject petition for your review. We kindly request you to amend 7 CFR Part 205 for inclusion of Terpene Polymers (Pinene Polymers) on the National List.

If there are further questions regarding the subject petition, please contact us.

Sincerely,

N. Bhushan Mandava

N. Bhushan Mandava, Ph.D., CPC, RAC
Miller Chemical & Fertilizer Corporation

cc: A.D. Vidyarthi

INTRODUCTION

Miller Chemical & Fertilizer Corporation (herein after referred to as "**Miller Chemical**" or "**Petitioner**") is submitting this petition to Agricultural Marketing Service (**AMS**), United States Department of Agriculture (**USDA**) for addition of Terpene Polymers (also referred to as Pinene Polymers) to National List under the provisions of Organic Foods Production Act of 1990, as amended (**Act**). Under the National Organic Program (**NOP**), additions and deletions of new substances to National List are handled through petition process. The National Organic Standards Board (**NOSB**) evaluates the petitions for recommendation to the Secretary of Agriculture for inclusion on or deletions from the National List in accordance with the Act.

In 2000, the USDA published the proposed rules in Federal Register [65 FR 43259, July 13, 2000] and in 2001 the final rule was published [65 FR 80637, December 21, 2001]. These were codified at 7 CFR Part 205 that deals with National Organic Program.

The petitioned substance for inclusion on the National List is a polymer known as terpene polymer that is also known as pinene polymer. We used this polymer in a plural form – terpene polymers or pinene polymers – to cover more than one substance. This is because pinene polymers is (are) a mixture of four chemical substances identified by EPA when approving them for use on food crops under 40 CFR §180.910. [They are identified as: (1) homopolymer of α-pinene (CAS #25766-18-1) (2) homopolymer of β-pinene (CAS #25719-60-2), (3) copolymer of α- and β-pinene (CAS #31393-98-3), and (4) terpenes and terpenoids, turpentine oil, α-pinene fraction polymerized (CAS #70750-57-1).]

These substances are manufactured from α-pinene and β-pinene that are the natural constituents of the deciduous pine tree. Turpentine oil is extracted from the sap of the pine tree followed by steam distillation. Fractionation of turpentine oil gives α-pinene and β-pinene which are polymerized to give dimmers, trimers and low molecular weight polymers (which are called pinene polymers or terpene polymers). The Environmental Protection Agency (**EPA**) included the above-mentioned four substances in pinene polymers for clearance under 40 CFR §180.910.

In support of the petition, we have submitted to you the required information on the terpene polymers (synonym: pinene polymers) that includes the identity of the polymers, physical and chemical properties, toxicity data, ecotoxicity data, environmental impact, human health (safety to humans) and other information.

Information Included in the Petition

The following two items must be included in the petition:

Item A

There are five categories in Item A. The subject petition is for inclusion of Terpene Polymer on the National List as a "**Synthetic substance allowed for use in organic crop production**".

Item B

The following information is provided in Item B:

1. The Substance's common name:

Terpene Polymers (a mixture of polymers)

Other Names: Pinene Polymers

2. The manufacturer's name, address and telephone number

Miller Chemical & Fertilizer Corporation,
120 Radio Road, P.O. Box 333,
Hanover, PA 17331
Telephone: 717-632-8921

3. The intended or current use of the substance such as use as a pesticide, animal feed additive, processing aid, nonagricultural ingredient, sanitizer or disinfectant.

Terpene Polymers (pinene polymers) are intended for use as an inert ingredient in pesticide formulations for use as Spreader-Sticker Spray adjuvant.

The monomers used in terpene polymers [terpene hydrocarbons (α- and β-pinene)] are reported to have insecticidal properties [**Attachments 29 and 30**], whereas the polymers derived from these monomers are inert and do not possess any pesticidal properties. Because of their strong adhesive and non-toxic properties, they are used as adjuvants in pesticide formulations applied to raw agricultural commodities.

4. A list of the crop, livestock or handling activities for which the substance will be used. If used for crops or livestock, the substance's rate and method of application must be described.

The petitioner uses terpene (pinene) polymers in a product known as SUSTAIN which is a proprietary blend consisting of the following ingredients:

CBI DELETED

All the three ingredients in SUSTAIN are included in EPA List 4 Inerts of minimal concern. (See Appendix 1 for inclusion of pinene polymers in List 4 Inerts.). Since they are List 4 Inerts, they are allowable for use in organic production [7 CFR §205.603(e)].

MILLER 6064 is the same as SUSTAIN.

SUSTAIN containing terpene (pinene) polymers can be incorporated in all pesticide products registered for agricultural (includes **all crops**), forestry, ornamental, industrial vegetation and non-cropland uses.

SUSTAIN containing terpene (pinene) polymers can be used on pesticide products for aerial and ground applications.

For aerial applications - all pesticides – apply SUSTAIN 4 to 16 oz per acre.
For ground applications – all pesticides – use SUSTAIN 4 oz to 1 pt of SUSTAIN per 100 gal of spray solution.
For soil applications – use SUSTAIN 1 to 2 pints per acre.

For additional information, see the enclosed label for SUSTAIN (**Attachment 1**). We have also provided the. Material Safety Data Sheet (MSDS) for SUSTAIN (**Attachment 2.3**). [We have also provided the MSDSs for PICCOLYTE® AO PINENE POLYMERS from Hercules, Inc. (**Attachment 2.1**) and SYLVARES TR A25 from Arizona Chemical (**Attachment 2.2**). They are the commercially available terpene polymers.]

Additionally, we have provided product formulation information on SUSTAIN in **Attachment 3.**

5. The source of the substance and a detailed description of its manufacturing or processing procedures from the basic component(s) to the final product. Petitioners with concerns for confidential business information can follow the guidelines in the instructions for submitting Confidential Business Information (CBI) listed in item B, #13.

Terpene (pinene) polymers are derived from natural crude turpentine, which is the volatile portion of the oleoresin found in conifer trees. After extraction of the turpentine (see details of the manufacture in **Attachment 4,** it is distilled to isolate pinenes (α- and β-pinene) which are regarded as the basic components (served as monomers). Polymerization of pinene monomers gives terpene polymers (pinene polymers).

See Attachment 4 for detailed manufacturing process for terpene (pinene) polymers.

monomers). Polymerization of pinene monomers gives terpene polymers (pinene polymers).

See Attachment 4 for detailed manufacturing process for terpene (pinene) polymers.
The petitioner provided Certificate of Analysis (CoA) for terpene polymers (Trade Name for formulated product: MILLER 6064) in **Attachment 5.**

6. A summary of any available previous reviews by state or private certification programs or other organizations of the petitioned substance.

In conjunction with potassium bicarbonate, the Organic Materials Review Institute (OMRI) recommended to use a combination product [potassium bicarbonate and pinolene based coating (which is a terpene polymer)] would be acceptable for organic growers [**Attachment 6**).
The EPA approved the use of pinene polymers (both α- and β-pinene polymers) under 40 CFR §180.910 for pre- and post-harvest uses. [See **Attachment 7.**1 for Notice of Petition Filing (63 FR 64494, November 20, 1998) and **Attachment 7.**2 for Final Rule (70 FR 28447, May 18, 2005).]
Because pinene polymers (terpene polymers) are cleared under 40 CFR §180.910, they are considered included in List 4 inerts. [See Appendix 1 for a copy of the letter from EPA regarding the inclusion of these substances in List 4 Inerts.]

7. Information regarding EPA, FDA, and state regulatory authority registrations, including registration numbers.

SUSTAIN is a product containing terpene polymers (pinene polymers) and is registered in California. Its Registration Number is 72-50015-AA.

See **Attachment 3** for Cal/EPA Confidential Statements of Formula for SUSTAIN.

We have submitted the composition information on SUSTAIN to EPA and this information is in the Master Files maintained by the Registration Division.

Products containing terpene polymers (e.g., SUSTAIN) are used in tank mix preparation with other registered pesticides (insecticides, fungicides, herbicides and plant growth regulators). See several pesticide products are tank mixed with SUSTAIN and NU-FILM® (that contain terpene polymers – see **Attachments 9-27**).

Terpene polymers (pinene polymers) are approved by EPA under Section 5 of the Toxic Substances Control Act (TSCA) and are considered to be UVCB substances for the TSCA Inventory purposes. The EPA cleared these substances (pinene polymers) under 40 CFR §180.910 for food uses
[See **Attachment 7.**1 for Notice of Petition Filing (63 FR 64494, November 20, 1998) and **Attachment 7.**2 for Final Rule (70 FR 28447, May 18, 2005).]
Because pinene polymers (terpene polymers) are cleared under 40 CFR §180.910, they are considered included in List 4 inerts of minimal concern. [See Appendix 1 for

a copy of the letter from EPA regarding the inclusion of these substances in List 4 Inerts.]

The FDA approved terpene polymers for use as a direct food additive under 21 CFR §172.615 (for use as a chewing gum base). The terpene polymers are also approved for use as indirect food additives (21 CFR §175.105, §177.1200 and §178.3930).

We have provided the complete information on the EPA and FDA approvals for terpene polymers (pinene polymers) in **Attachment 8**.

8. The Chemical Abstracts Service (CAS) number or other product numbers of the substance and labels of products that contains the petitioned substance.

As shown above, terpene polymers (also known as pinene polymers) are a mixture of polymers (copolymers and homopolymers). The EPA cleared the following substances (identified by CAS Registry Numbers) under 40 CFR §158.910 for crop production and crop protection (pre- and post-harvest uses).

Chemical Name	CAS Reg. No.
Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-, homopolymer (α-pinene, homopolymer)	25766-18-1
Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, homopolymer (β-pinene homopolymer)	25719-60-2
Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-, polymer with 6,6-dimethyl-2-methylenebicyco [3.1.1]heptane (Copolymer of α- and β-pinene)	31393-98-3
Terpenes and terpenoids, turpentine oil, α- pinene fraction Polymerized	70750-57-1

It is our opinion that "terpene polymers" is a synonym to "pinene polymers" because they are derived from not only α- and β-pinene, but also from terpenes, terpenoids and turpentine oil.

For additional information on terpene polymers and Chemical Abstracts Service (CAS) Registry Numbers, see **Attachments 7 and 8**.

9. The substance's physical properties and chemical mode of action including (a) chemical interactions with other substances, especially substances used in organic production; (b) toxicity and environmental persistence; (c) environmental impacts

from its use or manufacture; (d) effects on human health; (e) effects on soil organisms, crops or livestock.

Physical Properties:

Terpene polymers (also known as pinene polymers) have physical properties very similar to those of other polymers. The product is practically insoluble in water. Terpene polymers (Product Name: MILLER 6064) is a yellow (to amber colored) liquid with a terpenic odor. Miller 6064 has a specific gravity of 0.92 – 0.94 @ 20°C and its viscosity @ 20°C is 100 to 500 cps. Its pH (1% solution) ranges 6.0 to 8.0. The flash point is over 200°F.

For additional information, please see **Attachment 5.**

Chemical Mode of Action

We provide the following information in support of the chemical mode of action for terpene polymers (also known as pinene polymers):

a) Chemical interactions with other substances, especially substances used in organic production

Like other polymers, terpene polymers are the inert substances and do not react or interact with other substances (inorganic or organic). Terpene polymers are used to make the formulated products such as SUSTAIN and NU-FILM® which contain other inert ingredients approved by EPA for agricultural uses. The pesticide active ingredients (insecticides, fungicides, herbicides and plant growth regulators) are blended with these formulated products (such as SUSTAIN and NU-FILM® containing terpene polymers) to make the pesticide products. The ingredients (terpene polymers) in the pesticide products, **especially when used in organic production**, are not expected to interact with each other (because they are chemically inert) and they only exhibit certain (desired) physical properties [e.g., serving as a sticking agent (spreader-sticker) to make an film so as to protect the pesticide active ingredient from sunlight (ultraviolet inhibitor), to improve a better distribution of the product on the applied surface and to protect it from rain fall).

We have submitted several field study reports by various investigators in supporting that there are no known or reported chemical interactions of terpene polymers with other substances used in pesticide products used in agriculture and some of the products are used in organic crop production (**Attachments 9 to 28**).

b) Toxicity and environmental persistence

The monomers (α- and β-pinene and other terpenes) used in the manufacture of terpene polymers (pinene polymers) are not of high toxicity compounds to animals and aquatic organisms. The polymers (including dimmers and trimers) derived from these monomers are expected to have much lower toxicity as a result of decrease in absorption with increase in molecular weight. Although these polymers are not expected to degrade rapidly (especially the higher molecular weight species) in the soil, their presence in the environment is not of concern. They do not contaminate the air. They are unlikely contaminate the water (surface and ground water) because of their strong adhesive properties (stick to target areas). [**Attachment 33**].

We have summarized the available toxicity data on terpene monomers and their polymers as shown below:

Toxicity Data on Terpene Monomers [**Attachment 33**]

	α-Pinene	β-Pinene
Acute Oral LD_{50} (Rat)	3700 mg/kg	>5000 mg/kg
Acute Dermal LD_{50} (Rabbit)	>5000 mg/kg	>5000 mg/kg
Mutagenicity (Ames Assay/Microsome Assay/Reverse Mutation)	No Evidence of Mutagenicity	No Evidence of Mutagenicity

The terpene hydrocarbons (α- and β-pinenes) are not acutely toxic to laboratory animal through oral, dermal and inhalation routes of exposure. When tested with a mixture of terpene hydrocarbons, there was no evidence of maternal toxicity (NOAEL ~560 mg/kg bw/day) and developmental toxicity (NOAEL ~560 mg/kg bw/day) in mouse. There was no evidence of mutagenicity or genotoxicity when evaluated them in different assays.

Toxicity Data on Terpene Polymers **[Attachments 7 and 33]**

Acute oral LD_{50} (rat) :	>34.6 g/kg
Acute eye irritation (rabbit)	0.1 ml for 24 hr (Moderate Irritation)
Reproductive toxicity (rat)	NOAEL 10 g/100 g diet
90-day Subchronic toxicity (rat)	NOAEL 3967 mg//kg/day (37.5 mg/kg/day in another study)
2-yr Chronic toxicity (dog)	NOAEL 51 mg/kg/day
2-yr Chronic toxicity (rat)	NOAEL 3100 mg/kg/day

Source: Notice of Filing published in the Federal Register (63 FR 64494; November 20, 1998) for tolerance exemption for pinene polymers [**Attachment 7.2**].

In the Final Rule published in the Federal Register (70 FR 28447; May 18, 2005) for tolerance exemption for pinene polymers [**Attachment 7.1**], the EPA reviewed the toxicity data **NOT** only on the pinene polymers (see above for four pinene polymers with CAS Registry Numbers), **BUT ALSO** on the monomers (α- and β-pinene as the major components of turpentine) which are used to make the polymers (by a polymerization process to give dimers, trimers and polymers). In the supporting document (Science Assessment for α- and β-pinene chemicals) [**Attachment 33**], the Agency included physical and chemical properties, toxicity data (acute and chronic toxicity), mutagenicity and ecotoxicity data on both pinenes.

Based on the assessment of the data, the Agency concluded that α-pinene and β-pinene are not of high toxicity compounds. The processes used to form pinene polymers would increase the molecular weight. Greater molecular weight means decreased absorption. α - and/or β-Pinene dimers, trimers, or polymers should therefore be of even lower toxicity than pure α- pinene or β-pinene. Therefore, terpene polymers are considered safe to use in or on raw agricultural commodities. [See **Attachments 31-33** for additional information on the toxicity data on terpene polymers (pinene polymers).]

Ecotoxicity on terpene monomers and polymers:

According to EPA unpublished report (**Attachment #33**), terpene hydrocarbons (α- and β-pinene) are highly toxic to aquatic organisms on acute basis. The estimated values for these monomers are shown below:

	α- Pinene	β-Pinene
Freshwater fish	0.72 mg/L	0.62 mg/L
Marine/estuarine fish	0.51 mg/L	0.45 mg/L
Daphnia magna	0.93 mg/L	0.79 mg/L
Mysid shrimp	0.042 mg/L	0.034 mg/L
Algae	0.66 mg/L	0.0.56 mg/L
Chronic toxicity (Freshwater fish)	0.138 mg/L	0.117 mg/L
Bioconcentration factor (BCF)	2800	444

The EPA further noted that terrestrial animal toxicity based on the available rodent data would indicate that monomers (α- and β-pinene) are not toxic to wildlife on acute basis.

During the manufacture, α- and β-pinene undergoes polymerization to give dimers, trimers and polymers. The polymerized products (terpene polymers) are expected to show less toxic effects to aquatic organisms because of the gradual decrease in absorption as the molecular weight increases. In other words, the dimer will absorb less than a monomer, and trimer will absorb much less than a dimer. Likewise, the absorption of polymer (consisting of 4 or more monomers) is considerably less than its lower homologs. As the molecular weight of the polymer increases, there is a corresponding decrease in absorption, thus resulting in practically no absorption. As a result, terpene

polymers are expected to be non-toxic to aquatic organisms. Our data on Miller 6064 (containing about 70% terpene polymer) confirms that it is practically non-toxic (LC50 for Bluegill sunfish is more 100 mg/L) on acute basis.

Therefore, it is reasonable to conclude that terpene polymers are **not** expected to be toxic to aquatic organisms and wildlife.

Toxicity and Ecotoxicity Data on Formulated Product

Acute Toxicity **[Attachments 34-39]**

MILLER 6064 which is the same as SUSTAIN (containing terpene polymers) was evaluated for its acute toxic effects in laboratory animals.

Test Method	Dose	EPA Toxicity Category
Acute oral LD_{50} (rat)	Greater than 5000 mg/kg	IV
Acute dermal LD_{50} (rabbit)	Greater than 5000 mg/kg	IV
Acute inhalation LC_{50} (rat)	5.26 mg/L for 4 hr	IV
Acute dermal irritation (rabbit)	0.5 ml for 4 hr (PII=0.3)	IV
Acute eye irritation (rabbit)	0.1 ml for 24 hr	IV
Skin sensitization (Guinea pig)	----------	Not a skin sensitizer

Based on the animal test data, the test substance (MILLER 6064) is a non-toxic substance and belongs to EPA Toxicity Category IV [40 CFR §156.10(h) (1)]. The product (MILLER 6064) derived from terpene polymer does not require Signal Word such as **WARNING** or **DANGER** on the product label. The label for MILLER 6064 requires only CAUTION as the Signal Word. Because the product belongs to Toxicity Category IV, no precautionary statements are required for oral, dermal and inhalation as well as for skin and eye local effects. Therefore, terpene polymers are safe to handle when it is used in organic crop production based on the acute toxicity data.

Additional information on the acute toxicity studies on MILLER 6064 is provided in **Attachments 34 to 39.**

Aquatic Toxicity **[Attachments 40-42]**

MILLER 6064 (containing terpene polymers) was also evaluated for its acute toxic effects in aquatic organisms.

Test Method	Dose
Bluegill Sunfish LC50 (static 96-hr)	106.67 mg/L
(Lepomis macrochirus)	
Rainbow Trout LC50 (static 96-hr) *(Oncorhynchus mykiss)*	46.90 mg/L
Daphnia magna LC 50 (static 48-hr)	0.54 mg/L

According to EPA regulations for labeling of pesticide products for environmental hazards [40 CFR §156.10(h)(2)(ii)(B)], terpene polymers may not be toxic to aquatic organisms and may not require a statement "This Pesticide is Toxic to Fish".

Additional information on the aquatic acute toxicity studies on MILLER 6064 is provided in **Attachments 40 to 42.**

Summary of Toxicity Data on Terpene Polymers:
According to EPA, a few toxicity studies conducted with alpha- and/or beta-pinene polymers were located. The Agency performed a structure –activity-relationship (SAR) assessment that indicated an overall low concern. The toxicity information on α- and β-pinene indicates that these are not substances of high toxicity [**Attachment 33**].

As previously explained, processes used to form a α- and/or β-pinene polymer would increase the molecular weight. Greater molecular weight means decreased absorption. α- and/or β-pinene dimers, trimers, or polymers should therefore be of even lower toxicity than pure α- and β-pinene

Other environmental fate considerations: Terpene polymers (pinene polymers) do not readily hydrolyze in water and they do not bioaccumulate. They are not readily biodegradable [**Attachment 33**]. They are not toxic to aquatic organisms (see above and also the test data in **Attachments 31 to 33**) and unlikely to be toxic to wildlife based on animal toxicity data. They are unlikely to be toxic to aquatic plants (e.g., algae) because of low solubility.

c) Environmental impacts from its use or manufacture:

As mentioned above, terpene polymers are manufactured (see **Attachment 4** for the manufacture of pinene polymers) from monomers (α- and β-pinene) present in turpentine which is obtained from (1) Kraft pulping process (paper manufacture), (2) steam distillation of the resinous material from the trees (similar to maple syrup collection), and (3) extraction from pine stumps followed by distillation. The pinene monomers are polymerized via Fidel-Craft reaction to give the desired terpene polymers.

Terpene polymers are used in many formulated products for consumer products (oils, paints, inks and adhesives) and industrial uses. They are approved for use as food additives (regulated by FDA) and also for use as inert ingredients in pesticide products for agricultural uses (regulated by EPA).

The FDA approved terpene polymers for use as a direct food additive under 21 CFR Part §172.615 (for use as a chewing gum base). The terpene polymers are also approved for use as indirect food additives (21 CFR §175.105, §177.1200 and §178.3930 All the food additives (direct and indirect additives) are subject to environmental impact considerations (21 CFR Part 25). In other words, environmental assessments must have already been made for terpene polymers (pinene polymers) using format given at 21 CFR §25.31. [We believe that FDA must have obtained all the documents related environmental assessments for terpene polymers from the applicant(s)/petitioner(s) before approval of the additive(s).] Since the terpene polymers are approved by FDA, the environmental assessments documents are available from FDA.

Since the EPA cleared terpene polymers (pinene polymers) for use as an inert ingredient in pesticide formulations applied to growing crops or crops after harvest under 40 CFR §180.910 [FR 70 28447, May 18, 2005], we believe that the environmental impact assessments are readily available from EPA for the approved uses. Because the EPA has classified pinene polymers (same as terpene polymers) into List 4 Inerts, they are acceptable for use in organic crop production.

Since the environmental impact assessment documents for use and manufacture of terpene polymers are available from FDA and EPA, there is no need for Miller Chemical to make further assessments for terpene polymers for use in organic crop production.

d) **Effects on human health**

The effects of terpene polymers on human health are outlined in this section.
The routes of entry of terpene polymers include (1) dietary exposure (its use in the food as a food additive (direct and indirect additive), (2) food (residues in food when used as an inert ingredient), (3) drinking water (as a possible contaminant in ground water) and (4) non-dietary exposure.

In examining aggregate exposure, the EPA considers the available information concerning exposures from the pesticide residue in food and all other non-occupational exposures, including drinking water from ground water or surface water and exposure through pesticide use in gardens, lawns, or buildings (residential and other indoor uses).

1. Dietary exposure. Synthetic terpene resin (containing polymers of α-and β-pinene and/or dipentene with varying molecular weights and composition) is approved by the Food and Drug Administration for use as an ingredient in chewing gum base as a direct food additive under 21 CFR Part §172.615. The terpene polymers are also approved

for use as indirect food additives (21 CFR §175.105, §177.1200 and §178.3930 for food contact and packaging applications. It is reported that the existing food applications result in some small amount of dietary exposure to pinene monomers, oligomers, and polymers. This exposure can be expected to be quite small given that only a small amount, if any, of the synthetic terpene resin present in a food-contact article will migrate into food. It is estimated that this would result in a per capita consumption of α- and β-pinene repeating units of only 1.7 mg/kg body weight per day for a 60-kg adult. Actual intake will be significantly less than this number, given that not all synthetic terpene resin is used in food applications.

2. Food. For the proposed agricultural uses (as an inert ingredient), it is estimated that the resulting dietary exposure will not exceed 0.43 mg/kg body weight per day for a 60-kg adult. Actual intake will be less than his number. Please note that this intake is a subset of the worst-case aggregate exposure number, 1.7 mg/kg body weight per day.

3. Drinking water. Because of its relative insolubility, only trace amounts of pinene polymer, if any, will be found in drinking water. Some amount of pinene polymer will enter the soil in fields where it is applied as part of a pesticide formulation. Any pesticide chemical (including terpene polymers) present in the soil could potentially reach ground water, as is the case with agricultural chemicals generally. In the case of pinene polymers, one would expect that they adhere to the soil due to their strong adhesive properties and that they may biodegrade before reaching ground water. It is noted that any drinking water exposure will be within the worst-case aggregate exposure estimate, 1.7 mg/kg body weight per day.

4. Non-dietary exposure. Outside of food applications, pinene polymers are reported to be used in various adhesive applications including construction adhesives used, for example, to lay floor tile. Since pinene polymers present in adhesives are not volatile, they will therefore not be inhaled. The only human exposure will be that associated with accidental skin contact. It would be difficult to assign a numerical value to this non-occupational exposure for a typical person. Exposures from all sources cannot exceed 1.7 mg/kg body weight per day for a typical adult, given the total production volume of pinene polymers.

Cumulative Effects

No identified risks are associated with exposure to pinene polymers. The EPA has not made a common mechanism of toxicity finding as to α-pinene and β-pinene or any - α- and/or-βpinene polymers. These chemicals do not appear to produce a toxic metabolite produced by other substances. These are lower toxicity chemicals; therefore, the resultant risks separately and/or combined should also be low. The EPA did not consider that neither pinene monomers nor pinene polymers have a common mechanism of toxicity with other substances.

Determination of Safety for U.S. Population, and Infants and Children

For safety determination, the EPA considered the toxicity data derived using α-pinene and β-pinene, which exhibit low acute toxicity by the oral, dermal and inhalation routes, and low subchronic toxicity. Polymers composed of α-and β-pinene monomers, even those of low molecular weight, should be even less toxic than α- and β-pinene considering that their absorption is decreased. Based on the available information on toxicity and exposure, EPA concluded that there is a reasonable certainty of no harm from aggregate exposure to residues of α- pinene, β-pinene, and the pinene polymers.

The EPA concluded that pinene polymers (terpene polymers) as well as α-pinene and β-pinene (monomers) are safe for the general population including infants and children.

e) **Effects on soil organisms, crops or livestock.**
As stated before, terpene hydrocarbon (α-pinene and β-pinene) is found in the atmosphere. They result from emission from deciduous and coniferous forests. They are not persistent in the soil because they will readily volatilize from soil. On the other hand, pinene polymers are stable and resistant to environmental degradation. They adhere tightly to soil. The soil organisms are unlikely to be affected by polymers derived from α-pinene and β-pinene because of their innocuous or non-toxic nature. The animal and aquatic organisms' data fully support this conclusion (see **Attachments 31 to 42**).

Pinene polymers are cleared by EPA under 40 CFR §180.910 for use on food crops (pre- and postharvest uses). Because of the clearance status under 40 CFR §180.910 for pinene polymers, agricultural crops are not affected by them. The residues from pinene polymers are negligible and the treated crops are safe for human consumption. (**Attachment 33**). Since pinene polymers can be used for general agricultural use (food and non-food uses), it is unlikely that their exposure to non-target crops/plants will have adverse effects as a result of spray and dust drift during applications.

Livestock consume food crops exposed to pinene polymers. They also graze on the pinene polymers-treated areas. Since pinene polymers can be used for general agricultural use (food and non-food uses), they are safe to livestock.. Based on the animal toxicity data on pinene polymers and its monomers (α- pinene and β-pinene), exposure to these chemicals is unlikely to cause any harm to livestock.

10. Safety information about the substance including a Material Safety Data Sheet (MSDS) and a substance report from the National Institute of Environmental Health Studies

To our knowledge, there are no reports from the National Institute of Environmental Health Studies (NHIES) on terpene polymers. The petitioner, however, found the following reports on the monomers used to make the terpene polymers:
a) National Toxicology Program (NTP); Health and Safety Information for α-pinene (CAS No. 80-56-8)
http://ntp-db.niehs.nih.gov/htdocs/H&S_archive.zip

b) NTP; Turpentine, Toxicological Literature Review.
http://ntp.niehs.nih.gov/htdocs/Chem_Backgroun/ExSumPdf/turpentine.pdf
c) NTP; Testing Status; α-Pinene
http://ntp.niehs.nih.gov/INDEX.CFM?OBJECTID=07105185-B741-02BD-E4334DA286D3041

We have presented other safety studies – see science assessment for α-pinene and β-pinene chemical by EPA [**Attachment 33**].

For acute toxicity to laboratory animals and aquatic organisms, see attached toxicity study (Miller 6064) reports [**Attachments 34-42**] and the Material Safety Data Sheets for terpene polymers and the formulated product (SUSTAIN) [**Attachment 2**].

11. Research information about the substance, which includes comprehensive substance research, reviews and research bibliographies, including reviews and bibliographies, which present contrasting positions to those, presented by the petitioner in supporting the substance's inclusion on or removal from National List.

There are several published and unpublished reports on the use of our products (Pinolene®, Nu-Film® 17, Nu-Film® P) containing terpene polymers (pinene polymers) as an adjuvant in pesticide products. It is used as a spreader sticker for improved pest control. All the available information fully supports our claims that our products exhibit excellent adhesive (sticking) properties. Such properties are useful for extending the duration of pesticidal activity and protecting the pesticide active ingredient from rainfall, among others. As far as we are aware, there are no reports that present contrasting positions.

1) Our products containing terpene polymers are being utilized in a number of ongoing organic farm research projects to help develop new markets for organic crop production. One such project is being coordinated by the North Carolina Cooperative Extension Service [**Attachment 17**].
2) Over the past three years, our products containing terpene polymers have played a major role in improving organic disease prevention of apple scab and other secondary diseases. [**Attachments 18 and 19**].
3) Our products (Nu-Film® 17 and Nu-Film® P) containing terpene polymer were shown to inhibit the U.V light and provides excellent sticker-spreader properties. This was reported in Fruit Grower, March 1997, page 30. [**Attachment 14**].
4) A number of other organic producers and/or companies have recommended the use of our products (Pinolene®, Nu-Film® 17, Nu-Film® P) to improve the performance of their pesticide products. Examples include potassium bicarbonate and various Bt products such as Trident and Thuricide [**Attachments 6, 9, 10, 11 and 12**].
5) Several trails have been conducted with our products (containing terpene polymers) in combination with herbicides, insecticides and fungicides as shown in **Attachments 13-28.**

For complete information on the product efficacy (Pinolene®, Nu-Film® 17, and Nu-Film® P), see **Attachments 6, 9, 10-28**.

12. Petition Justification Statement (see next page)

PETITION JUSTIFICATION STATEMENT

Miller Chemical and Fertilizer Corporation ("**Miller Chemical**") has been working with terpene polymer-based products (derived from pine trees) since the late sixties under various trademarks including Pinolene®, Nu-Film Bt, Nu-Film® 17, Nu-Film® P. These products have been used worldwide on conventional crops. In 1991, the California Coalition for Organic Farmers (CCOF) also has used them in organic production after the initial approval. When the National Organic Program (NOP) was established in 2002, the standards and requirements for listing products changed. It was determined that the products marketed by Miller Chemical under the above-mentioned trade names (Pinolene®, Nu-Film®17 and Nu-Film®P) were not in compliance with the current NOP listed/approved products.

Approval of Terpene Polymers for Organic Production:

As stated above, Nu-Film® 17 and Nu-Film® P are the old products and have been used for over 40 years. The terpene polymers (synonymously called pinene polymers) are listed in TSCA Inventory as UVCB chemical substances. The terpene polymers have been identified with defined chemical structures and CAS Registry Numbers. They are approved by the Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA) for food uses. The FDA approved them as "terpene polymers" with product specifications to meet the criteria for use as direct food additive and also as indirect food additive. Previously, the EPA previously cleared only β-pinene polymer (which is one of the terpene polymers) for food use. Based on this clearance status, the EPA included only β-pinene in List 4 Inerts. Very recently, the EPA approved 4 pinene polymers (including β-pinene polymer) derived from α- and β-pinene for use on food crops. As a result of this clearance, all pinene polymers (terpene polymers) are now eligible for inclusion in List 4 Inerts. [See Appendix 1 for a copy of the letter from Ms. Kathryn Boyle, U.S. Environmental Protection Agency.]

The current NOP requirement is that the synthetic substances as defined by Section 2103 of 7 USC 6502 must be on 7 CFR §205.601. Please note that 7 CFR §205.601 includes List 4 Inerts among others allowable for use in organic production. Since pinene polymers (which are the same as terpene polymers) are included in List 4 Inerts, they are allowable for use in organic crop production.
[For further information, consult: (1) PR Notice 2003-1 for Labeling of Pesticide Products under the National Organic Program, 12 Pages; (2) List 4 Inerts at http://www.epa.gov/opprd001/inerts/inerts_list4.pdf; and (3) Appendix 1 for a copy of the letter from EPA.]

Registration of Terpene Polymers

Our products (SUSTAIN, Pinolene®, Nu-Film Bt, Nu-Film® 17, Nu-Film® P) containing terpene polymers (pinene polymers) have been in use for a number of years. They are used in pesticide formulations applied to raw agricultural commodities. They are

approved under 21 CFR §582.99 for use as adjuvants added to pesticide use dilutions by a grower or applicator prior to applications to raw agricultural commodity. SUSTAIN is registered (Registration Number: 72-50015) with the California Sate Department of Pesticide Regulation for use as an adjuvant for pre- and postharvest uses.

Current Research Programs and Field Trials on Terpene Polymers for Organic Production

1) Our products containing terpene polymers are being utilized in a number of ongoing organic farm research projects to help develop new markets for organic crop production. One such project is being coordinated by the North Carolina Cooperative Extension Service (**Attachment 17**).
2) Over the past three years, our products containing terpene polymers have played a major role in improving organic disease prevention of apple scab and other secondary diseases. (**Attachments 18 and 19**).
3) A number of other organic producers and/or companies have recommended the use of our products (Pinolene®, Nu-Film® 17, Nu-Film® P) to improve the performance of their pesticide products. Examples include potassium bicarbonate and various Bt products such as Trident and Thuricide (**Attachments 6, 9, 10, 11 and 12**).
4) Several trails have been conducted with our products containing terpene polymers as shown in Attachments 13-28.

Based on these examples plus a number of other uses (that cover both conventional and organic crop systems - see **Attachments 6 and 9 – 28**), we are submitting this petition to request that terpene polymers be added to NOP List.

Complement/Supplement the Cultural Practices with Synthetic Substances

Growers have other options, which they utilize in their organic cultural practices for pest control such as orchard sanitation, planting resistant varieties and the implementation of biodiversity measures. They also have biological control measures including but not limited to cover crops and the use of beneficial insects and natural fungi. In many situations these cultural solutions alone are not sufficient enough to allow growers to continue producing top quality fruits and vegetables, Therefore, it has been well established in organic systems to use approved substances on the National List (e.g., sulfur, copper products, *Bacillus thurengenesis, granulosis viruses, and botanicals).* All of these materials have limitations under the various environmental conditions where they are applied.

Are there other adjuvants that could be used in organic production?
Yes. There are a few adjuvants that are cleared by EPA for pre- and postharvest uses. We believe that they could be used in organic production provided that they meet the requirements under NOP. Although these adjuvants are cleared by EPA for use on food crops, it is likely that all of them are not included in List 4 Inerts. [To be allowed for use in organic production, the adjuvants must be in List 4 Inerts.]

Why terpene polymers are favored over other adjuvants in organic production?

They require adjuvant chemistry to work very effectively and our products containing terpene polymers are better suited than other adjuvants available for organic production because of the following unique properties they exhibit.

1) The liquid terpene polymer product has excellent sticking properties and adheres well to leaves and other plant parts (applied area). Too frequently there is a lack of control due to the loss of the organic fungicide washing away during heavy rains. The liquid terpene polymer product maintains a greater percentage of the organic active ingredient under rainfall or irrigation **[Attachment 15]**.
2) The liquid terpene polymer product protects compounds such as *Bacillus thurengenesis* against UV light on the leaf surface. There is no deleterious effect on the viability of the spores or the physiology of the cells germinating from the spores. The terpene polymer extends the active life of the Bt spores two to four weeks longer than previously observed in the field. Similar effects have also been observed with natural viral and fungal compounds **[Attachment 14]**.
3) The liquid terpene polymer product reduces volatilization from organic pesticide applications made under arid conditions. This increases deposition and reduced crystallization of compounds that need to stay in a liquid state to maintain active. Examples of this are the AQ10 fungus and Trichoderma fungus, which are susceptible to degradation under high and low humidity **[Attachment 18]**.
4) The liquid terpene polymer products benefits mentioned above are good for the environment because they help reduce the number of organic pesticide applications necessary for good control, which reduces the amounts of pesticides exposed to the environment **[Attachment 12]**.
5) The liquid terpene polymer is stable (does not break or depolymerize) in soil and hence there is no potential for run off and for seeping into the soil. There is no potential for contamination of surface and ground water **[Attachments 31 – 33]**.
6) The monomers in terpene polymers are naturally occurring substances (α- and β-pinene), which are present in the atmosphere, and the polymers are derived from these substances. They are found to be much safer than the natural substances because the toxicity is reduced as a result of decreased absorption of polymers with increasing chain length. These polymers can be formed by self polymerization (via thermal process) **[Attachment 33]**.
7) As shown in EPA Science Assessment document, pinenes (α- and β-form) are present in the environment. Total U.S. emissions of α-pinene from deciduous and coniferous forests are 6.6 mega tons/annum (emission rate: 1.84 x 10 to the power of –10 g/sq cm/sec); daily mean concentration varies from: 0.305 ppb to 0.147 depending on the area (0.147 ppm for suburban, 0.120 ppb for urban, 0.035 ppb for remote and 0.030 ppb for rural area). α-Pinene is a component of trees, fruits grasses, brushes, fungi, herbs and flowers. Since humans and animals are exposed to α- and β-pinene, any breakdown or degradation products of terpene polymers are not of any health and environmental concern **[Attachment 33]**.
8) Terpene polymers are safe to animals on acute basis. The product (Miller 6064 which is the same as SUSTAIN) proposed for organic food production is not

acutely toxic to animals. **[Attachments 34-39]**. Based on these studies, they are not expected to be acutely toxic to humans.

9) Terpene polymers are used as a direct food additive (used as a base for chewing gum) and also an indirect food additive for food packaging materials. Their safety to humans is fully established **[Attachment 8]**.
10) Because of their proven safety, several terpene polymers are used in consumer and industrial products as household caulking materials, inks and other consumer products. **[Attachment 2** contains MSDSs for terpene polymer resins used in consumer and industrial products.]
11) Terpene polymers are safe to aquatic organisms. The product (Miller 6064 which is the same as SUSTAIN) proposed for organic food production is not acutely toxic to fish. **[Attachments 40-42]**.

Beneficial Effects of Terpene Polymers:

The USDA should encourage the use of the terpene polymers (Pinene polymers) for organic crop production because:

- They are safe to use and provide excellent support to pesticide active ingredients (with sticking and adhesive properties) for target pests.
- They are safe to humans because of very low toxicity (as compared to the naturally occurring monomers (α- and β-pinene) which are present in the environment and used in cosmetics and fragrances. Therefore, they are approved for use as direct and indirect food additives).
- They are safe to use in consumer products such as inks, caulking materials, among others.
- They are considered to be safe to animals that graze on the polyterpene-treated areas and also safe to animals fed the treated agricultural crops.
- They are considered safe to beneficial insects and other soil organisms due to low toxicity and no biodegradation in the environment.
- They are also safe to fish and wildlife due to low toxicity to aquatic organisms and animals.
- They are safe to the environment since terpene polymers are unlikely to migrate from the treated areas into air, water (surface and ground water).
- They are safe to consume the treated crops. Because they are safe, the EPA has given the tolerance exemption to terpene (pinene) polymers.

With regards to the product costs, the price for terpene polymers is comparable to other products (adhesives) that are used in pesticide products for crop production.

We request the USDA to take into the above-cited benefits into consideration when evaluating the terpene polymers and approve them for inclusion on the National List.

EVALUATION CRITERIA FOR SUBSTANCES ADDED TO THE NATIONAL LIST

Category 1. Adverse impacts on humans or the environment? Substance TERPENE POLYMERS

Question	Yes	No	N/A[1]	Documentation (TAP; petition; regulatory agency; other)
1. Are there adverse effects on environment from manufacture, use, or disposal? [§205.600 b.2]			X	
2. Is there environmental contamination during manufacture, use, misuse, or disposal? [§6518 m.3]		X		1. Petition (Attachments 4 & 7) 2. Applicable Food GMPs No environmental contamination.
3. Is the substance harmful to the environment? [§6517c(1)(A)(i);6517(c)(2)(A)i]		X		Petition (Attachments 7 and 33)
4. Does the substance contain List 1, 2, or 3 inerts? [§6517 c (1)(B)(ii); 205.601(m)2]		X		Contains ONLY List 4 Inerts. See Appendix 1 in Petition.
5. Is there potential for detrimental chemical interaction with other materials used? [§6518 m.1]		X		The substance is an inert material. No interaction between chemicals is expected. See Attachment 5 to Petition.
6. Are there adverse biological and chemical interactions in agro-ecosystem? [§6518 m.5]		X		Petition (Attachments 7 & 33). EPA has considered them for tolerance exemption.
7. Are there detrimental physiological effects on soil organisms, crops, or livestock? [§6518 m.5]		X		See Section 9(e) of the Petition. Also Attachments 32 to 42 to Petition.
8. Is there a toxic or other adverse action of the material or its breakdown products? [§6518 m.2]		X		See Section 9(d) of the Petition and Attachments 7 & 33. It is unlikely that terpene polymers give breakdown products.
9. Is there undesirable persistence or concentration of the material or breakdown products in environment?[§6518 m.2]		X		See Section 9(c) of the Petition. Also see Attachments 7 & 33 to the Petition.
10. Is there any harmful effect on human health? [§6517 c (1)(A)(i) ; 6517 c(2)(A)i; §6518 m.4]		X		See Section 9(d) of the Petition. See also Attachments 7 & 33 to Petition.
11. Is there an adverse effect on human health as defined by applicable Federal regulations? [205.600 b.3]			X	
12. Is the substance GRAS when used according to FDA's good manufacturing practices? [§205.600 b.5]			X	
13. Does the substance contain residues of heavy metals or other contaminants in excess of FDA tolerances? [§205.600 b.5]			X	

[1]If the substance under review is for crops or livestock production, all of the questions from 205.600 (b) are N/A—not applicable

Category 2. Is the Substance Essential for Organic Production? Substance TERPENE POLYMERS

Question	Yes	No	N/A[1]	Documentation (TAP; petition; regulatory agency; other)
1. Is there a natural source of the substance? [§205.600 b.1]			X	
2. Is there an organic substitute? [§205.600 b.1]			X	
3. Is the substance essential for handling of organically produced agricultural products? [§205.600 b.6]			X	
4. Is there a wholly natural substitute product? [§6517 c (1)(A)(ii)]		X		Not aware of any wholly natural substitute product. Terpene polymers are made from naturally occurring substances.
5. Is the substance used in handling, not synthetic, but not organically produced? [§6517 c (1)(B)(iii)]		X		It is the synthetic substance (polymer) made from natural product (terpene hydrocarbons - alpha & beta-pinene).
6. Is there any alternative substances? [§6518 m.6]		X		Not aware of any adjuvanys alternative to the petitioned substance.
7. Is there another practice that would make the substance unnecessary? [§6518 m.6]		X		Very doubtful so long as the pesticides are recommended for pest control.

[1]If the substance under review is for crops or livestock production, all of the questions from 205.600 (b)are N/A—not applicable.

Category 3. Is the substance compatible with organic production practices?

Substance TERPENE POLYMERS

Question	Yes	No	N/A[1]	Documentation (TAP; petition; regulatory agency; other)
1. Is the substance compatible with organic handling? [§205.600 b.2]			X	
2. Is the substance consistent with organic farming and handling? [§6517 c (1)(A)(iii); 6517 c (2)(A)(ii)]	X			See Petition Justification Statement and other information (Attachments 6 and 17 to Petition).
3. Is the substance compatible with a system of sustainable agriculture? [§6518 m.7]	X			The petitioner believes that terpene polymers are compatible with a system of sustainable agriculture (see Petition.)
4. Is the nutritional quality of the food maintained with the substance? [§205.600 b.3]			X	
5. Is the primary use as a preservative? [§205.600 b.4]			X	
6. Is the primary use to recreate or improve flavors, colors, textures, or nutritive values lost in processing (except when required by law, e.g., vitamin D in milk)? [205.600 b.4]			X	
7. Is the substance used in production, and does it contain an active synthetic ingredient in the following categories: a. copper and sulfur compounds;		X		Terpene polymers (SUSTAIN) are used as an adjuvant - which is an inert ingredient. It does NOT contain the referenced active synthetic ingredient, or category of substances.
b. toxins derived from bacteria;		X		Same explanation as shown above.
c. pheromones, soaps, horticultural oils, fish emulsions, treated seed, vitamins and minerals?		X		Same explanation as stated above.
d. livestock parasiticides and medicines?		X		Same explanation as stated above.
e. production aids including netting, tree wraps and seals, insect traps, sticky barriers, row covers, and equipment cleaners?		X		Same explanation as shown above.

[1]If the substance under review is for crops or livestock production, all of the questions from 205.600 (b) are N/A—not applicable.

APPENDIX 1

List Classification Status
On Certain Pinene Polymers

July 15, 2005 Letter

From

Kathryn Boyle
Registration Division
U.S. Environmental Protection Agency

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

July 15, 2005

N. Bhushan Mandava
Agent for Miller Chemical and Fertilizer Corporation
Mandava Associates
1730 M St. NW
Suite 906
Washington, DC 20036

SUBJECT: List Classification Status of Certain Pinene Polymers

Dear Dr. Mandava:

In your email, dated June 16, 2005, you requested that the Office of Pesticide Programs provide information on the suitability of including pinene polymers in certified organic pesticide products. Only List 4 inert ingredients can be used in organic pesticide products.

The Agency recently completed its assessment of alpha- and beta-pinene, and certain polymers composed only of these two monomers. These polymers are listed below:

Common Chemical Name	CAS Nomenclature	CAS Reg. No.
alpha-pinene polymer	Bicyclo[3.1.1]hept-2-ene, ,6,6-trimethyl-, homopolymer	25766-18-1
beta-pinene polymer	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, homopolymer (9CI)	25719-60-2
copolymer of alpha- and beta-pinene	Bicyclo[3.1.1]hept-2-ene, ,6,6-trimethyl-, polymer with 6,6-dimethyl-2-methylenebicyclo[3.1.1] heptane (9CI)	31393-98-3
polymerized alpha- pinene fraction from turpentine	Terpenes and Terpenoids, turpentine oil, alpha-pinene fraction, polymd.	70750-57-1

These four polymers are now classified as List 4B. However, the publicly available lists of List 4B inert ingredients (see http://www.epa.gov/opprd001/inerts/lists.html) have not yet been updated to include these four chemicals. They will be included in the next List 4B update.

Kathryn Boyle

Kathryn Boyle, Inerts Team
Minor Use, Inerts and Emergency Response Branch
Registration Division (7505C)

LIST OF ATTACHMENTS

1. Label for SUSTAIN®

2. Material Safety Data Sheets

   2.1. MSDS for PICCOLYTE® AO PINENE POLYMERS from Hercules, Inc.

   2.2. MSDS for SYLVARES™ TR A25 from Arizona Chemical

   2.3. MSDS for SUSTAIN™ from Miller Chemical and Fertilizer Corporation

3. Product Formulation for SUSTAIN®

4. Manufacturing Process for Terpene (Pinene) Polymers

5. Certificate of Analysis for Terpene Polymers

6. Organic Materials Review Institute (OMRI) of Potassium Bicarbonate and Pinolene Based Coating

7. Tolerance Exemption for Pinene Polymers under 40 CFR §180.910

   7.1. Final Rule (70 FR 28447, May 18, 2005)

   7.2. EPA Notice of Filing of Pesticide Petition (63 FR 64494, November 20, 1998)

8. Copies of Letters to Mr. Kerry Leifer and Mr. A.D. Vidyarthi

   8.1. Letter to EPA dated May 09, 2002 re. Reassessment of Tolerance Exemption for ∃-Pinene Polymers under 180.1001(c)

   8.2. Letter to Mr. A.D. Vidyarthi of Miller Chemical re. Terpene Polymers and their Approval Status under EPA and FDA Regulations

9. Sandoz Product Development for TRIDENT™ Biological Insecticide

10. Miller Chemical NU-FILM® with Thuricide® and Dimilin®

11. Evaluation of Nu-Film 17 with Trident, Javelin and Dipel in Insecticide & Acaricide Tests [Reference: VEGETABLE CROPS, Volume 14 (pages 121 and 147)]

12. **Copy of the July 22, 1971 Letter from Norman R. Dubois of USDA to Mr. Charles H. Svec of Miller Chemical re. Application of Pinolene for extending the Bt residual activity for at least 4 weeks.**

13. **Efficacy of Nu-Film 17 in improving codling moth mortality from granulosis virus. CMGV TRIAL of DSIR Interim Report dated December 3, 1991**

14. **NuFilm 17 or NuFilm P as a UV Inhibitor/Sticker-Spreader. See "Another Weapon in the War on Worms", *FRUIT GROWER*, Page 30 F, March 1997..**

15. **Recommend Nu Film 17 for Preventing Washoff by Rainfall and for Using as a Sticker. See "Instructions for the Use of THURICIDE to Control Gypsy Moth, Oak Moth, and other Leaf Eating Worms." International Minerals & Chemical Corporation.**

16. **Nu-Film Bt has excellent sticker properties and has been recommended for use with THURICIDE**

17. **Letter from North Carolina State University Recommending the Registration of Terpene Resin Products Like Nu-Film 17 for Organic Production.**

18. **Nu-Film 17 Uses - Field Sprays of Bacillus subtilis and Fungicides for Control of Preharvest Fruit Diseases of Avacado in South Africa, *PLANT DISEASE*, May 1997. Pages 455-459**

19. **Nu-Film 17 Uses – Disease Management in Organic Cucurbit Crops. 2004 Research Project for the Ohio Vegetable and Small Fruit Research and Development Program**

20. **Nu-Film 17 with Insecticides for Horticultural Insects: A paper from the 54th Conference Proceedings (2001) of The New Zealand Plant Protection Society Incorporated. Horticultural Insects. *New Zealand Plant Protection. 54:10-14 (2001)***

21. **Nu-Film P – For Management of Lettuce Aphid, *Nasonovia ribisnigri* (Mosley) *in* Organic and Reduced-risk (IPM) leaf Lettuce. Pest Management Grants-Applied Research. Contract # CDPR-99-0224-CHANEY-03/01**

22. **SUSTAIN®. Evaluation of Tank-Mix Combinations of Prefar 4-E Selective Herbicide in Melons (Gowan Trial Number: PRE-05-02-T1)**

23. **SUSTAIN® - Terpene Polymer. Rice Trial Ratings. South Texas Ag Research Coastal. June 2004 Fax Transmission from Larry Emerson.**

24. SUSTAIN® Trial on Fall Lettuce. Report from Soil Serve, Yuma, Az, November 23, 2003

25. SUSTAIN® in Carrots 2003. FMG Ag Products

26. SUSTAIN® on Flue Cured Tobacco. FMC Ag Products Group. 2004 Field Trials.

27. Miller 6064 Rice Herbicide Retention Study. Sills Ag Consulting Group. Summer 2001.

28. Nu-Film 17, Pinolene B and SUSTAIN®. Evaluate the Efficacy of Different Surfactants with Pristine for Powdery Mildew on Squash. Glades Crop Care, Inc. Spring 2004.

29. α-Pinene and its Insecticidal Properties

30. β-Pinene and its Insecticidal Properties

31. α-Pinene Polymer. PAN Pesticide Database. Identification, Toxicity, Use, Water Pollution Potential, Ecological Toxicity and Regulatory Information

32. β--Pinene Polymer. PAN Pesticide Database. Identification, Toxicity, Use, Water Pollution Potential, Ecological Toxicity and Regulatory Information

33. EPA Scientific Assessment for alpha- and beta- Pinene Chemicals. Kathryn Boyle, Registration Division, EPA, April 11, 2003

34. Acute Oral Toxicity Study in Rats on Miller 6064

35. Acute Dermal Toxicity Study in Rabbits on Miller 6064

36. Acute Inhalation Toxicity Study in Rats on Miller 6064

37. Acute Dermal Irritation Study in Rabbits on Miller 6064

38. Acute Eye Irritation Study in Rabbits on Miller 6064

39. Skin Sensitization Study (Guinea Pig Maximization Test for Topically Applied Test Substance) on Miller 6064

40. Bluegill Sunfish (*Lepomis macrochirus*) Static 96-Hour Acute Toxicity Test on Miller 6064

41. **Rainbow Trout (*Oncorhynchus mykiss*) Static 96-Hour Acute Toxicity Study on Miller 6064**

42. ***Daphnia magna* Static 48-Hour Acute Toxicity Test on Miller 6064**

ATTACHMENT 1

Label for SUSTAIN®

SUSTAIN

NON-IONIC SPREADER-STICKER

PRINCIPAL FUNCTIONING AGENTS:
Pinene (terpene) Polymers, petrolatum, a-(p-Dodecylphenyl) – Omega-hydroxypoly (oxyethylene)..... 100%

EPA Reg. No. - Exempt | Proprietary Protected Technology | EPA Est. No. 72-PA-1
Calif. Reg. No. 72-50015-AA

GENERAL INFORMATION

Sustain is a non-ionic spreader-sticker adjuvant designed to improve the contact, wetting and adhesion of pesticides onto the plant surface. **Sustain** forms a soft film, which polymerizes protecting spray, deposits from rainfall erosion, volatility and ultraviolet (UV) degradation. Under most conditions, apply sprays containing **Sustain** at least one half hour, during daylight, before an anticipated rain. Sunlight, direct or indirect, for this time period is needed for the film to set.

DIRECTIONS FOR USE

Sustain may be used with all products registered for: Agricultural, Forestry, Ornamental, Industrial Vegetation and Non-Cropland uses.

Sustain may be applied by ground or aerial spray equipment in concentrate or dilute sprays.

GROUND: Dosage per 100 gallons of spray solution.

Fungicides, Insecticides, Plant Growth Regulators ..... 4 oz. to 1 pt.
Herbicides ..... 4 oz. to 1 pt.

AIR: Use 4 to 16 oz per acre.

SOIL APPLIED PESTICIDES: To stabilize, improve performance and retard photo-degradation apply **Sustain** at 1 to 2 pints per acre.

MIXING

Fill spray tank one-half full with water and begin agitation. Add pesticides as directed by label and continue filling. Add **Sustain** last and continue agitation.

Use this product in accordance with good agronomic practices, which include utilizing proven spray equipment set for proper coverage. Do not make applications when temperatures are too hot. Applications should be made at temperature levels and when other environmental conditions in your area are such that your experience indicates the application will be compatible and will accomplish the desired result.

PHYTOTOXICITY PRECAUTION: Under some environmental conditions, some pesticides or pesticide combinations may cause phytotoxicity on growing plants. Adjuvant products such as this product may increase the chance or the intensity of phytotoxicity. Use this product in a manner consistent with individual pesticide product recommendations.

LIMITED WARRANTY: The use of this material being beyond our control and involving elements of risk to human beings, animals, and vegetation, we do not make any warranty, express or implied, as to the effects of such use, when this product is not used in accordance with the directions as stated on this label

PA Right-To-Know: This product contains proprietary ingredient(s). This product is intellectual property of Miller Chemical & Fertilizer Corporation.

1/04M 5MP

KEEP OUT OF REACH OF CHILDREN

CAUTION

Manufactured By:
MILLER CHEMICAL & FERTILIZER CORPORATION
P.O. Box 333, Hanover, Pennsylvania 17331

Net Contents: 2.5 Gallons Liquid

ATTACHMENT 2

Material Safety Data Sheets

2.1. MSDS for PICCOLYTE® AO PINENE POLYMERS from Hercules, Inc.

2.2. MSDS for SYLVARES™ TR A25 from Arizona Chemical

2.3. MSDS for SUSTAIN™ from Miller Chemical and Fertilizer Corporation

ATTACHMENT 2.1

Material Safety Data Sheet for PICCOLYTE® AO PINENE POLYMERS from Hercules, Inc.

MATERIAL SAFETY DATA SHEET

Hercules Incorporated
Pinova Division
Hercules Plaza
1313 North Market Street
Wilmington, DE 19894-0001
(302) 594-5000 (24 HRS)

1 PRODUCT IDENTIFICATION

PRODUCT NAME	**PICCOLYTE® AO PINENE POLYMERS**
CHEMICAL/COMMON NAME	alpha-pinene/beta-pinene copolymer
CAS NUMBER	31393-98-3

2 COMPOSITION / INFORMATION ON INGREDIENTS

OSHA Hazardous Ingredient	CASRN	Amount
alpha-pinene/beta-pinene copolymer	31393-98-3	95 - 100 %

This product is shipped and delivered as a LIQUID or HOT LIQUID. THE HMIS HEALTH HAZARD RATING IN SECTION 16 IS FOR THE HOT MOLTEN or HOT LIQUID PRODUCT ONLY. The HMIS health hazard rating for the ambient temperature product is 0 minimal.

When shipped at elevated temperatures and delivered in HOT MOLTEN or HOT LIQUID form, refer to MSDS Sections 3, 4, 5, 7, and 8 for additional hazard information.

3 HAZARDS IDENTIFICATION

EMERGENCY OVERVIEW
DANGER!

HOT MOLTEN product.
Burns may cause irreversible eye injury and blindness.
Causes skin burns.
Inhalation of smoke or fumes may cause throat discomfort, coughing, or breathing difficulty.
Product may burn if ignited.

CAUTION!

LIQUID product at ambient temperature: May cause eye irritation.
Inhalation of mist may cause respiratory tract irritation.
Prolonged or repeated contact may cause skin irritation.

Refer to Section 5 for Hazardous Combustion Products, and Section 10 for Hazardous Decomposition/Hazardous Polymerization Products.

4 FIRST AID MEASURES

SKIN
HOT MOLTEN product: Immediately cool skin burns with water and cold packs for at least 15 minutes. Do NOT put ice directly on the skin. Do NOT attempt to remove solidified resin from the skin as severe tissue damage may result. Get immediate medical attention. See Note to Physician.
LIQUID product at ambient temperature: Wash thoroughly with soap and water. Get medical attention if irritation develops or persists.

EYE
HOT MOLTEN product: Cool burns with plenty of low-pressure water. Get immediate medical attention.
LIQUID product at ambient temperature: Remove contact lenses. Hold eyelids apart. Immediately flush eyes with plenty of low-pressure water for at least 15 minutes. Get immediate medical attention.

INHALATION
Remove to fresh air. Get medical attention if nasal, throat or lung irritation develops.

INGESTION
Not an ingestion hazard under anticipated conditions of use. For accidental ingestion: Do NOT induce vomiting. Get immediate medical attention.

NOTES TO PHYSICIAN
For HOT MOLTEN or HOT LIQUID product: Material should not be forcibly pulled from the skin. Mineral oil may be used to loosen and soften the material.

5 FIRE FIGHTING MEASURES

EXTINGUISHING MEDIA
Water spray, dry chemical, foam, carbon dioxide or clean extinguishing agents may be used on fires involving this product.

FIRE FIGHTING PROCEDURES
Wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH approved (or equivalent) and full protective gear when fighting fires involving this product.
Cool containers with water to prevent rupture.
Apply water to HOT MOLTEN resin fires from a safe, protected location to avoid body contact with hot resin.

CONDITIONS TO AVOID
None known.

HAZARDOUS COMBUSTION PRODUCTS
If heated to combustion, the following substances may be formed: carbon monoxide , carbon dioxide , aldehydes , carboxylic acids and smoke

FLASH POINT 270 ° F Cleveland Open Cup

6 ACCIDENTAL RELEASE MEASURES

Ventilate area. For small spills: Absorb spill with inert material (e.g., dry sand or earth), then place in a chemical waste container. Wash area with soap and water. For large spills: Dike to contain and pump into drums for use or disposal. In case of accidental spill or release, refer to Section 8, Personal Protective Equipment and General Hygiene Practices.

7 HANDLING & STORAGE

GENERAL MEASURES
For HOT MOLTEN or HOT LIQUID product: Use personal protective equipment as indicated in Section 8: Exposure Controls/Personal Protection.
Store in a cool, dry place: approximately 20° C (68° F).

MATERIALS OR CONDITIONS TO AVOID
Water contact with HOT MOLTEN or HOT LIQUID resin may result in foaming or spattering, which can cause eye or skin burns.

8 EXPOSURE CONTROLS / PERSONAL PROTECTION

WORK PRACTICES & ENGINEERING CONTROLS
Eyewash fountains and safety showers should be easily accessible.
Provide adequate ventilation.
If upper respiratory tract irritation occurs, use an approved dust/mist respirator to minimize exposure.

GENERAL HYGIENIC PRACTICES
Avoid contact with eyes, skin, and clothing.
Avoid breathing vapor, fumes or mist.
Handle in areas with adequate ventilation.
Avoid contamination of food, beverages, or smoking materials.
Wash thoroughly after handling, and before eating, drinking or smoking.
Remove contaminated clothing promptly and clean thoroughly before reuse.

RECOMMENDED EXPOSURE LIMITS
No exposure limit has been established. This product may irritate the upper respiratory tract if used under conditions that create dust or mist particulates.

PERSONAL PROTECTIVE EQUIPMENT
Safety glasses
Impervious gloves
Appropriate protective clothing
Wear approved dust/mist respirator if user operations create dust/mist that causes irritation.
Personnel exposed to HOT MOLTEN or HOT LIQUID material should wear protective clothing that provides protection against thermal burns. Required Protective Equipment: a) Longsleeved protective shirt, long pants, and work shoes; b) Hard hat and face shield; c) Long-cuff gloves (Gauntlet type-extending beyond the wrist); d) Lined rainsuit with protective hood or shoulder shroud or e) Full aluminized or thermal suit with hood.

PROTECTIVE MEASURES DURING REPAIR AND MAINTENANCE
Eliminate ignition sources.
Completely isolate and thoroughly clean all equipment, piping, or vessels before beginning maintenance or repairs.
Keep area clean. Product will burn.

9 PHYSICAL & CHEMICAL PROPERTIES

PHYSICAL STATE:	hot molten viscous liquid at 130-190° C (266-374° F); liquid
COLOR:	hot molten product: pale gray; liquid product: light amber
ODOR:	typical rosin
Specific Gravity	0.96
Percent Volatile	negligible at 68° F
Evaporation Rate	slower than butyl acetate
Solubility In Water	slightly soluble

10 STABILITY & REACTIVITY

HAZARDOUS DECOMPOSITION PRODUCTS
None known.

HAZARDOUS POLYMERIZATION
Not anticipated under normal or recommended handling and storage conditions.

GENERAL STABILITY CONSIDERATIONS
Stable under recommended handling and storage conditions.

INCOMPATIBLE MATERIALS
None known

11 TOXICOLOGICAL INFORMATION

CARCINOGENICITY INFORMATION
PRODUCT/SIMILAR PRODUCT - Not listed as a carcinogen by NTP. Not regulated as a carcinogen by OSHA. Not evaluated by IARC.

REPORTED HUMAN EFFECTS
PRODUCT/SIMILAR PRODUCT - No human toxicity studies have been carried out with this product.

REPORTED ANIMAL EFFECTS
PRODUCT/SIMILAR PRODUCT - Not toxic by OSHA/ANSI criteria based on acute animal peroral testing of this or a similar product (LD50 >500 mg/kg). Eye irritation (rabbit): mild. Ninety-day feeding studies of up to 50,000 ppm in the diet caused body weight depression at the highest dose due to poor palatability, and increase in liver size.

12 ECOLOGICAL INFORMATION

ECOTOXICOLOGICAL INFORMATION
PRODUCT/SIMILAR PRODUCT - No ecological studies have been carried out on this product.

13 DISPOSAL CONSIDERATIONS

WASTE DISPOSAL
Incineration in accordance with applicable regulations is the recommended disposal method. Landfilling in a permitted solid or hazardous waste facility is a suitable alternative after solidification to remove free liquids. Waste water may be sent to a sanitary sewer treatment facility in accordance with any local agreement, a permitted waste treatment facility, or discharged under a permit. Disposal should be in accordance with applicable Federal, State and local regulations.

14 TRANSPORT INFORMATION

GENERAL
This product is not subject to DOT regulations.

For specific information regarding transportation of this product, please call the Hercules representative at (905) 279-3338.

15 REGULATORY INFORMATION

CHEMICAL INVENTORIES
U.S. TSCA: The components of this product are included on the TSCA Inventory.

SARA TITLE III - SECTIONS 302/304
This product is not an Extremely Hazardous Substance subject to reporting under 40CFR355.

SARA TITLE III - SECTION 311 AND 312
HC-1: Acute health hazard
HC-3: Fire hazard

SARA TITLE III - SECTION 313
This product does not contain any chemicals subject to reporting under Section 313 of Title III of the Superfund Amendments and Reauthorization Act and 40CFR372.

CERCLA
This product does not contain any chemicals subject to reporting as a CERCLA Hazardous Substance under 40CFR302.4.

RCRA
This product is not a hazardous waste as listed in 40CFR261.33. It does not exhibit any of the hazardous characteristics listed in 40CFR261, Subpart C.

16 OTHER INFORMATION

HMIS RATINGS:

Health	3	Serious Hazard
Flammability	1	Slight Hazard
Reactivity	0	Minimal Hazard

LIST OF ACRONYMS

ACGIH: American Conferences of Governmental Industrial Hygienists
AIHA WEEL: American Industrial Hygienists Association-Workplace Environmental Exposure Level
ANSI: American National Standards Institute
CASRN: Chemical Abstracts Service Registry Number
CEPA: Canadian Environmental Protection Act
CERCLA: Comprehensive Emergency Response, Compensation and Liability Act
DSL: Domestic Substances List (Canadian)
HMIS: Hazardous Materials Identification System
IARC: International Agency for Research on Cancer
NDSL: Non-Domestic Substances List (Canadian)
NTP: National Toxicology Program
OSHA: Occupational Safety and Health Administration
PEL: Permissible Exposure Limit (OSHA)
RCRA: Resource Conservation and Recovery Act
RQ: Reportable Quantity
SARA: Superfund Amendment Reauthorization Act
STEL: Short-Term Exposure Limit
TLV: Threshold Limit Values (registered trademark of ACGIH)
TPQ: Threshold Planning Quantity
TSCA: Toxic Substance Control Act
TWA: Time Weighted Average

DISCLAIMER

The information and recommendations contained in this Material Safety Data Sheet have been compiled from sources believed to be reliable and to represent the most reasonable current opinion on the subject when the MSDS was prepared. No warranty, guaranty or representation is made as to the correctness or sufficiency of the information. The user of this product must decide what safety measures are necessary to safely use this product, either alone or in combination with other products, and determine its environmental regulatory compliance obligations under any applicable federal or state laws.

MSDS STATUS

Supersedes Date	MSDS Revision(s)
05/29/2003	Section 4

ATTACHMENT 2.2

Material Safety Data Sheet for SYLVARES™ TR A25 from Arizona Chemical

Arizona CHEMICAL

Material Safety Data Sheet

Section 1. Chemical Product and Company Identification

Product/Trade Name	**SYLVARES™ TR A25**
Supplier / Manufacturer	Arizona Chemical P.O. Box 550850 Jacksonville, FL 32255-0850 USA (800) 526-5294 / (904) 928-8700
Chemical Name	Terpene Resin

Code	TRA25
MSDS#	2198
Validation Date	1/31/2005
Print Date	1/31/2005

EMERGENCY PHONE
CHEMTREC: 1-800-424-9300
(transportation and medical)

Section 2. Composition and Information on Ingredients

Name	CAS #	% by Weight
1) Terpene Resin	Proprietary, NJTSRN-2198	> 99%

See Section 8 for Exposure Controls/ Exposure Limits/ Personal Protection information.

Section 3. Hazards Identification

EMERGENCY OVERVIEW

Product is a yellow, semi-solid or viscous liquid. *If product is frozen and ground to a fine dust:* Product may form explosive dust/air mixture if high concentration of product dust is suspended in air. Static electric charges created by emptying product from ungrounded containers in or near flammable vapors may cause flash fire. May cause eye irritation. Inhalation of vapors/fumes generated by heating this product may cause respiratory irritation with throat discomfort, coughing, or difficulty breathing.

HMIS

HEALTH:	1
FIRE:	1
REACTIVITY:	0
PPE:	see Section 8 of this MSDS.

0=Minimal; 1=Slight; 2=Moderate; 3=Serious; 4=Severe; (*)=Chronic health hazard.

Potential Health Effects

Eye Contact	Product may cause eye irritation. Rubbing may cause abrasion of the cornea. Symptoms may include irritation, redness, scratching of the cornea, and tearing. Vapors may also cause eye irritation. If heated product contacts the eye, thermal burns may result.
Skin Contact	Prolonged or repeated skin contact may cause irritation. When it is heated, this product may cause thermal burns.
Inhalation	Inhalation of vapors/fumes generated by heating this product may cause respiratory irritation with throat discomfort, coughing and difficulty breathing.
Ingestion	Ingestion of product may produce mild gastrointestinal disturbances.

Section 4. First Aid Measures

Eye Contact	Immediately flush eyes with flooding amounts of cool, low pressure water for at least 15 minutes. If irritation persists, get medical attention. If hot/molten product contacts eye, flush with water for at least 15 minutes and seek medical attention immediately.
Skin Contact	In case of skin contact, wash immediately with soap and water. If irritation develops or persists, seek medical attention. If hot product contacts skin, cool under running water and seek medical attention. Do not attempt to remove the hot, molten or cooled product from the skin.
Inhalation	Move person to non-contaminated air. If affected person is not breathing, apply artificial respiration. Seek medical attention.
Ingestion	If swallowed, contact a physician or poison control center immediately. DO NOT induce vomiting unless directed to do so by medical personnel.

Continued on Next Page

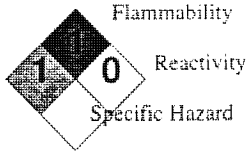
Notes to Physician	Provide general supportive measures and treat symptomatically. In case of ingestion, the decision of whether or not to induce vomiting should be made by the attending physician. If burn is present, treat as any thermal burn. Removing adhered product from burned skin may compromise the skin integrity and result in infection and/or more severe scarring.

****If victims of chemical over-exposure are taken for medical attention, give a copy of the label or MSDS to the physician/health professional.****

Section 5. Fire and Explosion Data

Flammability of the Product	Nonflammable.
Auto-Ignition Temperature	Not available.
Flash Point	CLOSED CUP: >121.11°C (250°F). (Setaflash Closed Cup.)
Flammable Limits	Not available.

NFPA 704



0=Minimal; 1=Slight; 2=Moderate; 3=Serious; 4=Severe

This information is for people trained in the National Fire Protection Association's (NFPA 704) identification of the Fire Hazards of Materials.

General Fire Hazards	*If product is frozen and ground into a fine dust:* may form explosive dust/air mixture if high concentration of product dust is suspended in air. Static electric charges created by emptying product from ungrounded containers in or near flammable vapors may cause flash fire. Product is not considered combustible. If heated above its flash point in the presence of air, product can support combustion.
Hazardous Decomposition Products	Smoke, carbon monoxide, carbon dioxide, and other products of combustion.
Extinguishing Media	Carbon dioxide, dry chemical or water.
Fire Fighting Equipment and Instructions	Wear full protective clothing, including self-contained positive pressure or pressure demand breathing apparatus, helmet, protective clothing and face mask. Use water to cool fire-exposed containers and to protect personnel.

Section 6. Accidental Release Measures

Containment	Contain the discharged material. If airborne dust is generated, eliminate all sources of ignition that may come into contact with the dust.
Clean-up Procedures	Wear appropriate protective equipment and clothing during clean-up. *If product is in dust form:* avoid generation of dust during clean-up. Wear an approved respirator if dust is generated above exposure limits. Attempt to reclaim free product, if this is possible. Shovel material into appropriate container for disposal. Follow all Local, State, Federal, and Provincial regulations for disposal.
Evacuation Procedures	Persons not wearing appropriate protective equipment should be excluded from area of spill until clean-up has been completed.
Special Instructions	Avoid contact with skin and eyes. Avoid skin contact with molten resins. Avoid inhalation of dust from spilled material. Avoid inhalation of fumes from molten product.

Section 7. Handling and Storage

Handling	Avoid eye and skin contact. *If product is in dust form:* avoid breathing dusts from this material. Avoid breathing fumes if product is used at high temperatures. Maintain good housekeeping to prevent dust accumulation. Flaked or crushed material may cause a dust problem. *If product is in dust form*, it is classified as a dust explosion hazard class II. Handling of product in dust form should be in accordance with NFPA. If handling with flammable or combustible materials, the explosion hazard may increase. Avoid ignition sources such as sparks and flame. In addition, when emptying bags where flammable vapors may be present, blanket vessel with inert gas; assure proper grounding (NFPA 69 - Explosion Prevention Systems; NFPA 70 - National Electric Code; NFPA 77 - Recommended Practices on Static Electricity; NFPA 654 - Standard for the Prevention of Fire and Dust Explosions in the Chemical, Dye, Pharmaceutical, and Plastics Industry), and pour material slowly into conductive grounded chutes. An explanation of dust explosions is available in Technical Bulletin #1. Please contact customer service to request a copy. Do not reheat product packaged in light metal containers. The light metal containers will not safely support the movement or transfer of the product in a hot, molten form. Do not chisel drums in areas where flammable liquids are stored or used. Wash thoroughly after handling. Never eat, drink, or smoke in work areas. Practice good personal hygiene after using this material, especially before eating, drinking, smoking, using the toilet or applying cosmetics.
Storage	Store at ambient temperature and atmospheric pressure. Guard against dust accumulation of this material. Flaked or crushed product may be prone to oxidation, therefore control inventory - use oldest material first. Suggest stainless steel construction for bulk storage.

Continued on Next Page

Section 8. Exposure Controls/Personal Protection

Engineering Controls

Provide local exhaust and general ventilation systems to maintain airborne concentrations below OSHA, ACGIH, and manufacturer recommended exposure limits. Local exhaust ventilation is preferred because it prevents contaminant dispersion into work areas by controlling it at its source. Local exhaust ventilation is recommended when generating excessive levels of airborne dust or vapors from handling or thermal processing. Use electrically grounded, explosion-proof equipment for ventilation or any handling of this product.

Personal Protection

Eye/Face:

Wear chemical goggles and face shield if handling molten material. Ensure compliance with OSHA's personal protective equipment (PPE) standard for eye and face protection, 29 CFR 1910.133.

Skin:

Use impervious gloves. Work clothing sufficient to prevent all skin contact should be worn, such as coveralls and long sleeves. For heated/molten product, use any type thermal insulating gloves and other clothing as necessary to protect from thermal burns. Ensure compliance with OSHA's personal protective equipment (PPE) standard, 29 CFR 1910.132 (general) and 138 (hand protection).

Respiratory:

Respirators should be selected by and used under the direction of a trained health and safety professional following requirements found in OSHA's respirator standard (29 CFR 1910.134) and ANSI's standard for respiratory protection (Z88.2-1992). A written respiratory protection program, including provisions for medical certification, training, fit testing, exposure assessments, maintenance, inspection, cleaning, and convenient, sanitary storage, must be implemented.

DUST/MIST: If concentrations are below the TLV and/or PEL, a NIOSH-approved disposable dust/mist respirator may be used for personal comfort. For concentrations above the TLV and/or PEL but less than 10 times these limits, a NIOSH-approved half-facepiece respirator equipped with dust-mist cartridges may be used. For concentrations greater than 10 times the TLV and/or PEL, consult the NIOSH respirator decision logic found in Publication No. 87-116 or ANSI Z88.2-1992. Note: ANSI Z88.2-1992 requires the use of a HEPA filter if the particle size distribution of the contaminant is unknown. Warning! Air-purifying respirators do not protect workers in oxygen-deficient atmospheres.

For molten/heated product:
GAS/VAPOR: For concentrations above the TLV and/or PEL but less than 10 times these limits, a NIOSH-approved half-face piece respirator equipped with appropriate chemical cartridges may be used. For concentrations greater than 10 times the TLV and/or PEL, consult the NIOSH respirator decision logic found in Publicaton No. 87-116 or ANSI Z88.2-1992. Warning! Air-purifying respirators do not protect workers in oxygen-deficient atmospheres.

General:

Use good industrial hygiene practices in handling this material. Eye wash fountains and emergency showers are recommended. Launder contaminated clothing before reuse.

If product is frozen and ground to a fine dust:
Observe exposure limits for Particulates (NOC):
ACGIH TLV TWA: 10 mg/m^3 Total dust; **ACGIH TLV TWA:** 5 mg/m^3 Respirable dust;
OSHA PEL TWA: 15 mg/m^3 Total dust; **OSHA PEL TWA:** 5 mg/m^3 Respirable dust.

Xylene is typically present at a residual level of < 200ppm. Observe exposure limits for xylene:
ACGIH TLV TWA: 100 ppm;
OSHA PEL TWA: 100 ppm;
OSHA STEL: 150 ppm.

Chemical Name or Product Name	CAS #	OSHA PEL	ACGIH TLV
1) Terpene Resin	Proprietary, NJTSRN-2198	Not established	Not established

NOTE: The 1989 OSHA PELs were vacated in 1993 and are not currently enforceable by Federal OSHA. However, some state OSHA programs may still enforce the 1989 limits.

Section 9. Physical and Chemical Properties

Physical state and appearance	Semi-solid or visocous liquid	Vapor Density	Not available
Odor	Odorless.	Percent Volatile (EPA Method 24)	3-5
Color	Yellow.	Solubility (water)	Negligible
Molecular Weight	314	Density (vs. water)	> 1
Specific Gravity	>1 (Water = 1)	Flash Point	CLOSED CUP: >121.11°C (250°F). (Setaflash Closed Cup.)

Continued on Next Page

Boiling Point	Not applicable	R/B Softening Point	22 - 28 °C
pH	Not applicable	Acid No. (per ASTM D-465)	Not available

Section 10. Stability and Reactivity Data

Chemical Stability	The product is stable.
Conditions to avoid	Avoid strong oxidizing agents.
Incompatibility	May react with strong oxidizing agents.
Hazardous Decomposition Products	Smoke, carbon monoxide, carbon dioxide, and other products of combustion.
Hazardous Polymerization	Hazardous polymerization will not occur.

Section 11. Toxicological Information

Toxicity to Animals	**Terpene resin**: ORAL, rats, LD50 = > 5,000 mg/kg; DERMAL, rabbits, LD50 = > 2,000 mg/kg; This product was found to be a minimal to mild skin irritant and not a significant eye irritant in rabbits.
Toxicity to Humans	Contact may cause skin or eye irritation. Ingestion may cause nausea, vomiting and diarrhea. Inhalation of vapors/fumes generated by heating this product may cause respiratory irritation with throat discomfort, coughing or difficulty breathing. **CARCINOGENIC EFFECTS**: None of this product's components are listed as carcinogens by ACGIH, IARC, NIOSH, NTP or OSHA. **MUTAGENIC EFFECTS**: Not available. **TERATOGENIC EFFECTS**: Not available. No information is available on the toxicity of this product to the reproductive system.

Section 12. Ecological Information

Ecotoxicity	No information is available.
Environmental Fate	No information is available.

Section 13. Disposal Considerations

Waste Disposal	Waste material must be tested using methods described in 40 CFR 261 to determine if it meets applicable definitions of hazardous waste. No EPA Waste Numbers are applicable for this product's components. Dispose of waste material according to Local, State, Federal, and Provincial Environmental Regulations. Write to the address listed in Section 1 for information on heavy metals analysis and other disposal information.

Section 14. Transport Information

DOT Classification	Not a DOT controlled material (United States).
Proper Shipping Name	None.
DOT Identification Number	None.
Packing Group	None.
Hazardous Substances Reportable Quantity	Not available.
Special Provisions for Transport	IF SHIPPED OVER 100°C (but less than product flash point): DOT Shipping Name: Elevated temperature liquid, n.o.s.; Hazard Class: 9; UN/NA Number: UN3257; Packing group III (bulk shipping requires "HOT" placard).
Additional Shipping Information	Not Determined

Continued on Next Page

International Transportation Regulations	Not Determined

Section 15. Regulatory Information

Federal and State Regulations

OSHA: Not hazardous by definition of Hazard Communication Standard (29 CFR 1910.1200).

SARA TITLE III:
SARA Section 302 (40 CFR 355 Appendix A): **None of this product's components are listed;**
SARA Section 311/312: **None;**
SARA Section 313 (40 CFR 372.65): **None of this product's components are listed;**
CERCLA (40 CFR 302.4): **None of this product's components are listed.**

TSCA Inventory: All of this product's components are listed.

International Inventories: All of this product's components are on or exempt from these inventories: Canada (DSL), Europe (EINECS or NLP), Japan (ENCS), China (IECS), Korea (ECL), Philippines (PICCS), Australia (AICS)..

This product contains antioxidant(s).

State Lists: None of this product's components are listed in CA, FL, MA, MN, NJ, or PA.

This product may contain trace levels of ethylbenzene, a component of xylene, which is currently on the California List of Known Carcinogens and Reproductive Toxins.

Section 16. Other Information

Key/Legend

ACGIH = American Conference of Governmental Industrial Hygienists. ANSI = American National Standards Institute. ASTM = American Society for Testing and Materials. CERCLA = Comprehensive Environmental Response, Compensation and Liability Act. DOT = Department of Transportation. EPA = Environmental Protection Agency. IARC = International Agency for Research on Cancer. LD = Lethal Dose. NIOSH = National Institute of Occupational Health and Safety. NTP = National Toxicology Program. OSHA = Occupational Safety and Health Administration. PEL = Permissible Exposure Limit. SARA = Superfund Amendments and Reauthorization Act. TLV = Threshold Limit Value. TSCA = Toxic Substance Control Act.

Validated by Cindy Smith on 1/31/2005.	**Verified by Product Regulatory Affairs.**
	Printed 1/31/2005.

Supersedes Date 05/10/04 **Reason for Revision** **Updated Section 15.**

ATTACHMENT 2.3

Material Safety Data Sheet for SUSTAIN™ from Miller Chemical and Fertilizer Corporation

CHEMICAL & FERTILIZER CORPORATION

MATERIAL SAFETY DATA SHEET

IDENTITY **SUSTAIN™**

Section I

Manufacturer's Name	Emergency Telephone Number
MILLER CHEMICAL & FERTILIZER CORP.	CHEMTREC: 1-800-424-9300 717-632-8921
Address BOX 333, RADIO ROAD HANOVER, PA 17331	Telephone Number for Information 717-632-8921 Date Prepared 12/1/01

Section II - Hazardous Ingredients/Identity Information

NAME	CAS #	OSHA PEL	ACGIH TLV	OTHER LIMITS RECOMMENDED
THIS PRODUCT HAS BEEN TESTED AS A WHOLE TO DETERMINE IT'S HAZARDS	NA	ND	ND	245mg/m3

Recommended NFPA Rating: HEALTH: NE FIRE: NE REACTIVITY: NE

THE NFPA RATING HAS NOT BEEN ESTABLISHED FOR THIS PRODUCT

PA Right-to-Know: This product contains proprietary ingredients.

This product contains the following chemical(s) subject to the reporting requirements of Section 313 of Title III of the Superfund Amendments and Reauthorization Act of 1986 and 40 CFR part 372 (the corresponding CAS number and typical percent by weights are also provided).

NONE

Section III - Physical/Chemical Characteristics

Percent Volatile	No data available	Specific Gravity (H20=1)	0.92-0.94 @ 20c
Vapor Pressure (mm Hg @ 25c)	No data available	Melting Point	No data available
Vapor Density (AIR=1)	No data available	Evaporation Rate (Butyl Acetate=1)	ND
Solubility in Water	EMULSIFIES	pH(5% solution)	6.5 - 7.5
Appearance and Odor	YELLOW TO AMBER LIQUID: MODERATE ODOR		

Section IV - Fire and Explosion Hazard Data

Flash Point	> 200 c	Flammable Limits	ND	LEL	ND	UEL ND

Extinguishing Media: FOAM, CARBON DIOXIDE, DRY CHEMICAL, WATERSPRAY OR SAND/EARTH

Special Fire Fighting Procedures: USE WATER SPRAY TO KEEP FIRE-EXPOSED CONTAINERS COOL. USE SUPPLIED AIR BREATHING APPARATUS EQUIPMENT. USE WATER SPRAY TO DISPERSE VAPORS.

Unusual Fire and Explosion Hazards: EVACUATE PEOPLE DOWNWIND FORM FIRE. CONTROL RUNOFF WATER.

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid	EXCESSIVE HEAT, SOURCES OF IGNITION, STRONG OXIDIZERS
	Stable	X		

Incompatability(materials to avoid): STRONG OXIDIZERS

Hazardous Decomposition or Byproducts: OXIDES OF CARBON UNDER FIRE CONDITIONS

Hazardous Polymerization	May Occur		Conditions to Avoid	NONE KNOWN
	Will Not Occur	X		

NA - Not Available or Not Applicable
ND - Not Determined

Section VI - Health Hazard Data

Route(s) of Entry	Inhalation?	YES	Skin?	YES	Ingestion? YES
Health Hazards (Acute and Chronic)	ORAL TOXICITY: RATS >5050 mg/kg.				
DERMAL TOXICITY RATS >5050 mg/kg			INHALATION TOXICITY: LC50 >5.26 mg/L		
THIS PRODUCT IS CLASSIFIED AS A NON SKIN SENSITIZER (GUINEA PIG)					
THIS PRODUCT MAY CAUSE SLIGHT EYE IRRITATION					
THIS PRODUCT MAY CAUSE MILD EYE, SKIN AND THROAT IRRITATION					
CHRONIC: NO DATA AVAILABLE					
Carcinogenicity: NTP?	NO	IARC Monographs?	NO	OSHA Regulated?	NO
Signs and Symptoms of Exposure	NO DATA AVAILABLE				
Medical Conditions Generally Aggravated by Exposure	CONTACT MAY CAUSE IRRITATION				
Emergency and First Aid Procedures	**INGESTION:** DO NOT INDUCE VOMITING, CALL A PHYSICIAN.				
EYES:	IRRIGATE WITH WATER FOR AT LEAST 15 MINUTES, SEEK MEDICAL ATTENTION.				
SKIN:	REMOVE ANY CONTAMINATED CLOTHING AND WASH SKIN WITH SOAP AND WATER.				
INHALATION:	REMOVE VICTIM TO FRESH AIR, CALL A PHYSICIAN				

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in Case Material is Released or Spilled	ELIMINATE ALL SOURCES OF IGNITION. DIKE OR IMPOUND TO KEEP PRODUCT OUT OF SEWERS AND WATERCOURSES. ABSORB SPILL WITH INERT MATERIAL, SHOVEL INTO WASTE CONTAINERS. WASH AREA WITH WATER. ABSORB WATER WITH INERT MATERIAL. CONTINUE THIS PROCEDURE UNTIL NO ODOR REMAINS.
Waste Disposal Method	DISPOSE OF WASTE AND WASTE CONTAINERS IN ACCORDANCE WITH LOCAL/STATE/FEDERAL REGULATIONS.
Precautions to be Taken in Handling and Storing	KEEP CONTAINERS CLOSED WHEN NOT IN USE. KEEP FROM SOURCES OF IGNITION. DO NOT CONTAMINATE WATER, FOOD, FEED BY STORAGE OR DISPOSAL. FOLLOW GOOD INDUSTRIAL HYGIENE PRACTICES. STORE BETWEEN 40.5 F AND 120.5 F.
Other Precautions	KEEP FROM CHILDREN AND ANIMALS. AFTER WORKING WITH THIS PRODUCT, THOROUGHLY CLEAN EQUIPMENT. WASH THOROUGHLY, CHANGE CLOTHING, AND CLEAN PROTECTIVE GEAR.

Section VIII - Control Measures

Respiratory Protection	A RESPIRATOR APPROVED BY NIOSH/MSHA SHOULD BE WORN WHERE VAPOR INHALATION COULD OCCUR.				
Ventilation	Local Exhaust	NA	Special	NA	
	Mechanical	PREFERRED	Other	NA	
Protective Gloves	CHEMICAL RESISTANT (e.g. rubber)			Eye protection	CHEMICAL SPLASH GOGGLES
Other Protective Clothing or Equipment	CHEMICAL RESISTANT APRON, CLEAN BODY-COVERING CLOTHING, BOOTS, HAT				
Work/Hygienic Practices	PREVENT EATING, DRINKING, TOBACCO USAGE AND COSMETIC APPLICATION TO PREVENT EXPOSURE.				

THE SUBMISSION OF THIS MSDS MAY BE REQUIRED BY LAW BUT THIS IS NOT AN ASSERTION THAT THIS SUBSTANCE IS HAZARDOUS WHEN USED IN ACCORDANCE WITH PROPER SAFETY PRACTICES AND NORMAL HANDLING PROCEDURES.

THE INFORMATION HEREIN IS GIVEN IN GOOD FAITH, BUT NO WARRANTY, EXPRESSED OR IMPLIED, IS MADE.

NA - Not Available or Not Applicable
ND - Not Determined

ATTACHMENT 3

Product Formulations for SUSTAIN®

Registered with the

California Department of Pesticide Regulation

California Department of Pesticide Regulation

Product Formulation Information

DPR Form 39-030 (rev. 3-99)
Page 3 of 6

1. Brand Name: SUSTAIN

3. U.S. EPA/Calif. Reg. No. (if assigned): Calif. Reg. No. 72-

2. Firm Name: MILLER CHEMICAL & FERTILIZER CORP

4. pH (if water soluble liquid)

5. Active Ingredient Give common chemical name for each active ingredient listed on the label. Microbials should show genus, species, and strain.	6. Chemical Abstracts Service (CAS) (or ATCC) No.	7. Brand name of **source product** for active ingredient	8. EPA Reg. No. of **source product**	9. Percent by weight of **source product** in formulated product.	10. Percent by weight of **active ingredient** in formulated product.

11. Inert Ingredient (common chemical name)	12. Chemical Abstracts Service (CAS) No.	13. Brand name of **source product**.	14. Purpose in formulation.	15. Percent by weight of **source product** in formulated product.	16. Percent by weight of **inert ingredient** in formulated product.
				Total Columns 9 + 15 =100.00%	Total Columns 10+16 =100.00%

If space is not sufficient, attach additional pages. Inert ingredients information given on this form is considered to be confidential business information and is protected from disclosure under the California Public Records Act (Gov. Code §62[illegible])). You may submit a copy of your USEPA Confidential Statement of Formula in lieu of this page.

CBI DELETED

"CBI DELETED"
Page 55

California Department of Pesticide Regulation

Product Formulation Information

Alternate Formula 1

DPR Form 39-030 (rev. 3-99)
Page 3 of 6

1. Brand Name: SUSTAIN

3. U.S. EPA/Calif. Reg. No. (if assigned): Calif. Reg. No. 72-

2. Firm Name: MILLER CHEMICAL & FERTILIZER CORP.

4. pH (if water soluble liquid)

5. Active Ingredient Give common chemical name for each active ingredient listed on the label. Microbials should show genus, species, and strain.	6. Chemical Abstracts Service (CAS) (or ATCC) No.	7. Brand name of **source product** for active ingredient	8. EPA Reg. No. of **source product**	9. Percent by weight of **source product** in formulated product	10. Percent by weight of **active ingredient** in formulated product

11. Inert Ingredient (common chemical name)	12. Chemical Abstracts Service (CAS) No.	13. Brand name of **source product**.	14. Purpose in formulation.	15. Percent by weight of **source product** in formulated product	16. Percent by weight of **inert ingredient** in formulated product
				Total _____ Columns 9 + 15 =100.00%	Total _____ Columns 10+ =100.00%

If space is not sufficient, attach additional pages. Inert ingredients information given on this form is considered to be confidential business information and is protected from disclosure under the California Public Records Act (Gov. Code §6254.2(l)). You may submit a copy of your USEPA Confidential Statement of Formula in lieu of this page.

CBI DELETED

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California Department of Pesticide Regulation

DPR Form 39-030 (rev. 3-99)
Page 3 of 6

Product Formulation Information

Alternate Formula 2

1. Brand Name: SUSTAIN

3. U.S. EPA/Calif. Reg No. (if assigned): Calif. Reg. No. 72-

2. Firm Name: MILLER CHEMICAL & FERTILIZER CORP.

4. pH (if water soluble liquid) _____

5. Active Ingredient Give common chemical name for each active ingredient listed on the label. Microbials should show genus, species, and strain.	6. Chemical Abstracts Service (CAS) (or ATCC) No.	7. Brand name of **source product** for active ingredient	8. EPA Reg No. of **source product**	9. Percent by weight of **source product** in formulated product.	10. Percent by weight of **active ingredient** in formulated product.
CBI DELETED					

11. Inert Ingredient (common chemical name)	12. Chemical Abstracts Service (CAS) No.	13. Brand name of **source product**.	14. Purpose in formulation.	15. Percent by weight of **source product** in formulated product.	16. Percent by weight of **inert ingredient** in formulated product.
CBI DELETED					
				Total _____ Columns 9 + 15 =100.00%	Total _____ Columns 10+ =100.00%

If space is not sufficient, attach additional pages. Inert ingredients information given on this form is considered to be confidential business information and is protected from disclosure under the California Public Records Act (Gov. Code §6254.2(l)). You may submit a copy of your USEPA Confidential Statement of Formula in lieu of this page.

California Department of Pesticide Regulation

Product Formulation Information

Alternate Formula 3

1. Brand Name: SUSTAIN 3. U.S. EPA/Calif. Reg. No. (if assigned) Calif. Reg. No. 72-

2. Firm Name: MILLER CHEMICAL & FERTILIZER CORP. 4. pH (if water soluble liquid)

DPR Form 39-030 (rev. 3-99)
Page 3 of 6

5. Active Ingredient Give common chemical name for each active ingredient listed on the label. Microbials should show genus, species, and strain.	6. Chemical Abstracts Service (CAS) (or ATCC) No.	7. Brand name of **source product** for active ingredient	8. EPA Reg. No. of **source product**	9. Percent by weight of **source product** in formulated product	10. Percent by weight of **active ingredient** in formulated product.

11. Inert Ingredient (common chemical name)	12. Chemical Abstracts Service (CAS) No.	13. Brand name of **source product.**	14. Purpose in formulation.	15. Percent by weight of **source product** in formulated product	16. Percent by weight of **inert ingredient** in formulated product.
				Total _____ Columns 9 + 15 =100.00%	Total _____ Columns 10+ =100.00%

If space is not sufficient, attach additional pages. Inert ingredients information given on this form is considered to be confidential business information and is protected from disclosure under the California Public Records Act (Gov. Code §6254.2(i)). You may submit a copy of your USEPA Confidential Statement of Formula in lieu of this page.

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ATTACHMENT 4

Manufacturing Process for Terpene (Pinene) Polymers

"CBI DELETED"

MILLER® CHEMICAL & FERTILIZER CORPORATION

ANDREW G. SMITH
COMPLIANCE OFFICER
P.O. BOX 333, 120 RADIO ROAD
HANOVER, PENNSYLVANIA 17331 U.S.A.
TELEPHONE. 717-632-8921 EXT. 245
FAX NO. 717-646-1104

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ATTACHMENT 5

Certificate of Analysis for Terpene Polymers

MILLER® CHEMICAL & FERTILIZER CORPORATION

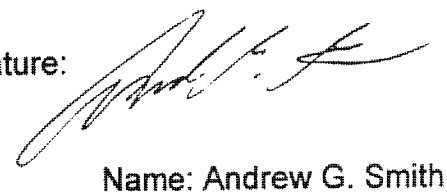
P.O. BOX 333, 120 RADIO ROAD
HANOVER, PENNSYLVANIA 17331 U.S.A.
TELEPHONE: 717-632-8921
FAX NO.: 717-632-4581

1/18/01

CERTIFICATE OF ANALYSIS

Product Name: MILLER 6064
Quantity Produced: 7.4 kilograms
Batch Number: 150386
Date of Formulation: 12/13/00

Appearance: Yellow to Amber Liquid
Odor: Terpenic
Moisture: Negligble (non-aqueous)
Specific Gravity: 0.92 - 0.94 @ 20c
Viscosity @ 20c: 100 to 500 cps
pH(1% solution): 6.0 to 8.0
Solubility in Water: Insoluble (Emulsifies)
Flash Point: over 200 f
Purity: 100%

Signature: 

Name: Andrew G. Smith
Title: Compliance Officer

ATTACHMENT 6

Report by

The Organic Materials Review Institute (OMRI)

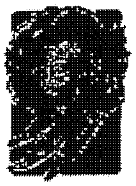
For

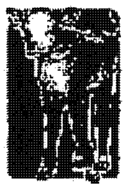
Potassium Bicarbonate and Pinolene Based Coating

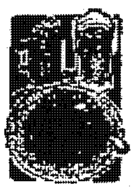
TO: Mr. Krienke

The Organic Materials Review Institute

Brand Name List · Generic Lists · Product Evaluations · Subscribe to OMRI · Technical Papers · GMO Issues · USDA Final Rule · Press Releases







Potassium Bicarbonate

Crops

Identification

Chemical Names:
Potassium bicarbonate

Other Names:
Potassium acid carbonate; potassium hydrogen carbonate

CAS Numbers:
298-14-6

Other Codes:
None

Characterization

Composition:
$KHCO_3$

Properties:
White granules, crystals or powder. Melting point 100°C; specific gravity 2.17. Appreciable solubility.

How Made:
Produced by carbonating potassium hydroxide to K2CO3 which is then carbonated to KHCO3. Carbonation is accomplished by injecting carbon dioxide gas into an aqueous solution of potassium hydroxide. Potassium hydroxide is formed by the electrolysis of potassium chloride.

Specific Uses:
Disease control of powdery mildew (Sphaerotheca fuliginea) and early blight (Alternaria cucumerina) on cucurbits and tomatoes respectively. Also experimentally as disease control on grapes.

Action:
Bicarbonate ion has been identified as the probable cause of growth inhibition in some bacteria and fungi. The bicarbonate causes the collapse of hyphal walls and shrinkage of conidia (different parts of the fungus). In addition, pH elevation may

also play a significant role.

Combinations:
Recommended to be used with a coating polymer to help provide uniform coverage of leaf surfaces. Oil and pinolene based coatings would be acceptable for organic growers and are much more effective than bicarbonate alone. Potassium bicarbonate is also sometimes mixed with sodium bicarbonate and inert ingredients in formulations.

Status

OFPA
The list of exemptions for synthetics on the National List in OFPA 6517(1)(B)(i) does not mention a material such as this, except to the extent that it could be considered a production aid.

Regulatory
The EPA has registered products for disease control just in the past few years. Registered for control of powdery mildew on grapes, cucumbers, strawberries, tobacco, and roses.

Status among Certifiers
none

Historic Use
none in organics because there were no registered products.

International
Not mentioned in IFOAM or CODEX.

OFPA 2119(m) Criteria

1. The potential of such substances for detrimental chemical interactions with other materials used in organic farming systems.

   The substance is stable and hazardous polymerization will not occur.

2. The toxicity and mode of action of the substance and of its breakdown products or any contaminants, and their persistence and areas of concentration in the environment.

   Decomposition products are potassium carbonate, water, and carbon dioxide. These materials readily dissipate in the environment. In concentrations of greater that 1%, potassium bicarbonate can have phytotoxic effects on the plants in the form of beige necrotic spots on the leaves (Ziv, 1992). These can be alleviated by using a 1% or less concentration and by atomizing the spray so there are no big droplets.

3. The probability of environmental contamination during manufacture, use, misuse or disposal of such substance.

   There are some impurities created in the manufacture of potassium

bicarbonate. The main ones are chlorine, sulfate and water, which are impurities primarily from the formation of potassium hydroxide from potassium chloride as the precursor material. The chlorine level is not exceeding 0.5% and the sulfate level is not exceeding 0.045%. Both of these impurities are common in nature and have biological processes which transform them into stable materials, although chlorine has well known concerns elaborated in the bleach TAP review from 1994.

4. The effect of the substance on human health.

No carcinogenicity. No effects of overexposure were documented.
--Mild alkaline irritant to respiratory system. Coughing, sneezing, possible breathing difficulty in acute cases.
--Mild eye irritant, possible reddening due to alkaline effect or abrasion.
--No LD50 information found relating to normal routes of occupational exposure.

5. The effects of the substance on biological and chemical interactions in the agroecosystem, including the physiological effects of the substance on soil organisms (including the salt index and solubility of the soil), crops and livestock.

Is known to inhibit the growth of bacteria and yeasts in agar media under certain conditions. There has been a little study of the bicarbonate ion (mostly from ammonium bicarbonate) on soil-borne pathogens. A suppression effect was found (Ziv, 1992). Its use may result in pH elevation, which will have a myriad of effects on the soil.

6. The alternatives to using the substance in terms of practices or other available materials. Potential alternative cultural, biological, natural, and existing organic controls also include:
    1. Crop rotation.
    2. Selection and cultivation of disease resistant varieties.
    3. Nutrient management, particularly correcting Nitrogen:Calcium and Nitrogen:Potassium ratio balances.
    4. Water management, including humidity control and air movement management for crops grown in greenhouses.
    5. Planting density.
    6. Trellising and pruning for improved air movement.
    7. Sanitation: pruning and removal of diseased tissue.
    8. Foliar application of non-synthetic materials:
        a) Compost tea extracts.
        b) Microbial fungicides and antagonists
    9. Foliar application of synthetic materials recommended for inclusion on the National List
        a) Copper based materials.
        b) Sulfur.
        c) Suffocating oils.

These organic controls are not very effective and are not applicable in many cropping situations. The specific microbial fungicides are still in development and not yet approved for organic production. The diseases targeted by potassium carbonate are difficult to control organically, and the

chemicals are now running into resistant strains of the disease organisms. Potassium carbonate may in many situations be more environmentally sound and safer for applicators and other farmworkers than the other synthetic alternatives.

7. Its compatibility with a system of sustainable agriculture. The NOSB has already recommended that this substance be allowed as an ingredient in food labeled as organic. See discussion below for compatability with sustainable agriculture.

Discussion

Condensed Reviewer Comments
None of the reviewers has a commercial or financial interest in potassium bicarbonate.

Reviewer 1
The diseases that this material can control are widespread and hard to manage with currently approved organic materials or even conventional chemicals. This material may be safer than already allowed materials such as sulfur to people and / or plants.

To evaluate this material, I believe it is important to look at the assumptions behind the organic law which allows natural materials and prohibits synthetic ones. One primary assumption is that natural materials are safer than synthetic ones AND their residue is safer for the consumer to ingest. Part of the assumption is that humans have coevolved with natural materials and our bodies have the ability to handle them without adverse effects. Another part of the assumption is the inability to handle synthetic or novel materials that can have an adverse effect on our bodies.
. . .

It is far easier to define a material as being synthetic or natural than it is to determine its safety. Defining safety is expensive and ultimately based on the value system of the evaluators. . . . Banning safe alternatives to toxic chemicals used in conventional agriculture solely because they are synthetic is counter productive to the organic movement as a whole. The result will be less crops being produced in more limited regions and because of that, the overall impact on the environment and for the people will be negative in general. There will be less acreage under organic stewardship and more adverse effects from people growing crops . . . using conventional methods.

Potassium bicarbonate should be allowed for organic production under a category of synthetic - safe.

Reviewer 2
The compound, although synthetic, appears quite benign and should be compatible with organic production.

Potassium bicarbonate should be added to the National List of Allowed Synthetics. This material appears to be a least toxic, agronomically desirable material, with greater efficacy for controlling powdery mildew or late blight than does the currently available organic options. It is also available in different formulations which are grower and environmentally friendly.

Reviewer 3
The material is compatible with organic production when it is used for foliar disease control in non-fertilizer amounts. The only caution is that growers must not use it at fertilizer rates. However, if growers apply it foliarly in solution concentrations much above 0.5% potassium bicarbonate, they will cause phytotoxicity to their crop. Potassium bicarbonate should be allowed for foliar disease control when used at rates recommended for disease control. It should not be allowed at fertilizer rates.

Conclusion
The diseases which are controlled with potassium bicarbonate, powdery mildew and early blight, are very difficult to control with any acceptable organic practices or materials. In fact these diseases have influenced the ability to grow susceptible crops in certain environments at some seasons. While sodium bicarbonate, which is natural, does have some effect, it does not have enough of a control by itself to inspire product development on it.

The data available on this material points to it being safe and benign to the environment when used at recommended concentrations. It is, however, synthetic. It does not fit strictly within the itemized categories in OFPA pertaining to synthetics on the National List. Therefore the choice of whether it should be allowed in organic agriculture becomes a choice between the agronomic and precautionary approach vs. the regulatory one.

References

Corral, L.G.; Post, L.S. Montville, T.J. 1988. Antimicrobial activity of sodium bicarbonate. J Food Sci Off Publ Inst Food Technol., Chicago, IL: The Institute. May/June v. 53(3) p. 981-982.

Kirk-Othmer Encyclopedia of Chemical Technology, 3rd. edition, 1982. John Wiley & Sons.

Tisdale, S.L. , W.L. Nelson, and J.D. Beaton. Soil Fertility and Fertilizers. Eds.1985. Macmillan Pub. Co., NY.

Ziv, O. 1992. Effects of bicarbonates and film-forming polymers on cucurbit foliar diseases. Plant Disease.

Zitter, T.A. & Drennan, J.L. 1994. Comparison of Hydraulic and Electrostatic Sprayers for Fungicidal Control of Early and Late Blight in Tomato, 1993. Fungicide and Nematocide Tests Vol. 49:174.

Zitter, T.A. & Drennan, J.L. 1995. Early Blight Control in Susceptible and Resistant Tomato. Fungicide and Nematocide Tests Vol. 50:182.

Ziv, O. & Zitter, T.A. 1992. Effects of Bicarbonates and Film-Forming Polymers on Cucurbit Foliar Diseases. Plant Disease 76:513-517.

For more information on OMRI

including ordering publications and having products reviewed for use in organic production, contact
Organic Materials Review Institute, Box 11558, Eugene, OR 97440-3758 USA
or call (541) 343-7600 • Fax (541) 343-8971
or e-mail us at info@omri.org

ATTACHMENT 7

Tolerance Exemption for Pinene Polymers under 40 CFR §180.910

7.1. Final Rule (70 FR 28447, May 18, 2005)

7.2. EPA Notice of Filing of Pesticide Petition (63 FR 64494, November 20, 1998)

ATTACHMENT 7.1

Final Rule

Tolerance Exemption for Pinene Polymers under 40 CFR §180.910

(70 FR 28447, May 18, 2005)

the distribution of power and responsibilities between the Federal Government and Indian tribes." This rule will not have substantial direct effects on tribal governments, on the relationship between the Federal Government and Indian tribes, or on the distribution of power and responsibilities between the Federal Government and Indian tribes, as specified in Executive Order 13175. Thus, Executive Order 13175 does not apply to this rule.

XIII. Congressional Review Act

The Congressional Review Act, 5 U.S.C. 801 *et seq.*, as added by the Small Business Regulatory Enforcement Fairness Act of 1996, generally provides that before a rule may take effect, the agency promulgating the rule must submit a rule report, which includes a copy of the rule, to each House of the Congress and to the Comptroller General of the United States. EPA will submit a report containing this rule and other required information to the U.S. Senate, the U.S. House of Representatives, and the Comptroller General of the United States prior to publication of this final rule in the **Federal Register**. This final rule is not a "major rule" as defined by 5 U.S.C. 804(2).

List of Subjects in 40 CFR Part 180

Environmental protection, Administrative practice and procedure, Agricultural commodities, Pesticides and pests, Reporting and record keeping requirements.

Dated: April 29, 2005.

Lois Rossi,

Director, Registration Division, Office of Pesticide Programs.

■ Therefore, 40 CFR chapter I is amended as follows:

PART 180—[AMENDED]

■ 1. The authority citation for part 180 continues to read as follows:

Authority: 21 U.S.C. 321(q), 346a and 371.

■ 2. In § 180.950, the table in paragraph (e) is amended by adding alphabetically the following entry to read as follows:

§ 180.950 Tolerance exemptions for minimal risk active and inert ingredients.

* * * * *

(e)* * *

Chemical Name	CAS No.
* * * *	*
Red cabbage color, expressed from edible red cabbage heads via a pressing process using only acidified water.	None
* * * *	*

[FR Doc. 05–9482 Filed 5–17–05; 8:45 am]

BILLING CODE 6560–50–S

ENVIRONMENTAL PROTECTION AGENCY

40 CFR Part 180

[OPP–2005–0110; FRL–7710–3]

Pinene Polymers; Exemption from the Requirement of a Tolerance

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: This regulation establishes exemptions from the requirement of a tolerance for residues of several alpha- and/or beta-pinene polymers when used as inert ingredients in or on growing crops and when applied to raw agricultural commodities after harvest. Hercules, Inc. submitted a petition to EPA under the Federal Food, Drug, and Cosmetic Act (FFDCA), as amended by the Food Quality Protection Act of 1996 (FQPA), requesting an exemption from the requirement of a tolerance. This regulation eliminates the need to establish a maximum permissible level for residues of alpha and/or beta-pinene polymers.

DATES: This regulation is effective May 18, 2005. Objections and requests for hearings must be received on or before July 18, 2005.

ADDRESSES: To submit a written objection or hearing request follow the detailed instructions as provided in Unit XI. of the **SUPPLEMENTARY INFORMATION**. EPA has established a docket for this action under Docket identification (ID) number OPP–2005–0110. All documents in the docket are listed in the EDOCKET index at *http://www.epa.gov/edocket*. Although listed in the index, some information is not publicly available, i.e., CBI or other information whose disclosure is restricted by statute. Certain other material, such as copyrighted material, is not placed on the Internet and will be publicly available only in hard copy form. Publicly available docket materials are available either electronically in EDOCKET or in hard copy at the Public Information and Records Integrity Branch (PIRIB), Rm. 119, Crystal Mall #2, 1801 S. Bell St., Arlington, VA. This docket facility is open from 8:30 a.m. to 4 p.m., Monday through Friday, excluding legal holidays. The docket telephone number is (703) 305–5805.

FOR FURTHER INFORMATION CONTACT: Kathryn Boyle, Registration Division (7505C), Office of Pesticide Programs, Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington, DC 20460–0001; telephone number: (703) 305–6304; e-mail address: *boyle.kathryn@epa.gov*.

SUPPLEMENTARY INFORMATION:

I. General Information

A. Does this Action Apply to Me?

You may be potentially affected by this action if you are an agricultural producer, food manufacturer, or pesticide manufacturer. Potentially affected entities may include, but are not limited to:

- Crop production (NAICS code 111)
- Animal production (NAICS code 112)
- Food manufacturing (NAICS code 311)
- Pesticide manufacturing (NAICS code 32532)

This listing is not intended to be exhaustive, but rather provides a guide for readers regarding entities likely to be affected by this action. Other types of entities not listed in this unit could also be affected. The North American Industrial Classification System (NAICS) codes have been provided to assist you and others in determining whether this action might apply to certain entities. If you have any questions regarding the applicability of this action to a particular entity, consult the person listed under **FOR FURTHER INFORMATION CONTACT**.

B. How Can I Get Electronic Documents and Other Related Information?

In addition to using EDOCKET at (*http://www.epa.gov/edocket/*), you may access this **Federal Register** document electronically through the EPA Internet under the "**Federal Register**" listings at *http://www.epa.gov/fedrgstr/*. A frequently updated electronic version of 40 CFR part 180 is available on E-CFR Beta Site Two at *http://www.gpoaccess.gov/ecfr/*.

II. Background and Statutory Findings

In the **Federal Register** of November 20, 1998 (63 FR 64494) (FRL–6027–6), EPA issued a notice pursuant to section 408 of FFDCA, 21 U.S.C. 346a, as amended by FQPA (Public Law 104–170), announcing the filing of a pesticide petition (PP 6E4782) by Hercules, Inc. 1313 North Market St., Wilmington, DE 19894–0001. The petition requested that 40 CFR part 180 be amended by establishing an

exemption from the requirement of a tolerance for residues of alpha- and/or beta-pinene polymers for use as an inert ingredient in pesticide products. That notice included a summary of the petition prepared by the petitioner.

The Agency interpreted the petitioner's request for an exemption for alpha- and/or beta-pinene polymers, as a request to amend the existing exemption for beta-pinene polymers to include alpha- and/or beta-pinene polymers. In the Notice of Filing the petitioner used only the generalized term alpha- and/or beta-pinene polymers and did not specifically identify the chemicals by CAS Reg. No. or Name. The alpha- and/or beta-pinene polymers considered by the Agency are in the following Table.

CHEMICALS CONSIDERED

Common chemical name	CAS Nomenclature	CAS No.
Alpha-pinene polymer	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-, homopolymer	25766–18–1
Beta-pinene polymer	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, homopolymer (9CI)	25719–60–2
Copolymer of alpha- and beta-pinene	Bicyclo[3.1.1]hept-2-ene, 2,6,6trimethyl, polymer with 6,6- dimethyl-2-methylenebicyclo [3.1.1]heptane (9CI)	31393–98–3
Polymerized alpha- pinene fraction from turpentine	Terpenes and Terpenoids, turpentine oil, alpha-pinene fraction, polymd.	70750–57–1

There were no comments received in response to the notice of filing.

Section 408(b)(2)(A)(i) of the FFDCA allows EPA to establish an exemption from the requirement for a tolerance (the legal limit for a pesticide chemical residue in or on a food) only if EPA determines that the tolerance is "safe." Section 408(b)(2)(A)(ii) of the FFDCA defines "safe" to mean that "there is a reasonable certainty that no harm will result from aggregate exposure to the pesticide chemical residue, including all anticipated dietary exposures and all other exposures for which there is reliable information." This includes exposure through drinking water and in residential settings, but does not include occupational exposure. Section 408(b)(2)(C) of the FFDCA requires EPA to give special consideration to exposure of infants and children to the pesticide chemical residue in establishing a tolerance and to "ensure that there is a reasonable certainty that no harm will result to infants and children from aggregate exposure to the pesticide chemical residue * * *."

EPA performs a number of analyses to determine the risks from aggregate exposure to pesticide residues. First, EPA determines the toxicity of pesticides. Second, EPA examines exposure to the pesticide through food, drinking water, and through other exposures that occur as a result of pesticide use in residential settings.

III. Inert Ingredient Definition

Inert ingredients are all ingredients that are not active ingredients as defined in 40 CFR 153.125 and include, but are not limited to, the following types of ingredients (except when they have a pesticidal efficacy of their own): Solvents such as alcohols and hydrocarbons; surfactants such as polyoxyethylene polymers and fatty acids; carriers such as clay and diatomaceous earth; thickeners such as carrageenan and modified cellulose; wetting, spreading, and dispersing agents; propellants in aerosol dispensers; microencapsulating agents; and emulsifiers. The term "inert" is not intended to imply nontoxicity; the ingredient may or may not be chemically active. Generally, EPA has exempted inert ingredients from the requirement of a tolerance based on the low toxicity of the individual inert ingredients.

IV. Toxicological Profile

Consistent with section 408(b)(2)(D) of the FFDCA, EPA has reviewed the available scientific data and other relevant information in support of this action and considered its validity, completeness and reliability and the relationship of this information to human risk. EPA has also considered available information concerning the variability of the sensitivities of major identifiable subgroups of consumers, including infants and children. The nature of the toxic effects caused by alpha- and/or beta-pinene polymers are discussed in this unit.

Alpha- and beta-pinene are bicyclic terpene hydrocarbons. They are the major components of turpentine. The two chemicals are closely related, having the same empirical formula of $C_{10}H_{16}$ and the same basic ring structure. Alpha- and/or beta-pinene polymers are manufactured by various processes that increase the molecular weight beyond that of alpha- or beta-pinene and include formation of a dimer (two "pinenes" in a single molecule), formation of a trimer (three "pinenes" in a single molecule), or polymerization.

The data considered in this assessment included information submitted by the petitioner, and information located by OPP on the internet, primarily information prepared by the National Toxicology Program (NTP) and the robust summaries for bicyclic terpene hydrocarbons submitted in 2002 to EPA by the Terpene Consortium of the Flavor and Fragrance High Production Volume Consortia (FFHPVC). The Agency evaluated first the toxicity of the alpha- and beta-pinene chemicals.

The toxicity of alpha- and beta-pinene is defined by studies from open-literature conducted with alpha-pinene, beta-pinene and various alpha- and beta-pinene mixtures. There is also a structure-activity-relationship (SAR) assessment for alpha-pinene. The findings of the SAR assessment are consistent with the studies from open-literature. The overall conclusions are the following; however, greater detail on the Agency's review and evaluation of the submitted studies and articles from open literature are in the Alpha- and Beta-Pinene Science Assessment in EDOCKET at (*http://www.epa.gov/edocket/*).

Alpha- and beta-pinene are of low acute toxicity via the oral, dermal and inhalation routes. Both alpha-and beta-pinene are irritants to the skin, eye and mucous membranes. Alpha- and beta-pinene are well-absorbed by all routes of exposure.

The subchronic toxicity of alpha- and beta-pinene compounds appears to be low. A subchronic oral toxicity study indicated minor changes in liver and thryoid weights at the two higher dose levels, which were not considered treatment related. There were no effects at approximately 800 mg/kg/day.

developmental/reproductive toxicants. Therefore, EPA has not used a safety factor analysis to assess the risk. For the same reasons a tenfold safety factor is unnecessary.

VIII. Determination of Safety for U.S. Population, and Infants and Children

The database considered for this action included mostly toxicity data derived using alpha- and beta-pinene. Alpha- and beta-pinene exhibit low acute toxicity by the oral, dermal and inhalation routes, and low subchronic toxicity. Polymers composed of alpha and beta-pinene monomers, even those of low molecular weight, should be even less toxic than alpha- and beta-pinene considering that their absorption is decreased. Based on the available information on toxicity and exposure, EPA concludes that there is a reasonable certainty of no harm from aggregate exposure to residues of alpha-pinene, beta-pinene, and alpha- and/or beta-pinene polymers. EPA finds that amending the existing exemption from the requirement of a tolerance for beta-pinene polymers to include alpha- and/or beta-pinene polymers will be safe for the general population including infants and children.

IX. Other Considerations

A. Endocrine Disruptors

FQPA requires EPA to develop a screening program to determine whether certain substances, including all pesticide chemicals (both inert and active ingredients), "may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or such other endocrine effect * * *" EPA has been working with interested stakeholders to develop a screening and testing program as well as a priority setting scheme. As the Agency proceeds with implementation of this program, further testing of products containing alpha-pinene, beta-pinene, or any alpha- and/or beta-pinene polymers for endocrine effects may be required.

B. Analytical Method(s)

An analytical method is not required for enforcement purposes since the Agency is establishing an exemption from the requirement of a tolerance without any numerical limitation.

C. Existing Exemptions

There is an existing tolerance exemption for B-pinene polymers in 40 CFR 180.910 when applied to growing crops or to raw agricultural commodities after harvest.

D. International Tolerances

The Agency is not aware of any country requiring a tolerance for alpha- and/or beta-pinene polymers nor have any CODEX Maximum Residue Levels (MRLs) been established for any food crops at this time.

X. Conclusions

Therefore, exemptions from the requirement for a tolerance are established for alpha-pinene polymer (CAS Reg. No. 25766–18–1), beta-pinene polymer (CAS Reg. No. 25719–60–2), copolymer of alpha- and beta-pinene (CAS Reg. No. 31393–98–3), and terpenes and terpenoids, turpentine oil, alpha-pinene fraction, polymd. (CAS Reg. No. 70750–57–1).

XI. Objections and Hearing Requests

Under section 408(g) of FFDCA, as amended by FQPA, any person may file an objection to any aspect of this regulation and may also request a hearing on those objections. The EPA procedural regulations which govern the submission of objections and requests for hearings appear in 40 CFR part 178. Although the procedures in those regulations require some modification to reflect the amendments made to the FFDCA by FQPA, EPA will continue to use those procedures, with appropriate adjustments, until the necessary modifications can be made. The new section 408(g) of FFDCA provides essentially the same process for persons to "object" to a regulation for an exemption from the requirement of a tolerance issued by EPA under new section 408(d) of FFDCA, as was provided in the old FFDCA sections 408 and 409 of FFDCA. However, the period for filing objections is now 60 days, rather than 30 days.

A. What Do I Need to Do to File an Objection or Request a Hearing?

You must file your objection or request a hearing on this regulation in accordance with the instructions provided in this unit and in 40 CFR part 178. To ensure proper receipt by EPA, you must identify docket ID number OPP–2005–0110 in the subject line on the first page of your submission. All requests must be in writing, and must be mailed or delivered to the Hearing Clerk on or before July 18, 2005.

1. *Filing the request.* Your objection must specify the specific provisions in the regulation that you object to, and the grounds for the objections (40 CFR 178.25). If a hearing is requested, the objections must include a statement of the factual issues(s) on which a hearing is requested, the requestor's contentions on such issues, and a summary of any evidence relied upon by the objector (40 CFR 178.27). Information submitted in connection with an objection or hearing request may be claimed confidential by marking any part or all of that information as CBI. Information so marked will not be disclosed except in accordance with procedures set forth in 40 CFR part 2. A copy of the information that does not contain CBI must be submitted for inclusion in the public record. Information not marked confidential may be disclosed publicly by EPA without prior notice.

Mail your written request to: Office of the Hearing Clerk (1900L), Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington, DC 20460–0001. You may also deliver your request to the Office of the Hearing Clerk in Suite 350, 1099 14th St., NW., Washington, DC 20005. The Office of the Hearing Clerk is open from 8 a.m. to 4 p.m., Monday through Friday, excluding legal holidays. The telephone number for the Office of the Hearing Clerk is (202) 564–6255.

2. *Copies for the Docket.* In addition to filing an objection or hearing request with the Hearing Clerk as described in Unit XI.A., you should also send a copy of your request to the PIRIB for its inclusion in the official record that is described in **ADDRESSES**. Mail your copies, identified by docket ID number OPP–2005–0110, to: Public Information and Records Integrity Branch, Information Resources and Services Division (7502C), Office of Pesticide Programs, Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington, DC 20460–0001. In person or by courier, bring a copy to the location of the PIRIB described in **ADDRESSES**. You may also send an electronic copy of your request via e-mail to: *opp-docket@epa.gov*. Please use an ASCII file format and avoid the use of special characters and any form of encryption. Copies of electronic objections and hearing requests will also be accepted on disks in WordPerfect 6.1/8.0 or ASCII file format. Do not include any CBI in your electronic copy. You may also submit an electronic copy of your request at many Federal Depository Libraries.

B. When Will the Agency Grant a Request for a Hearing?

A request for a hearing will be granted if the Administrator determines that the material submitted shows the following: There is a genuine and substantial issue of fact; there is a reasonable possibility that available evidence identified by the requestor would, if established resolve one or more of such issues in favor of the requestor, taking into account

uncontested claims or facts to the contrary; and resolution of the factual issues(s) in the manner sought by the requestor would be adequate to justify the action requested (40 CFR 178.32).

XII. Statutory and Executive Order Reviews

This final rule establishes an exemption from the tolerance requirement under section 408(d) of the FFDCA in response to a petition submitted to the Agency. The Office of Management and Budget (OMB) has exempted these types of actions from review under Executive Order 12866, entitled *Regulatory Planning and Review* (58 FR 51735, October 4, 1993). Because this rule has been exempted from review under Executive Order 12866 due to its lack of significance, this rule is not subject to Executive Order 13211, *Actions Concerning Regulations That Significantly Affect Energy Supply, Distribution, or Use* (66 FR 28355, May 22, 2001). This final rule does not contain any information collections subject to OMB approval under the Paperwork Reduction Act (PRA), 44 U.S.C. 3501 *et seq.*, or impose any enforceable duty or contain any unfunded mandate as described under Title II of the Unfunded Mandates Reform Act of 1995 (UMRA) (Public Law 104–4). Nor does it require any special considerations under Executive Order 12898, entitled *Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations* (59 FR 7629, February 16, 1994); or OMB review or any Agency action under Executive Order 13045, entitled *Protection of Children from Environmental Health Risks and Safety Risks* (62 FR 19885, April 23, 1997). This action does not involve any technical standards that would require Agency consideration of voluntary consensus standards pursuant to section 12(d) of the National Technology Transfer and Advancement Act of 1995 (NTTAA), Public Law 104–113, section 12(d) (15 U.S.C. 272 note). Since tolerances and exemptions that are established on the basis of a petition under section 408(d) of the FFDCA, such as the exemption in this final rule, do not require the issuance of a proposed rule, the requirements of the Regulatory Flexibility Act (RFA) (5 U.S.C. 601 *et seq.*) do not apply. In addition, the Agency has determined that this action will not have a substantial direct effect on States, on the relationship between the national government and the States, or on the distribution of power and responsibilities among the various levels of government, as specified in Executive Order 13132, entitled *Federalism* (64 FR 43255, August 10, 1999). Executive Order 13132 requires EPA to develop an accountable process to ensure "meaningful and timely input by State and local officials in the development of regulatory policies that have federalism implications." "Policies that have federalism implications" is defined in the Executive Order to include regulations that have "substantial direct effects on the States, on the relationship between the national government and the States, or on the distribution of power and responsibilities among the various levels of government." This final rule directly regulates growers, food processors, food handlers and food retailers, not States. This action does not alter the relationships or distribution of power and responsibilities established by Congress in the preemption provisions of section 408(n)(4) of the FFDCA. For these same reasons, the Agency has determined that this rule does not have any "tribal implications" as described in Executive Order 13175, entitled *Consultation and Coordination with Indian Tribal Governments* (65 FR 67249, November 6, 2000). Executive Order 13175, requires EPA to develop an accountable process to ensure "meaningful and timely input by tribal officials in the development of regulatory policies that have tribal implications." "Policies that have tribal implications" is defined in the Executive Order to include regulations that have "substantial direct effects on one or more Indian tribes, on the relationship between the Federal Government and the Indian tribes, or on the distribution of power and responsibilities between the Federal Government and Indian tribes." This rule will not have substantial direct effects on tribal governments, on the relationship between the Federal Government and Indian tribes, or on the distribution of power and responsibilities between the Federal Government and Indian tribes, as specified in Executive Order 13175. Thus, Executive Order 13175 does not apply to this rule.

XIII. Congressional Review Act

The Congressional Review Act, 5 U.S.C. 801 *et seq.*, as added by the Small Business Regulatory Enforcement Fairness Act of 1996, generally provides that before a rule may take effect, the agency promulgating the rule must submit a rule report, which includes a copy of the rule, to each House of the Congress and to the Comptroller General of the United States. EPA will submit a report containing this rule and other required information to the U.S. Senate, the U.S. House of Representatives, and the Comptroller General of the United States prior to publication of this final rule in the **Federal Register**. This final rule is not a "major rule" as defined by 5 U.S.C. 804(2).

List of Subjects in 40 CFR Part 180

Environmental protection, Administrative practice and procedure, Agricultural commodities, Pesticides and pests, Reporting and recordkeeping requirements.

Dated: April 27, 2005.

Betty Shackleford,

Acting Director, Registration Division, Office of Pesticide Programs.

■ Therefore, 40 CFR chapter I is amended as follows:

PART 180—[AMENDED]

■ 1. The authority citation for part 180 continues to read as follows:

Authority: 21 U.S.C. 321(q), 346a and 371.

■ 2. In § 180.910, the table is amended by removing the current B-pinene polymer entry and by alphabetically adding the following entries to read as follows:

§ 180.910 Inert ingredients used pre- and post-harvest; exemptions from the requirement of a tolerance.

* * * * *

Inert ingredients	Limits	Uses
* * *	* *	* *
Bicyclo[3.1.1]hept–2–ene, 2,6,6–trimethyl–, homopolymer (Alpha-pinene, homopolymer)(CAS Reg. No. 25766–18–1),.	.....	Surfactants, related adjuvants of surfactants
Bicyclo[3.1.1]heptane, 6,6–dimethyl–2–methylene–, homopolymer (Beta-pinene, homopolymer) (CAS Reg. No. 25719–60–2),.	.....	Surfactants, related adjuvants of surfactants

Inert ingredients	Limits	Uses
Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-, polymer with 6,6-dimethyl-2- methylenebicyclo [3.1.1] heptane (Copolymer of alpha- and beta-pinene) (CAS Reg. No. 31393-98-3),		Surfactants, related adjuvants of surfactants
Terpenes and terpenoids, turpentine oil, alpha-pinene fraction, polymd. (CAS Reg. No. 70750-57-1).		Surfactants, related adjuvants of surfactants

* * * * *

[FR Doc. 05-9479 Filed 5-17-05; 8:45 am]
BILLING CODE 6560-50-S

ENVIRONMENTAL PROTECTION AGENCY

40 CFR Part 180

[OPP-2005-0095; FRL-7711-9]

Fludioxonil; Pesticide Tolerance

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: This regulation establishes a tolerance for residues of fludioxonil (4-(2,2-difluoro-1,3-benzodioxol-4-yl)-*H*-pyrrole-3-carbonitrile) in or on pomegranate. Interregional Research Project Number 4 (IR-4) requested this tolerance under the Federal Food, Drug, and Cosmetic Act (FFDCA), as amended by the Food Quality Protection Act of 1996 (FQPA).

DATES: This regulation is effective May 18, 2005. Objections and requests for hearings must be received on or before July 18, 2005.

ADDRESSES: To submit a written objection or hearing request follow the detailed instructions as provided in Unit VI. of the **SUPPLEMENTARY INFORMATION**. EPA has established a docket for this action under docket identification (ID) number OPP-2005-0095. All documents in the docket are listed in the EDOCKET index at *http://www.epa.gov/edocket*. Although listed in the index, some information is not publicly available, i.e., CBI or other information whose disclosure is restricted by statute. Certain other material, such as copyrighted material, is not placed on the Internet and will be publicly available only in hard copy form. Publicly available docket materials are available either electronically in EDOCKET or in hard copy at the Public Information and Records Integrity Branch (PIRIB), Rm. 119, Crystal Mall #2, 1801 S. Bell St., Arlington, VA. This docket facility is open from 8:30 a.m. to 4 p.m., Monday through Friday, excluding legal holidays. The docket telephone number is (703) 305-5805.

FOR FURTHER INFORMATION CONTACT: Shaja R. Brothers, Registration Division (7505C), Office of Pesticide Programs, Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington, DC 20460-0001; telephone number: (703) 308-3194; e-mail address: *brothers.shaja@epa.gov*.

SUPPLEMENTARY INFORMATION:

I. General Information

A. Does this Action Apply to Me?

You may be potentially affected by this action if you are an agricultural producer, food manufacturer, or pesticide manufacturer. Potentially affected entities may include, but are not limited to:

- Crop production (NAICS 111), e.g., agricultural workers; greenhouse, nursery, and floriculture workers; farmers.
- Animal production (NAICS 112), e.g., cattle ranchers and farmers, dairy cattle farmers, livestock farmers.
- Food manufacturing (NAICS 311), e.g., agricultural workers; farmers; greenhouse, nursery, and floriculture workers; ranchers; pesticide applicators.
- Pesticide manufacturing (NAICS 32532), e.g., agricultural workers; commercial applicators; farmers; greenhouse, nursery, and floriculture workers; residential users.

This listing is not intended to be exhaustive, but rather provides a guide for readers regarding entities likely to be affected by this action. Other types of entities not listed in this unit could also be affected. The North American Industrial Classification System (NAICS) codes have been provided to assist you and others in determining whether this action might apply to certain entities. If you have any questions regarding the applicability of this action to a particular entity, consult the person listed under **FOR FURTHER INFORMATION CONTACT**.

B. How Can I Access Electronic Copies of this Document and Other Related Information?

In addition to using EDOCKET (*http://www.epa.gov/edocket/*), you may access this **Federal Register** document electronically through the EPA Internet under the "**Federal Register**" listings at *http://www.epa.gov/fedrgstr/*. A frequently updated electronic version of 40 CFR part 180 is available at E-CFR Beta Site Two at *http://www.gpoaccess.gov/ecfr/*.

II. Background and Statutory Findings

In the **Federal Register** of March 17, 2004 (69 FR 12680) (FRL-7347-3), EPA issued a notice pursuant to section 408(d)(3) of FFDCA, 21 U.S.C. 346a(d)(3), announcing the filing of a pesticide petition (PP 3E6803) by IR-4, 681 US Highway #1 South, North Brunswick, NJ 08902-3390. The petition requested that 40 CFR 180.516 be amended by establishing a tolerance for residues of the fungicide fludioxonil (4-(2,2-difluoro-1,3-benzodioxol-4-yl)-*H*-pyrrole-3-carbonitrile) in or on pomegranate at 2.0 parts per million (ppm). This petition has subsequently been amended to propose pomegranate (post-harvest) at 5.0 ppm. That notice included a summary of the petition prepared by Syngenta Crop Protection, the registrant. There were no comments received in response to the notice of filing.

Section 408(b)(2)(A)(i) of FFDCA allows EPA to establish a tolerance (the legal limit for a pesticide chemical residue in or on a food) only if EPA determines that the tolerance is "safe." Section 408(b)(2)(A)(ii) of FFDCA defines "safe" to mean that "there is a reasonable certainty that no harm will result from aggregate exposure to the pesticide chemical residue, including all anticipated dietary exposures and all other exposures for which there is reliable information." This includes exposure through drinking water and in residential settings, but does not include occupational exposure. Section 408(b)(2)(C) of FFDCA requires EPA to give special consideration to exposure of infants and children to the pesticide chemical residue in establishing a tolerance and to "ensure that there is a reasonable certainty that no harm will result to infants and children from aggregate exposure to the pesticide chemical residue * * *."

ATTACHMENT 7.2

Tolerance Exemption for Pinene Polymers under 40 CFR §180.910

Notice of Filing of Pesticide Petition (63 FR 64494, November 20, 1998)

common mechanism of toxicity with other substances.

E. Safety Determination

1. *U.S. population.* In the meeting of September 30, 1993, the OPP RfD Peer Review Committee recommended that the RfD for this chemical be based on a NOAEL of 20 mg/kg/day for a dose-related increase in size and altered tinctorial properties of centrilobular hepatocytes in males and females at 60 and 200 mg/kg/day in a chronic toxicity study in rats. An uncertainty factor (UF) of 100 was used to account for the inter-species extrapolation and intra-species variability. On this basis, the RfD was calculated to be 0.20 mg/kg/day. The TMRC from existing tolerances is 0.001845 mg/kg/day. Existing tolerances utilize >1% of the RfD. It should be noted that no regulatory value has been established for this chemical by the World Health Organization (WHO) up to this date. The committee classified picloram as a "Group E" chemical, no evidence of carcinogenicity for humans.

Using the conservative exposure assumptions described above and based on the completeness and reliability of the toxicity data, it is concluded that aggregate exposure to picloram will utilize approximately 1% of the RfD for the U.S. population. Generally, exposures below 100% of the RfD are of no concern because the RfD represents the level at or below which daily aggregate dietary exposure over a lifetime will not pose appreciable risk to human health. Thus, there is a reasonable certainty that no harm will result from aggregate exposure to picloram residues.

2. *Infants and children.* In assessing the potential for additional sensitivity of infants and children to residues of picloram, data from developmental toxicity studies in the rat and rabbit and a 2-generation reproduction study in the rat were considered. The developmental toxicity studies are designed to evaluate adverse effects on the developing organism during prenatal development resulting from pesticide exposure to one or both parents. Reproduction studies provide (1) information relating to effects from exposure to the pesticide on the reproductive capability of mating animals and (2) data on systemic toxicity.

Developmental toxicity was studied using rats and rabbits. The developmental study in rats resulted in a developmental NOAEL of >298 mg/kg/day and a maternal toxicity NOAEL of 280 mg/kg/day. A study in rabbits resulted in a maternal NOAEL of 34 mg/kg/day and a developmental NOAEL of 344 mg/kg/day. Based on all of the data for picloram, there is no evidence of developmental toxicity at dose levels that do not result in maternal toxicity.

In a 2-generation reproduction study in rats, The NOAEL for parental systemic toxicity is 200 mg/kg/day. There was no effect on reproductive parameters at 1,000 mg/kg/day nor was there an adverse effect on the morphology, growth or viability of the offspring; thus, the reproductive NOAEL is 1,000 mg/kg/day.

FDCA section 408 provides that EPA may apply an additional safety factor for infants and children in the case of threshold effects to account for pre- and post-natal toxicity and the completeness of the database. Based on the current toxicological data requirements, the database relative to pre- and post-natal effects for children is complete. Therefore, it is concluded that an additional uncertainty factor is not warranted and that the RfD at 0.2 mg/kg/day is appropriate for assessing aggregate risk to infants and children.

Using the conservative exposure assumption previously described, it is concluded that the percent of the RfD that will be utilized by aggregate exposure to residues of picloram will be less than 4% of the RfD for all populations and subgroups. Since this estimate represents the 'worst case' exposure for a given population (Non-nursing infants, >1 year old), exposures will be less for all other sub-populations e.g. children, 1-6 years. Therefore, based on the completeness and reliability of the toxicity data and the conservative exposure assessment, it is concluded that there is a reasonable certainty that no harm will result to infants and children from aggregate exposure to picloram residues.

F. International Tolerances

There are no Codex maximum residue levels established for residues of picloram.

G. Other Considerations

Data Gaps. Residue data for sorghum aspirated grain fractions is currently being generated. Based on the toxicological data and the levels of exposure, EPA has determined that the proposed tolerances will be safe.

[FR Doc. 98-31067 Filed 11-19-98; 8:45 am]

BILLING CODE 6560-50-F

ENVIRONMENTAL PROTECTION AGENCY

[PF-832; FRL-6027-6]

Notice of Filing of Pesticide Petitions

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice.

SUMMARY: This notice announces the initial filing of pesticide petitions proposing the establishment of regulations for residues of certain pesticide chemicals in or on various food commodities.

DATES: Comments, identified by the docket control number PF-832, must be received on or before December 21, 1998.

ADDRESSES: By mail submit written comments to: Public Information and Records Integrity Branch (7502C), Information Resources and Services Division, Office of Pesticides Programs, Environmental Protection Agency, 401 M St., SW., Washington, DC 20460. In person bring comments to: Rm. 119, CM #2, 1921 Jefferson Davis Highway, Arlington, VA.

Comments and data may also be submitted electronically to: opp-docket@epamail.epa.gov. Follow the instructions under "SUPPLEMENTARY INFORMATION." No confidential business information should be submitted through e-mail.

Information submitted as a comment concerning this document may be claimed confidential by marking any part or all of that information as "Confidential Business Information" (CBI). CBI should not be submitted through e-mail. Information marked as CBI will not be disclosed except in accordance with procedures set forth in 40 CFR part 2. A copy of the comment that does not contain CBI must be submitted for inclusion in the public record. Information not marked confidential may be disclosed publicly by EPA without prior notice. All written comments will be available for public inspection in Rm. 119 at the address given above, from 8:30 a.m. to 4 p.m., Monday through Friday, excluding legal holidays.

FOR FURTHER INFORMATION CONTACT: By mail: Bipin Gandhi, Registration Division (7505C), Office of Pesticide Programs, Environmental Protection Agency, 401 M St., SW., Washington, DC 20460. Office Location, telephone number, and e-mail address: Rm. 707A, CM #2 1921 Jefferson Davis Hwy., Arlington, VA 22202, (703-8380, e-mail: gandhi.bipin@epamail.epa.gov.

SUPPLEMENTARY INFORMATION: EPA has received pesticide petitions as follows

proposing the establishment and/or amendment of regulations for residues of certain pesticide chemicals in or on various food commodities under section 408 of the Federal Food, Drug, and Comestic Act (FFDCA), 21 U.S.C. 346a. EPA has determined that these petitions contain data or information regarding the elements set forth in section 408(d)(2); however, EPA has not fully evaluated the sufficiency of the submitted data at this time or whether the data supports granting of the petition. Additional data may be needed before EPA rules on the petition.

The official record for this notice of filing, as well as the public version, has been established for this notice of filing under docket control number [PF–832] (including comments and data submitted electronically as described below). A public version of this record, including printed, paper versions of electronic comments, which does not include any information claimed as CBI, is available for inspection from 8:30 a.m. to 4 p.m., Monday through Friday, excluding legal holidays. The official record is located at the address in "ADDRESSES" at the beginning of this document.

Electronic comments can be sent directly to EPA at:

opp-docket@epamail.epa.gov

Electronic comments must be submitted as an ASCII file avoiding the use of special characters and any form of encryption. Comment and data will also be accepted on disks in Wordperfect 5.1/6.1 or ASCII file format. All comments and data in electronic form must be identified by the docket control number [PF–832] and appropriate petition number. Electronic comments on this notice may be filed online at many Federal Depository Libraries.

List of Subjects

Environmental protection, Agricultural commodities, Food additives, Feed additives, Pesticides and pests, Reporting and recordkeeping requirements.

Dated: October 22, 1998.

James Jones,

Director, Registration Division, Office of Pesticide Programs.

Summaries of Petitions

Petitioner summaries of the pesticide petitions are printed below as required by section 408(d)(3) of the FFDCA. The summaries of the petitions were prepared by the petitioners and represent the views of the petitioners. EPA is publishing the petition summaries verbatim without editing them in any way. The petition summary announces the availability of a description of the analytical methods available to EPA for the detection and measurement of the pesticide chemical residues or an explanation of why no such method is needed.

1. EDM Corp

PP 8E4968

EPA has received a pesticide petition (8E4968) from EDM Corp 2278 So. Indiana Porterville, CA 93257 proposing pursuant to section 408(d) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. 346a(d), to amend 40 CFR part 180 to establish an exemption from the requirement of a tolerance for Yucca Extract in or on the raw agricultural commodity when used in accordance with good agriculture practice as an inert ingredient in pesticide formulations applied to growing crops, the EPA has determined that the petition contains data or information regarding the elements set forth in section 408(d)(2) of the FFDCA; however, EPA has not fully evaluated the sufficiency of the submitted data at this time or whether the data supports granting of the petition. Additional data may be needed before EPA rules on the petition.

A. Residue Chemistry

1. *Plant metabolism.* No plant metabolism studies have been submitted in support of this tolerance exemption petition since yucca extract, a sarsasaponin is present in most plant life.

2. *Analytical method.* Since the petitioner has requested a tolerance exemption, a residue analytical method is not required.

3. *Magnitude of residues.* No yucca extract residue studies were conducted since yucca extract is naturally found at significant levels (> .68 ppm) in many different types of food. In addition, residue trials are not practical since it is very difficult to distinguish Sarsaponin residues naturally occurring versus sapsaponin residues from yucca extract.

B. Toxicological Profile

1. *Acute toxicity— Study #6176-P320 acute oral toxicity.* The acute oral LD^{50} for a 70% solution of yucca extract is > 5,000 milligrams/kilogram (mg/kg). Accordingly, yucca extract relatively non-toxic by the oral route.

The petitioner has requested that the Agency waive all sub-chronic, chronic/oncogenicity, mutagenicity, developmental and reproductive toxicity study requirements for yucca extract. There is an overwhelming lack of evidence for any chronic effects induced by dietary ingestion of yucca extract.

C. Aggregate Exposure

1. *Food.* The FDA title 21 under CFR 172.510, FEMA #3121, No Limitations. Food. Sarsasaponin is naturally found in several types of foods, such as fruits and vegetables,(asparagrus, legumes ect) at various levels.

2. *Drinking water.* Degradation of sarsasaponin in water.

D. Cumulative Effects.

No cumulative adverse effects are expected from long-term exposure to yucca extract.

E. Safety Determination

1. *U.S. population.* Yucca has been approved for uses in food and beverages by the FDA title 21 CFR 172.510, FEMA number 3121, with no limits. Approval of this petition will not increase dietary exposure to yucca extract. Accordingly, there is reasonable certainty that no harm will result from aggregate exposure of the U.S. population to yucca extract.

2. *Infants and children.* Since yucca extract is also an additive in soft drinks, root beer etc, the daily exposure to children is anticipated to be trivial, no adverse effects on infants or children are expected.

F. International Tolerances

There are no approved CODEX maximum residue levels (MRLS) established for residues of yucca extract.

Previously submitted Yucca extract data:

1. THERM-70 Study #6176-P320 Acute Oral Toxicity.

2. Regarding the use of the inert ingredient Yucca extract:

A-350 tons raw materials are used for all usese in the United States.

B- 300,000 lbs of raw material makes 4,630 gallons of THERMX-70 for pesticidal uses.

C- CELLU-CON, INC. Received raw material in 1997 from Mexico (85%) and U.S. 15%.

D- Yucca already approved for uses in food and beverages by the FDA title 21 CFR 172.510, FEMA number 3,121, no limits.

E- We would like to waive Yucca (Schidigera) to be approved under title 40 CFR in section 180.1001 as an Inert Ingredient.

3. This is to advise you regarding EDM's use of Yucca. We will not be using more than 6% THERMX-70 as a wetting in our product MIRAGE.

Enclosed is a packet of information to assist you in studying this material.

A- FDA 21 CFR 172.510
B- COMMERCIAL FEED LICENSE
C- THERMX-70 label
D- THERMX-70 MSDS sheet
E- Sarsaponin (Micro-Aid)

4. DESERT PRIDE label Yucca Herbal Food Tablets has been sold in stores since 1974.

2. Hercules, Incorporated

PP 6E4782

EPA has received a pesticide petition (PP 6E4782) from Hercules, Incorporated, 1313 North Market Street, Wilmington, Delaware, proposing pursuant to section 408(d) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. 346a(d), to amend 40 CFR part 180 to establish an exemption from the requirement of a tolerance for polymers of α-pinene and/or *B*-pinene in or on raw agricultural commodities. EPA has determined that the petition contains data or information regarding the elements set forth in section 408(d)(2) of the FFDCA; however, EPA has not fully evaluated the sufficiency of the submitted data at this time or whether the data supports granting of the petition. Additional data may be needed before EPA rules on the petition.

A. Toxicological Profile

1. *Acute toxicity.* An acute oral intubation test was conducted. Two male and two female rats were administered four dose levels of oligomeric copolymer ranging from 10.2 to 34.6 g/kg. No deaths resulted. The oral LD_{50} in rats is therefore >34.6 g/kg. An acute eye irritation study was conducted. Two rabbits were treated with 0.1 milliliter (ml) of undiluted oligomeric copolymer material instilled in each eye. One eye of each animal was rinsed with running water after one minute. The unwashed eye showed moderate irritation to the iris and conjunctiva which persisted for 4 days after treatment. Irritation in the washed eyes was mild and persisted for 3 days after treatment.

2. *Reproductive and developmental toxicity.* Petitioner has not identified a reproduction study in which the test substance was an α-pinene based polymer. In the interest of complete disclosure, Petitioner is aware of a limited reproduction study dated 1960 that was conducted at the LaWall & Harrisson Laboratories in connection with a larger 2-year feeding study. The test substance was Hercules Piccolyte S125 Polyterpene Resin, a *B*-pinene-based resin which is derived from the polymerization of a terpene feedstock containing a minimum *B*-pinene content of 80% and an α-pinene content of between 5% and 9%. Groups of six female Sprague-Dawley rats were fed the test substance at 0%, 3%, or 10% of the diet. After 4 months of exposure, the rats were mated with similarly treated males. All females bore litters except one from the untreated control group. All litters were normal in size and a few stillborn pups were noted in each group. There were some deaths among the pups, but survival to weaning was equal in all groups. Indices of reproductive and developmental performance were not calculated. The dietary level of 10% was considered the no-observed-adverse-effect level (NOAEL) in this limited reproduction study.

3. *Subchronic toxicity— i. Study No. 1.* In a study conducted in 1968, groups of 10 male and 10 female Charles River rats were fed diets containing 0%, 1%, 3%, or 5% of an α-pinene based resin for 3 months. Criteria of evaluation for possible toxic effects included general appearance and behavior, growth, food consumption, survival, clinical laboratory results, absolute and relative organ weights, and gross and microscopic pathology. Effects seen at the 5% dietary concentration include increases in relative liver weight in both sexes, and absolute liver weight in females only. Increased relative thyroid weight in males was noted at the 5% and 3% dosage levels. In the absence of histopathological alterations, these changes are regarded as adaptive and not of toxicological significance. The dietary level of 5%, equivalent to an overall average of 3,967 milligrams/kilogram/day (mg/kg/day) is considered the NOAEL in this study.

ii. *Study No. 2.* Groups of ten male and ten female Sprague-Dawley rats were fed diets containing 0%, 0% (i.e., two untreated controls), 0.01%, 0.05%, 0.2%, 1%, or 5% of Terpene AP for 90 days. Criteria of evaluation included appearance and behavior, growth, survival, hematology and urinalysis, organ weights and gross and microscopic pathological evaluation. A paired feeding study was conducted in conjunction with the main study to evaluate the significance of diet rejection vs. compound-related toxicity in weight gain reduction associated with high concentrations of Terpene AP. In the paired feeding study, each rat fed 5% Terpene AP (Test Group) was matched with a rat of the same sex and similar weight. Each of the Paired Feeding Control Group received the same amount of diet in each 24-hour period as the corresponding treated rat during the preceding reference 24-hour period, but without the test material. Two deaths occurred during the study. They were not dosage-related and were attributed to respiratory infection and not to compound-related toxicity. Decreased body weight gain and increased liver weight were consistent findings. Final body weights were reduced 16% in males and 11% in females at the highest dosage level. The paired-feeding study demonstrated that the effect was due to food rejection based on poor palatability and not due to systemic toxicity of the test material. Liver weight, as absolute weight and liver/brain weight ratios, increased in a dosage-related fashion. At the 5% dietary levels, 39% and 83% absolute weight increases were noted in males and females, respectively. Lesser increases were noted at the 1% and 0.2% dietary levels of the test material. Liver weight/body weight ratios were increased artifactually because of the growth depression. Since there were no adverse histological findings associated with the liver weight increases, the finding is attributed to generalized physiologic stress and not to organ-specific toxicity. Thyroid hyperplasia noted in some rats at the 5% and 1% levels is a secondary effect of the liver weight increase. The dietary concentration of 0.05% Polyterpene was a NOAEL in this 90-day study. Because food consumption was not evaluated, an equivalent mg/kg/day NOAEL could not be calculated in this study. Based on analyses of food consumption data from similar studies, an approximate dosage equivalent would be 37.5 mg/kg/day.

4. *Chronic toxicity— i. Study No. 3.* A terpene resin was fed to beagle dogs, three per sex per group, at dietary levels of 0%, 0.2%, 1% and 5% for 2 years. Criteria of effect included appearance and behavior, growth and survival, food consumption, hematology, clinical chemistry, urinalysis, absolute and relative organ weights and gross and microscopic pathology. Effects seen at the 5% dietary level included moderate reduction in growth and increased absolute and relative liver weight at 1 year and 2 years, and minimal hepatocellular fatty changes at 1 year but not 2 years. Similar liver effects were seen at the 1% dietary concentration. The dietary levels of 0.2% terpene resin equivalent to an overall average of 51 mg/kg/day, a NOAEL in this 2-year study.

ii. *Study No. 4.* Groups of 30 male and 30 female Sprague Dawley rats were fed diets containing 0%, 0.2%, 1%, or 5% terpene resin for 2 years. The terpene resin was a copolymer of α- and *B*-pinene. No differences from controls were noted in any test groups with respect to appearance and behavior,

food consumption, growth, survival, tumor incidence, hematology and urinalysis. All means were within the range of normal variation. Significant elevations of absolute and relative liver weight were noted in females after 12 months on the 1% and 5% diets. In males, absolute liver weight was elevated at the 5% level and relative liver weights were elevated at both the 1% and 5% levels. After 24 months of treatment, relative liver weights were elevated in males at 5% and in females at 1% and 5%. Histological examinations after 2 years showed only effects anticipated in untreated animals. Liver enlargement in the absence of histopathological changes results from compensatory effects. The highest dietary concentration of 5% terpene resin, equivalent to an overall average of 3,100 mg/kg body weight per day, is regarded as the NOAEL in this study.

5. *Endocrine disruption.* A comprehensive literature search has revealed no reports associating pinene monomers or polymers with endocrine effects. Petitioner has not undertaken any testing to explore further the possibility that pinene polymers or monomers could cause endocrine effects and understands that EPA will implement a screening program for endocrine effects in the future.

C. Aggregate Exposure

1. *Dietary exposure.* Synthetic terpene resin, consisting of polymers of α-pinene, *B*-pinene, and/or dipentene, is currently cleared by the Food and Drug Administration for use as an ingredient of chewing gum base and for use in a variety of food-contact or food packaging applications. The range of materials that are used in these applications under the name "synthetic terpene resin" will vary in composition and molecular weight. These existing food applications result in some small amount of dietary exposure to pinene monomers, oligomers, and polymers. This exposure can be expected to be quite small given that only a small amount, if any, of the synthetic terpene resin present in a food-contact article will migrate into food. Similarly, the insoluble gum base portion of chewing gum is ordinarily discarded after chewing, and like the other components of gum base, synthetic terpene resin is not extracted to any significant degree by saliva. Petitioner has presented calculations showing very roughly that even if the total annual U.S. production volume of terpene resins were incorporated directly into the diet, this would result in a per capita consumption of α-pinene and α-pinene repeating units of only 1.7 mg/kg body weight per day for a 60-kg adult. Actual intake will be significantly less than this number, given that not all synthetic terpene resin is used in food applications, and that very little migration and ingestion can be attributed to the existing food-contact and chewing gum applications.

2. *Food.* Petitioner does not manufacture sticker formulations and therefore has not conducted studies to show the actual quantity of pinene polymers that will remain on harvested food crops. Based on the conservative assumption that all pinene polymer will remain on food crops at the time of harvest, Petitioner has presented calculations showing that the resulting dietary exposure will not exceed 0.43 mg/kg body weight per day for a 60-kg adult. Actual intake will be less than his number. Petitioner notes that this intake is a subset of the worst-case aggregate exposure number, 1.7 mg/kg body weight per day.

3. *Drinking water.* Due to its relative insolubility, only trace amounts of pinene polymer, if any, will be found in drinking water. Some amount of pinene polymer will enter the soil in fields where it is applied as part of a pesticide formulation. Any pinene polymer present in the soil could potentially reach ground water, as is the case with agricultural chemicals generally. In the case of pinene polymers, Petitioner notes that they can be expected to adhere to the soil due to their adhesive properties and that they may biodegrade before reaching ground water. Petitioner further notes that any drinking water exposure will be within the worst-case aggregate exposure estimate, 1.7 mg/kg body weight per day.

4. *Non-dietary exposure.* Outside of food applications, pinene polymers are used in various adhesive applications including construction adhesives used, for example, to lay floor tile. Pinene polymers present in adhesives are not volatile and will therefore not be inhaled. The only human exposure will be that associated with accidental skin contact. It would be difficult to assign a numerical value to this non-occupational exposure for a typical person. Exposures from all sources cannot exceed 1.7 mg/kg body weight per day for a typical adult, given the total production volume of α-pinene polymers.

D. Cumulative Effects

No identified risks are associated with exposure to pinene polymers. The mechanism or mode of action associated with pinene polymers is simply that the substance is physically sticky.

E. Safety Determination

1. *U.S. population.* Petitioner estimates that exposure to α-pinene polymers and repeating units attributable to the requested action will be less than 0.43 mg/kg body weight per day in a 60-kg adult. This number is based on a set of conservative assumptions, and actual exposure is expected to be much less. In no event will aggregate exposure, by all routes and from all sources, exceed 1.7 mg/kg body weight, given the total production volume of α-pinene polymers. In several of the available animal feeding studies, the NOAEL was found to be 5% or more of the diet (greater than 3,000 mg/kg body weight per day). The lowest reported NOAEL of which the petitioner is aware is 37.5 mg/kg body weight, which is somewhat of an outlying value.

2. *Infants and children.* Infants and children will not experience higher levels of exposure to pinene polymers than the rest of the population as a result of the action requested in this petition. Furthermore, no chronic or acute effects are associated with pinene polymers, for which infants and children could be particularly sensitive. Petitioner expects pesticide sticker formulations containing pinene polymers to be used on a variety of food crops, which will lead to low levels of residues distributed evenly throughout the food supply. Considering this variety of uses, exposure should be spread evenly over the entire population and not concentrated in any particular sub-population. Dietary exposure in adults will not exceed 0.43 mg/kg body weight per day from the requested application, and aggregate exposure from all sources and routes cannot exceed 1.7 mg/kg body weight per day. These estimates correspond to an adult weighing 60 kg and consuming 1,500 grams of solid food per day. The numbers can be adjusted to account for the weight of a child. For example a child weighing 30 kg and consuming 1,000 g of solid food per day will be exposed to no more than 0.56 mg/kg body weight per day from the requested application and no more than an aggregate of 3.3 mg/kg body weight per day from all routes and all sources. Exposure estimates thus adjusted for children compare favorably with the NOAEL reported in the animal feeding studies.

[FR Doc. 98-31063 Filed 11-19-98; 8:45 am]

BILLING CODE 6560-50-F

ATTACHMENT 8

Copies of Letters to Mr. Kerry Leifer and Mr. A.D. Vidyarthi

8.1 Letter to EPA dated May 09, 2002
re. Reassessment of Tolerance Exemption for
β-Pinene Polymers under 180.1001(c)

8.2. Letter to Mr. A.D. Vidyarthi of Miller Chemical
re. Terpene Polymers and their Approval Status
under EPA and FDA Regulations

ATTACHMENT 8.1

Letter to Mr. Kerry Leifer at EPA

Letter to EPA dated May 09, 2002
re. Reassessment of Tolerance Exemption for
β-Pinene Polymers under 180.1001(c)

MANDAVA ASSOCIATES

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VIA FACSIMILE 703-305-0599

May 9, 2002

Mr. Kerry B. Leifer
Minor Use Inerts and Emergency Response Branch
Registration Division (7505C)
U,S, Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460

SUBJECT: Reassessment of Tolerance Exemption for β-Pinene Polymers under 180.1001(c)

Requestor: Miller Chemical and Fertilizer Corporation

Dear Kerry:

On behalf of Miller Chemical & Fertilizer Corporation ("**Miller Chemical**"), we request the Agency to reassess the existing regulation under 40 CFR 180.1001(c) for tolerance exemption for β-pinene polymers for use as surfactant, related adjuvant of surfactant in pesticide formulations for pre- and post harvest applications.

The reason for our request is that:

- There are **NO** β-pinene polymers that are commercially available and all the polymer products contain both the isomers (α- and β-pinenes); and,
- The β-pinene polymers are **NOT** identified by CAS Registry Number(s) (see Table 1 and Table 2).
  [We can not readily distinguish/differentiate the polymers made from α-pinene and β-pinene.]

We request the Agency to broaden the regulation to include α-pinene polymers and β-pinene polymers under terpene resins (terpene polymers) or pinene polymers.

The bases for amendment are the following:

1) All terpene polymers are listed in TSCA inventory (Table 1 and Table 2).

2) Several terpene polymers (except the last three entries in Table 1) are made from α- and β-pinene which are derived from pinene fraction of the turpentine oil).

3) Terpene polymers (synonym: terpene resins) derived from α-pinene and β-pinene are widely used in building and packaging materials as well as in some consumer products.

4) The FDA has approved terpene polymers for use in food (direct food additive approval under 21 CFR §172.615 for use a chewing gum base) and also in food contact materials (Table 3).

We have provided additional information on the terpene resins (terpene polymers) in Appendix 1.

Based on the above considerations, Miller Chemical and Fertilizer Corporation requests the Agency to amend the subject regulation to read as "Terpene Polymers".

If our request is granted, we will submit a proposed regulation for your consideration and approval.

Thanks for your understanding and cooperation.

Sincerely,

N. Bhushan Mandava

N. Bhushan Mandava, Ph.D.
Miller Chemical and Fertilizer Corporation

Enclosure
cc: A.D. Vidyarthi

APPENDIX 1

TERPENE RESINS (TERPENE POLYMERS)

This appendix contains information on terpene resins (terpene polymers) which include the status of regulatory approvals by EPA and FDA.

1. Background Information:

Terpenoids are a group of natural products derived from plant sources. They are classified into monoterpenes, sesquiterpenes, and di- and triterpenes. Monoterpenes consist of 10 carbon atoms and sesquiterpenes are made up of 15 carbon atoms . Essential oils are the major source for both these types (mono- and sesquiterpenes).

The terpene polymers are also referred to as terpene resins. α- and β-Pinenes are commonly used as the monomers for the manufacture of terpene polymers (Table 2). Turpentine oil appears to be the rich source for α- and β-pinenes. Other monomers [including other terpenes (e.g., limonene, caryophyllene, and myrcene) and non-terpenoid compounds (e.g., isoprene, phenol, diethylene glycol and formaldehyde) are also mixed with α- and β-pinenes for the manufacture of terpene resins. (See Tables 1 and 2 for TSCA approved terpene polymers).

2. Clearance Status for Terpene Polymers Under TSCA:

There are several polymers derived from α- and β-pinenes and other terpenoids which are cleared under TSCA as shown in Tables 1 and 2. The Agency listed all of these terpene polymers as UVCB chemicals. Please note that the reported terpene polymers under TSCA (Table 2) are only the copolymers except two homopolymers (monomers in homopolymers: α-pinene or β-pinene). These polymers are manufactured from terpenoids of vegetable origin.

3. Approval Status for Use as Direct Food Additives and Indirect Food Additives:

Terpene resins are approved for use as a direct food additive (in chewing gum) and also as a indirect food additive (in packaging materials) as shown in Table 3.

4. Summary and Conclusions:

We have evaluated the regulatory status for terpene polymers (Synonym: terpene resins) which are manufactured from α- and β-pinenes and other monomers (terpenes and non-terpenes). We found that several terpene polymers (pinene-based) are approved by EPA under Section 5 of the TSCA. They are considered as the UVCB compounds for the TSCA Inventory purpose. Only one terpene resin (β-pinene polymer) is cleared for use in pesticide products under 40 CFR 180.1001(c). The FDA approved the terpene resins for use as direct food additives under 21 CFR §172.615. The FDA also approved the terpene polymers as indirect food additives for use as components of adhesives (21 CFR §175.105), cellophane (21 CFR §177.1200) and polyolefin (including polypropylene) film (21 CFR §178.3930) which are used for the manufacture of articles intended for use in packaging the food. Because of their approvals (by FDA), the terpene polymers are regarded as safe.

Table 1. Terpene Polymers and Condensates Derived From Alpha- and Beta-Pinenes and Other Terpenoids*

CAS Reg. No.	Chemical Name of the Terpene Polymer
68334-41-8	Terpenes and terpenoids, limonene fraction, polymers with terpene oligomers and turpentine-oil β-pinene fraction terpenes
70085-54-6	Terpenes and terpenoids, limonene fraction, polymers with turpentine-oil β-pinene fraction terpenes
68334-42-9	Terpenes and terpenoids, oligomers, polymers with turpentine oil limonene fraction terpenes and turpentine-oil β-pinene fraction terpenes
70750-56-0	Terpenes and terpenoids, turpentine-oil, limonene fraction, polymer with turpentine-oil β-pinene fraction terpenes
70750-57-1	Terpenes and terpenoids, turpentine-oil, α-pinene fraction polymerized
70750-58-2	Terpenes and terpenoids, turpentine-oil, β-pinene fraction polymerized
70750-59-3	Terpenes and terpenoids, turpentine-oil, α-pinene fraction, polymer with turpentine-oil β-pinene fraction terpenes
68555-27-1	Terpenes and terpenoids, turpentine-oil, terpinolene fraction, polymer with diethylene glycol and maleic acid
70750-53-7	Terpenes, 80% or greater limonene fraction, polymerized
68188-04-5	Terpenes and terpenoids, clove-oil, reaction products with formaldehyde

* Except the last three entries, all other polymers contain either α-pinene or β-pinene or both pinenes (which are derived from turpentine-oil). Please note that all the aforementioned terpene polymers (also known as terpene resins) are derived from pinene fraction of the turpentine oil.

Table 2. Terpene Resins Derived from Alpha- and Beta-Pinene (Homo- and Copolymers of Alpha- and Beta-Pinene)

CAS Reg. No.	Chemical Name of the Terpene Polymer
65733-79-1	α-Pinene, limonene, phenol polymer
31424-98-3	α-Pinene, dicycopentadiene polymer
25359-84-6	α-Pinene, phenol polymer
29565-96-6	α-Pinene, polymer with formaldehyde
68240-07-3	α-Pinene, β-pinene, phenol polymer
68240-09-5	β-Pinene, α-pinene, diterpene, β-phyllandrene polymer
68921-48-2	β-Pinene, formaldehyde condensate
68911-52-4	β-Pinene, phenol, formaldehyde, phthalic anhydride, glycerin, linseed oil, dehydrated castor oil polymer
25766-18-1	2-Pinene, polymers [also called α-pinene polymer or pinene resin]
31693-85-3	2-Pinene, polymer with isoprene
31393-98-3	2-Pinene, polymer with 2(10)-pinene
25719-60-2	2(10)-pinene, polymers [also known as β-pinene polymer or β-pinene resin]
18172-67-3	(-)-2(10)-pinene, polymers

Table 3. Terpene Resins (From Alpha- and Beta-Pinenes and Other Terpenoids) Approved by FDA

	Direct Food Additives:
	Approval under 21 CFR §172.615 for use as a chewing gum base
Synthetic Resin:	Consisting of polymers of α-pinene, β-pinene, and/or diterpene
Natural Resin:	Consisting of polymers of α-pinene
	Indirect Food Additives:
	Approvals for use as indirect food additives in packaging materials
21 CFR §175.105	Terpene resins (α- and β-pinenes) homopolymers, copolymers, and condensates with phenol, formaldehyde, coumarone and/or indene are approved as component(s) of adhesives for use in packaging, transporting or holding food.
21 CFR §177.1200	Terpene resins identified in 21 CFR §172.615 (see above for food additive clearance) are approved as component(s) of cellophane that may be used for packaging food.
21 CFR §178.3930	Terpene resins - consisting of the hydrogenated polymers of terpene hydrocarbons obtainable from sulfate turpentine.
	Terpene resins - consisting of polymers of beta-pinene

ATTACHMENT 8.2

Letter to Mr. A.D. Vidyarthi

Letter to Mr. A.D. Vidyarthi at Miller Chemical
re. Terpene Polymers and their Approval Status

MANDAVA ASSOCIATES

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VIA FACSIMILE 717-632-4581

November 7, 1997

Mr. A.D. Vidyarthi
Vice President - Manufacturing
Miller Chemical and Fertilizer Corporation
P.O. Box 333, Radio Road
Hanover, PA 17331

RE: Terpene Polymers and Their Approval Status under EPA and FDA Regulations

Dear Mr. Vidyarthi:

This letter is in response to your October 30, 1997 telephone request for information on the approval status for terpene polymers. We understand that, more specifically, you are interested in finding out the status of the terpene polymers under the FDA's Food Additive Regulations, and the EPA's Regulations for Pesticides and Commercial Chemicals.

1. General Information:

The EPA has the authority to regulate chemical substances including terpene polymers under the Toxic Substances Control Act (TSCA) and the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). The FDA's authority to regulate the subject polymers comes from the Federal Food, Drug and Cosmetic Act (FFDCA).

Terpenoids are a group of natural products derived from plant sources. They are classified into monoterpenes, sesquiterpenes, and di- and triterpenes. Monoterpenes consist of 10 carbon atoms and sesquiterpenes are made up of 15 carbon atoms . Essential oils are the major source for both these types (mono- and sesquiterpenes).

The terpene polymers are also referred to as terpene resins. α- and β-Pinenes are commonly used as the monomers for the manufacture of terpene polymers (Table 2). Turpentine oil appears to be the rich source for α- and β-pinenes. Other monomers [including other terpenes (e.g., limonene, caryophyllene, and myrcene) and non-terpenoid compounds (e.g., isoprene, phenol, diethylene glycol and formaldehyde) are also mixed with α- and β-pinenes for the manufacture of terpene resins. (See Tables 1 and 2 for TSCA approved terpene polymers).

Mr. A.D. Vidyarthi
November 7, 1997

2. Clearance Status for Terpene Polymers Under TSCA:

There are several polymers derived from α- and β-pinenes and other terpenoids which are cleared under TSCA as shown in Tables 1 and 2. The Agency listed all of these terpene polymers as UVCB chemicals. Please note that the reported terpene polymers under TSCA (Table 2) are only the copolymers except two homopolymers (monomers in homopolymers: α-pinene or β-pinene). These polymers are manufactured from terpenoids of vegetable origin.

3. Approval Status for Use as Ingredients in Pesticide Formulations:

Based on the available information, we found that none of the terpene resins (terpene polymers) are approved under 40 CFR §180.1001 for use as inert ingredients in pesticide formulations applied to raw agricultural commodities (viz., applications to growing crops, crops after harvest or food animals).

4. Approval Status for Use as Direct Food Additives and Indirect Food Additives:

The FDA approves chemical substances for use as direct food additives (intended for use in food, thereby they will become the components of the food for human consumption) and also as indirect food additives (that come in contact with food).

4.1. Direct Food Additives:

Terpene resins are approved by FDA for use as a direct food additive under 21 CFR Part 172. They are approved under 21 CFR §172.615 for use as a chewing gum base if they meet the following specifications and limitations, used in amounts not to exceed those required to produce the intended physical or other technical effect. They are considered as masticatory substances which meas that they are of the natural (coagulated or concentrated lattices) of vegetable origin:

Synthetic Resin:	Consisting of polymers of α-pinene, β-pinene, and/or diterpene; acid value less than 5; saponification number less than 5, and color less than 4 on the Gardner scale as measured in 50 percent mineral spirit solution.
Natural Resin:	Consisting of polymers of α-pinene; softening point minimum 155°C determined by U.S.P. closed-capillary method, United States Pharmacopeia XX (1980) (page 961).

Mr. A.D. Vidyarthi
November 7, 1997

4.2. Indirect Food Additives:

Terpene resins are approved for use as indirect food additives in packaging materials that come in contact with food. They are used as components of adhesives, cellophane and polyolefin (including polypropylene) film as noted below:

21 CFR §175.105	Terpene resins (α- and β-pinenes) homopolymers, copolymers, and condensates with phenol, formaldehyde, coumarone and/or indene are approved as component(s) of adhesives for use in packaging, transporting or holding food.
21 CFR §177.1200	Terpene resins identified in 21 CFR §172.615 (see above for food additive clearance) are approved as component(s) of cellophane that may be used for packaging food.
21 CFR §178.3930	Terpene resins - consisting of the hydrogenated polymers of terpene hydrocarbons obtainable from sulfate turpentine and meeting the following specifications: Drop-softening point of 118°-138°C; iodine value less than 20 - are approved as components of polypropylene film intended for use in contact with food. Terpene resins - consisting of polymers of beta-pinene and meeting the following specifications: Acid value less than 1; color less than 4 on the Gardner scale as measured in 50 percent mineral spirits solution - are approved as components of polyolefin film intended for use in contact with food.

5. Summary and Conclusions:

We have evaluated the regulatory status for terpene polymers (Synonym: terpene resins) which are manufactured from α- and β-pinenes and other monomers (terpenes and non-terpenes). We found that several terpene polymers (pinene-based) are approved by EPA under Section 5 of the TSCA. They are considered as the UVCB compounds for the TSCA Inventory purpose. Terpene resins are not cleared for use in pesticide products under the FIFRA. Based on the safety evaluations, the FDA approved the terpene resins for use as direct food additives under 21 CFR §172.615 provided they meet the specifications and other limitations. The FDA also approved the terpene polymers as indirect food additives for use as components of adhesives

Mr. A.D. Vidyarthi
November 7, 1997

(21 CFR §175.105), cellophane (21 CFR §177.1200) and polyolefin (including polypropylene) film (21 CFR §178.3930) which are used for the manufacture of articles intended for use in packaging the food. Because of their approvals by EPA and FDA, the terpene polymers are regarded as safe to humans and the environment.

If there are further questions regarding the information provided in this letter, please contact us at 202-223-1424.

Thanks and best regards.

Sincerely,

N. Bhushan Mandava

N. Bhushan Mandava, Ph.D., CPC, RAC

Mr. A.D. Vidyarthi
November 7, 1997

Table 1. Terpene Polymers and Condensates Derived From Alpha- and Beta-Pinenes and Other Terpenoids*

CAS Reg. No.	Chemical Name of the Terpene Polymer
68334-41-8	Terpenes and terpenoids, limonene fraction, polymers with terpene oligomers and turpentine-oil β-pinene fraction terpenes
70085-54-6	Terpenes and terpenoids, limonene fraction, polymers with turpentine-oil β-pinene fraction terpenes
68334-42-9	Terpenes and terpenoids, oligomers, polymers with turpentine oil limonene fraction terpenes and turpentine-oil β-pinene fraction terpenes
70750-56-0	Terpenes and terpenoids, turpentine-oil, limonene fraction, polymer with turpentine-oil β-pinene fraction terpenes
70750-57-1	Terpenes and terpenoids, turpentine-oil, α-pinene fraction polymerized
70750-58-2	Terpenes and terpenoids, turpentine-oil, β-pinene fraction polymerized
70750-59-3	Terpenes and terpenoids, turpentine-oil, α-pinene fraction, polymer with turpentine-oil β-pinene fraction terpenes
68555-27-1	Terpenes and terpenoids, turpentine-oil, terpinolene fraction, polymer with diethylene glycol and maleic acid
70750-53-7	Terpenes, 80% or greater limonene fraction, polymerized
68188-04-5	Terpenes and terpenoids, clove-oil, reaction products with formaldehyde

* Except the last three entries, all other polymers contain either α-pinene or β-pinene or both pinenes (which are derived from turpentine-oil). Please note that all the aforementioned terpene polymers (also known as terpene resins) are derived from pinene fraction of the turpentine oil.

Mr. A.D. Vidyarthi
November 7, 1997

Table 2. Terpene Resins Derived from Alpha- and Beta-Pinene (Homo- and Copolymers of Alpha- and Beta-Pinene)

CAS Reg. No.	Chemical Name of the Terpene Polymer
65733-79-1	α-Pinene, limonene, phenol polymer
31424-98-3	α-Pinene, dicycopentadiene polymer
25359-84-6	α-Pinene, phenol polymer
29565-96-6	α-Pinene, polymer with formaldehyde
68240-07-3	α-Pinene, β-pinene, phenol polymer
68240-09-5	β-Pinene, α-pinene, diterpene, β-phyllandrene polymer
68921-48-2	β-Pinene, formaldehyde condensate
68911-52-4	β-Pinene, phenol, formaldehyde, phthalic anhydride, glycerin, linseed oil, dehydrated castor oil polymer
25766-18-1	2-Pinene, polymers [also called α-pinene polymer or pinene resin]
31693-85-3	2-Pinene, polymer with isoprene
31393-98-3	2-Pinene, polymer with 2(10)-pinene
25719-60-2	2(10)-pinene, polymers [also known as β-pinene polymer or β-pinene resin]
18172-67-3	(-)-2(10)-pinene, polymers

Table 3. Terpene Resins (From Alpha- and Beta-Pinenes and Other Terpenoids) Approved by FDA

	Direct Food Additives:
	Approval under 21 CFR §172.615 for use as a chewing gum base
Synthetic Resin:	Consisting of polymers of α-pinene, β-pinene, and/or diterpene
Natural Resin:	Consisting of polymers of α-pinene
	Indirect Food Additives:
	Approvals for use as indirect food additives in packaging materials
21 CFR §175.105	Terpene resins (α- and β-pinenes) homopolymers, copolymers, and condensates with phenol, formaldehyde, coumarone and/or indene are approved as component(s) of adhesives for use in packaging, transporting or holding food.
21 CFR §177.1200	Terpene resins identified in 21 CFR §172.615 (see above for food additive clearance) are approved as component(s) of cellophane that may be used for packaging food.
21 CFR §178.3930	Terpene resins - consisting of the hydrogenated polymers of terpene hydrocarbons obtainable from sulfate turpentine.
	Terpene resins - consisting of polymers of beta-pinene

ATTACHMENT 9

Sandoz Product Development for TRIDENT™ Biological Insecticide

DEVELOPMENTS

Information from Sandoz Product Development

FEBRUARY, 1989

TO: University Entomologists
Extension Specialists
Sandoz Crop Protection Ag Sales Representatives
Key Dealers/Distributors
Key Crop Consultants

FROM: D.G. Eastman, Field Scientist, Sandoz Crop Protection Corporation

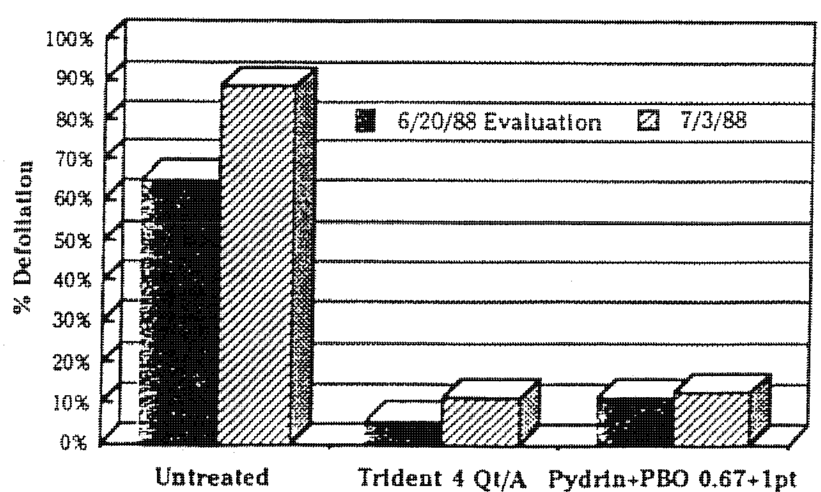
SUBJECT: **TRIDENT™ Biological Insecticide**

CHB, Picked this up last week. New product by Sandoz. See page 3. Mike

Introduction:

Trident biological insecticide, a control agent for Colorado potato beetle larvae, is derived from the naturally occurring bacteria *Bacillus thuringiensis* variety *tenebrionis*. It has been tested in university, grower and in-house field trials on potatoes, tomatoes and eggplants from 1986-88. These trials have demonstrated that Trident is an effective means of controlling both resistant and non-resistant Colorado potato beetle larvae when applied properly in scouted fields.

Trident has specific activity on Colorado potato beetle larvae. When the larva ingests Trident, spores and crystal pro-toxins enter the gut. There the crystal is dissolved and a toxin released which attaches to the gut wall of the larva. The gut membrane ceases to function normally and eventually erodes. **The insect stops feeding within 30 minutes to 4 hours after ingesting Trident.** Spores from the bacteria pass through the gut membrane and reproduce in the bloodstream of the insect. **The insect dies in two to five days from the combined effects of starvation and erosion of the gut.**



G. Ghidiu; Rutgers Univ.;Bridgeton, NJ

DIAGRAM 1

Trident Colorado Potato Beetle Control in Potato

Use Rates and Additives
Normal use rate is 4 quarts per acre. Up to 6 quarts may be applied in heavy infestations of larvae or when it is difficult to get good coverage. A minimum of 3 quarts per acre may be used for ground applications in light populations of young Colorado potato beetle larvae and following a previous application of an effective contact insecticide.

The addition of a spreader sticker, such as pinolene, has shown improved control of young beetle larvae.

Pinolene is the active ingredient in NuFilm

Other Insect Pests
Trident is effective on young Colorado potato beetle larvae. It may be rotated with or mixed with other insecticides that are effective on other pests. (e.g. aphids, leafhoppers and flea beetles).

Example Program Approach
1. Use an effective soil applied systemic or foliar contact insecticide to control Colorado potato beetle adults.

2. Apply Trident plus a spreader sticker two to three days after first egg hatch.

3. Make second application of Trident four to seven days after the first. Use shorter interval in heavy egg-hatch situations or following rainfall.

4. Apply an effective contact insecticide to control escaped large larvae and secondary potato pests. Include Trident in the mix if egg hatching continues.

This sequence may be repeated on subsequent generations. Close monitoring of insect pressure and development is important for making good decisions on the time to apply any control measure.

PRODUCT STORAGE

Activity may be impaired if stored above 90F. Protect from freezing.

EFFECTS ON BENEFICIAL INSECTS, MAN AND THE ENVIRONMENT

Trident is not harmful to most beneficial insects. It contains no ingredients that are a health hazard to man. Trident has no food tolerance, re-entry restrictions or minimum preharvest interval. It should always be used in a manner corresponding to the label.

ATTACHMENT 10

Miller Chemical NU-FILM® with Thuricide® and Dimilin®

MILLER CHEMICAL & FERTILIZER CORPORATION

P.O. BOX 333, RADIO ROAD
HANOVER, PENNSYLVANIA 17331 U.S.A.
TELEPHONE: 717 - 632-8921
FAX NO: 717 - 632-4581
TELEX: 840-448 (MILLER CH HNVR)
CABLE ADDRESS - MICO

NU-FILM® with Thuricide® and Dimilin®

FOREST RESEARCH INSTITUTE - YUGOSLAVIA - DEPT. FOR PLANT PROTECTION
Researcher: Dr. Miroslav Harapin - March 1983

Nu-Film-17 is a sticking agent which, applied together with pesticides forms a protecting film on needles and leaf surfaces. By its application, it: 1) protects a pesticide from sunlight (ultraviolet); 2) improves a better distribution of the product on the surface; and, 3) protects from rain fall.

The most important pest in the Adriatic coastal area and on the islands is a pine processionary moth (Thaumetopoea pityocampa Schiff.). This is controlled only with biologic preparations like: Dipel, Bactucide, Bactospein, Thuricide and Dimilin. Very often the results are not satisfactory.

I. LABORATORY TREATMENT

For the laboratory treatment, 600 caterpillars were put into 6 cages, 100 in each. In 2 cages, e.g. 200 caterpillars were fed with needles treated by Thuricide HP. In the other 2 cages, another 200 caterpillars were fed with needles treated by Thuricide HP plus Nu-Film-17. The last 200 caterpillars were fed with non-treated needles.
The experiment was laid on October 11, 1982. Ten days after the experiment laying, the caterpillars were put in cages again (the experiment was repeated), but the needles which were used were taken from the previously treated trees.

RESULTS

Number of Cages	Pesticide	Mortality Percentage After Days 5	10	20
1	Thuricide	63	87	61
2		59	72	72
3	Thuricide + Nu-Film-17	94	100	97
4		87	95	100
5	Control	3	5	6

II. FIELD TREATMENT

The experimental plots on the island of Hvar in the pine culture (Pinus halepensis Mill.) were chosen for the treatement. The trees height was about 6 meters. The forest was severely attacked by the pine processionary moth caterpillars. They were in their third development phase. Four plots were chosen for treatment, each of 1 hectare, separated from one another. The treatment was carried out on October 28, 1982, by ground equipment.

Plots of 1 hectare were marked with Latin numerals I, II, III, and IV.
On plot I Dimilin WP 25 with 200 g per hectare was applied.

On plot II Dimilin WP 25 with 200 g per hectare plus Nu-Film-17, 1 kilo per hectare was applied.

(OVER)

On plot III Thuricide HP, 1 kilo per hectare was applied.

On plot IV Thuricide HP, 1 kilo per hectare plus Nu-Film-17, 1 kilo per hectare was applied.

Ten trees were marked on each plot on which nests with caterpillars were registered.

RESULTS

On each plot, on 10 chosen and marked trees, the nests with survived caterpillars were registered.

On plot I (Dimilin) out of 39 registered nests, caterpillars survived in 7 of them, or 17.9% e.g. mortality was 82.1%.

On plot II (Dimilin + Nu-Film-17) out of 25 nests, there were no caterpillars survived. Mortality was 100%.

On plot III (Thuricide) out of 23 nests, the caterpillars survived in 8 of them, or 34.78%. Mortality here was 66.22%.

On plot IV (Thuricide + Nu-Film-17) there were no caterpillars survived. Mortality was 100%.

CONCLUSION

The laboratory and field experiment results show that Nu-Film-17, as an additive to pesticides has characteristics mentioned above, and can be highly recommended in the forest protection.

Nu-Film-17 - Registered trademark Miller Chemical & Fertilizer Corporation
Dimilin - Registered trademark Uniroyal Chemical Co., Inc.
Thuricide - Registered trademark Sandoz Corp.

ATTACHMENT 11

Evaluation of

Nu-Film 17

With

Trident, Javelin and Dipel

in

Insecticide & Acaricide Tests

Reference:
VEGETABLE CROPS
Volume 14 (pages 121 and 147)

CORN (SWEET): *Zea mays* L. 'Sweet Sue'
European corn borer (ECB): *Ostrinia nubilalis* (Hübner)

Donald C. Weber, David N. Ferro, and Rolf B. Parker
Department of Entomology
University of Massachusetts
Amherst, MA 01003
(413) 545-2283 (52E)

CONTROL ON SWEET CORN, 1988: Corn was planted 7 Jun in South Deerfield, Mass., in a randomized complete block design with 4 replicates. Rows were 3 ft apart, 25 ft long, and plants were spaced 9 inches apart in the row with 4 rows/plot. Insecticides were applied using a tractor-drawn boom sprayer equipped with hollow-cone-type drop nozzles (5 nozzles/row), at 90 psi and in 90 gal water/acre. Scouting of 200 green tassels on 1 Aug showed no damage by Lepidoptera. The first treatment was applied 2 d after full silk. Treatments were applied on 5, 10, and 15 Aug. Pheromone trap captures showed peak ECB flights during silk, but very low corn earworm and fall armyworm populations in the area. On 19 Aug, 50 ears were harvested from each plot, and the number of ears infested with larvae of each pest was recorded. Only ears with damage to kernels were scored as damaged.

Larvin at both rates controlled ECB to a commercially marketable level of <5%. Plots treated with the high rate of Javelin DF and Nufilm sticker had a 65% reduction in ECB ear damage.

Treatment	Rate form/acre	% Infested
Larvin 3.2 EC	20 oz	1.5a
Larvin 3.2 EC	30 oz	3.5a
Javelin DF + Nu-Film 17	1.25 lb + 16 oz	6.5ab
Ambush 2 EC	12 oz	9.5abc
Javelin DF	1.25 lb	11.0abc
Javelin DF	0.5 lb	14.0bc
Javelin L + Nu-Film 17	64 oz + 16 oz	14.5bc
Dipel ES + Nu-Film 17	64 oz + 16 oz	16.0bc
Control	—	18.5c

Arcsine transformed data; means followed by the same letter are not significantly different ($P = 0.05$, DMRT).

CORN (SWEET): *Zea mays* L. 'Silver Queen'
European corn borer (ECB): *Ostrinia nubilalis* (Hübner)

J. Whalen and R. Breeding
Department of Entomology
University of Delaware
Newark, DE 19717
(302) 451-2526 (53E)

CONTROL ON SWEET CORN, 1988: Sweet corn was planted on 20 Apr near Georgetown, Del. Four-row plots, 20 ft long on 30-inch centers, were replicated 4 times in a randomized complete block design. Granular treatments were applied with a hand shaker in a 7-inch band over the whorl on 22 Jun. Liquid treatments were applied with a CO_2-pressurized wheelbarrow sprayer delivering 40 gal/acre at 40 psi on 22 Jun. All plant whorls were removed from the center two rows of each plot, unrolled, and the number of infested plants counted on 27 Jun.

Pressure from ECB was moderate. All treatments provided significantly better control when compared with the untreated plots. No phytotoxicity was observed.

		% Infested whorls	
Treatment	Rate lb (AI)/acre	Pretreatment 22 Jun	Posttreatment 27 Jun
Fortress 10 G	0.300	22.10a	6.50bc
Fortress 10 G	0.400	39.52a	9.10bc
Fortress 10 G	0.500	30.90a	1.20c
Dipel 10 G	1.000	31.80a	15.55b
Dursban 15 G	1.000	42.22a	8.43bc
Thiodan 15 G	1.000	28.17a	6.98bc
Karate 1 EC	0.015	42.12a	16.03b
Asana 0.66 XL	0.03	39.15a	16.63b
Orthene 75 SP	1.00	33.95a	18.63b
Control	—	20.87a	41.25a

Means followed by the same letter are not significantly different ($P = 0.05$, DMRT).

Treatment	Rate lb (AI)/acre	Total larvae (% control) First instars	Second instars	Third instars	Fourth instars	% Defoliated plants
Sprayed 23 Aug and evaluated 25 Aug						
Thimet 20 G	3	0.6b (98.2)	6.8bc (87.4)	51.6b (56.6)	58.7b (47.5)	25b
Temik 15 G	2	2.2b (91.3)	10.8bc (80.1)	25.0bc (79.0)	34.4bc (69.2)	10bc
Pydrin 2.4 EC	0.1	0.0b (100.0)	0.0c (100.0)	0.0c (100.0)	2.6c (97.7)	5bc
Capture 2 EC	0.02	0.0b (100.0)	0.0c (100.0)	7.2bc (93.9)	0.0c (100.0)	0c
Asana 1.9 EC	0.025	0.2b (99.4)	0.0c (100.0)	5.4bc (95.5)	0.0c (100.0)	0c
M-one 4.5%	3 qt form.	0.4b (98.8)	1.4c (97.4)	5.4bc (95.5)	0.6c (99.5)	5bc
M-one 4.5%	4 qt form.	1.0b (96.9)	3.6bc (93.4)	9.6bc (91.9)	4.6c (95.9)	5bc
M-one 4.5%	6 qt form.	0.0b (100.0)	7.8bc (85.6)	12.6bc (89.4)	21.8c (80.5)	10bc
Trident + Nu-film 17	4 qt form. + 1 qt form.	0.8b (97.5)	5.2bc (90.4)	7.8bc (93.4)	6.4c (94.3)	5bc
Trident	4 qt form.	0.4b (98.8)	9.0bc (83.4)	21.0bc (82.4)	9.8c (91.2)	10bc
Pyronone	3 pt form.	0.0b (100.0)	0.0c (100.0)	36.8bc (69.0)	3.8c (96.6)	1c
Pydrin 2.4 EC + Butacide 8 EC	0.05 + 0.12	0.0b (100.0)	0.0c (100.0)	0.8bc (99.3)	7.8c (93.0)	0c
Pydrin 2.4 EC + Butacide 8 EC	0.05 + 0.25	0.0b (100.0)	0.0c (100.0)	0.0c (100.0)	0.0c (100.0)	0c
Kryocide 96 W	11	0.2b (99.4)	0.4c (99.2)	2.4bc (98.6)	1.8c (93.6)	2bc
Margosan-O 0.3%	20 ppm	1.0b (96.9)	14.0b (28.4)	111.0a (6.7)	128.0a (−14.5)	65a
Control	—	32.0a (0.0)	54.2a (0.0)	119.0a (0.0)	111.8a (0.0)	60a

Means followed by the same letter are not significantly different ($P = 0.05$; DMRT).

Treatment	Rate lb (AI)/acre	Total larvae (% control) First instars	Second instars	Third instars	Fourth instars	% Defoliated plants
Sprayed 3 Sep and evaluated 5 Sep						
Thimet 20 G	3	1.4b (68.2)	2.4b (82.1)	6.3bc (86.2)	8.3c (84.0)	32b
Temik 15 G	2	0.0b (100.0)	1.0b (92.5)	5.6bcd (87.8)	7.0cd (86.5)	15bc
Pydrin 2.4 EC	0.1	0.0b (100.0)	0.0b (100.0)	0.0e (100.0)	0.0d (100.0)	12bc
Capture 2 EC	0.02	0.0b (100.0)	0.0b (100.0)	0.0e (100.0)	0.0d (100.0)	0d
Asana EC	0.025	0.0b (100.0)	0.0b (100.0)	0.0e (100.0)	0.0d (100.0)	0d
M-one 4.5%	3 qt form.	0.0b (100.0)	0.4b (97.0)	1.4e (96.9)	1.2cd (97.7)	8bc
M-one 4.5%	4 qt form.	0.0b (100.0)	0.0b (100.0)	0.4e (99.1)	0.4d (99.2)	5bc
M-one 4.5%	6 qt form.	0.0b (100.0)	0.2b (98.5)	1.0e (97.8)	0.6d (98.8)	10bc
Trident + Nu-film 17	4 qt form. + 1 qt form.	0.0b (100.0)	0.4b (97.0)	2.2cde (95.2)	1.4cd (97.3)	8bc
Trident	4 qt form.	1.6b (63.6)	2.4b (82.1)	8.6b (81.2)	7.4cd (85.7)	15bc
Pyronone	3 pt form.	0.0b (100.0)	0.0b (100.0)	0.0e (100.0)	0.0d (100.0)	0d
Pydrin 2.4 EC + Butacide 8 EC	0.05 + 0.12	0.0b (100.0)	0.0b (100.0)	0.0e (100.0)	0.0d (100.0)	0d
Pydrin 2.4 EC + Butacide 8 EC	0.05 + 0.25	0.0b (100.0)	0.0b (100.0)	0.0e (100.0)	0.0d (100.0)	0d
Kryocide 96 W	11	0.0b (100.0)	0.0b (100.0)	0.0e (100.0)	0.0d (100.0)	1cd
Margosan-O 0.3%	20 ppm	0.0b (100.0)	3.0b (77.6)	46.0a (−0.4)	19.0b	70a
Control	—	4.4a (0.0)	13.4a (0.0)	45.8a (0.0)	51.8a (0.0)	75a

Means followed by the same letter are not significantly different ($P = 0.05$; DMRT).

POTATO: *Solanum tuberosum* L. 'Russet Burbank'
Colorado potato beetle (CPB); *Leptinotarsa decemlineata* (Say)

Abdelaziz Lagnaoui and Edward B. Radcliffe
Department of Entomology
University of Minnesota
St. Paul, MN 55108

(87E)

CONTROL ON POTATO IN THE RED RIVER VALLEY OF MINNESOTA AND NORTH DAKOTA, 1988: Potatoes were planted 4 May at the University of Minnesota Northwest Experimental Station, Crookston, and at the Red River Valley Grower's Association Research Farm, Grand Forks. Experimental plots were 25 ft long with 36-inch row spacing. Treatments were randomized in complete blocks with 3 replications. Insecticidal sprays were applied 23 Jun with a CO_2-powered hand sprayer delivering 36 gal/acre. Posttreatment evaluations were done 24 h, 72 h, and 168 h later. They consisted of direct counts on all second and third instars on 10 randomly selected plants/plot.

CPB populations were very high. Total numbers of CPB larvae were significantly different among treatments. The percentage of control was calculated based on comparison with the average of 4 untreated control plots, corrected by Abbott's formula. Control ranged from 3.9 to 99.7%. Addition of Butacide (piperonyl butoxide) to Pydrin sprays appreciably enhanced CPB control.

ATTACHMENT 12

Copy of the July 22, 1971 Letter
From Norman R. Dubois of USDA
to Mr. Charles H. Svec of Miller Chemical
re. Application of Pinolene for extending the Bt Residual
Activity for at Least 4 Weeks.

UNITED STATES DEPARTMENT OF AGRICULTURE
FOREST SERVICE
NEFES, Forest Insect and Disease Laboratory
151 Sanford Street, Hamden, Connecticut 06514

4500-FS-NE-2202

July 22, 1971

Mr. Charles H. Svec
Vice President Pinolene Market Development
Miller Chemical and Fertilizer Corporation
Box 333
Hanover, Pennsylvania 17331

Dear Mr. Svec:

In reply to your letter of July 12, 1971, in 1964 we investigated the possibility of using Pinolene materials as a sticker for Bacillus thuringiensis suspensions. Enclosed is a report on the effect of various concentrations of Pinolene #1674 and #1773 on the physiology of this microorganism.

In addition to this laboratory study, in field test of B. thuringiensis (Thuricide 90T Flowable®) against gypsy moth larvae, in 1967 we found that a 2.5% concentration of Pinolene #1773 incorporated into the suspension, extended the residual activity of the B. thuringiensis by at least four weeks. This activity was monitored by, a) cutting the field sprayed foliage at weekly intervals and feeding it to caged larvae in the lab, and b) by making leaf presses on agar (Trypticase Soy Agar) and determining the number of B. thuringiensis colonies developing on the agar as representatives of drops deposited per unit area of leaf surface. We did not at the time have a satisfactory solvent to wash leaf surfaces to determine B. thuringiensis spore recovery from washed leaves, however I understand your company does have an excellent solvent to remove the Pinolene from foliage surfaces.

I hope I have provided you and your customer some information on our work with Pinolene and if I can be of further assistance please don't hesitate to call or write.

Sincerely,

Normand R. Dubois

NORMAND R. DUBOIS
Microbiologist

Enclosure

6200-11 (1/69)

ATTACHMENT 13

Efficacy of Nu-Film 17 in Improving Codling Moth Mortality from granulosis virus.

CMGV TRIAL of DSIR , New Zealand

Interim Report Dated December 3, 1991

NEW ZEALAND DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH

DSIR Plant Protection

CMGV TRIAL - ALEXANDRA, CENTRAL OTAGO
NEW ZEALAND, 1990-91
INTERIM REPORT 12.3.91

Objective: To determine the efficacy of Nufilm 17 in improving codling moth mortality from granulosis virus.

Products/Rates:

Madex (Andermatt, Switzerland): 16.7 mls/100l
Sugar : 50g/100l
Milk powder :100g/100l
Nufilm 17 (Key Industries Ltd): 50g/100l

Application method:

Hand lance from tractor-drawn sprayer 100-200 kp, 7-10 litres per tree.

Application frequency:

Every two weeks to test the ability of additives to improve efficacy. Dates: 16.11,29.11, 15.12, 28.12, 11.1, 25.1, 7.2, 21.2, and 8.3.

Replicates:

Single trees; 10.
Cultivars used for replication: Golden Delicious (1), Red Delicious (1), Lord Lambourne (2), Granny Smith (3), Spartan (1), Ballarat (2).

Site:

DSIR Earnscleugh Nursery, Earnscleugh Road, RD 1. Alexandra. Unsprayed for about 10? years. High codling moth population.

Sampling methods:

1. Pheromone traps (2)
2. Pre-entry larval mortality
3. Post-entry larval mortality - Windfalls (all)
   - Harvest (sample) plus total crop count.

Treatments:

1. Madex + Sugar + Nufilm 17.
2. Madex + sugar + milk powder.
3. Madex + sugar.
4. Untreated control.

Initial preliminary results:

1. Pheromone traps confirm high codling moth population. Flight October to March. Single generation plus small partial second generation.

2. Pre-entry larval mortality (minimum 100 hatched eggs/treatment).

		Red Del.	Gold. Del.	GrSm/LL	Ballarat
Treatment	1	83.3%	70.0	70.0	71.4
	2	60.0	53.3	56.5	47.4
	3	45.8	47.8	52.6	42.9
	4	21.4	18.8	17.6	16.7

3. Post-entry larval mortality as shown by percentage of harvested crop infested by "deep" entries.

		Percentage deep entries Lord Lambourne	
		Rep 6	Rep 7
Treatment	1	1.3	1.0
	2	3.1	2.5
	3	6.1	3.1
	4	35.6	21.4

Other cultivars have yet to be harvested.

C.H.Wearing
Scientist - Integrated Pest Management
DSIR Plant Protection

ATTACHMENT 14

NuFilm 17 or NuFilm P
as a UV Inhibitor/Sticker-Spreadrer.

See "Another Weapon in the War on Worms",
***FRUIT GROWER*, Page 30 F, March 1997.**

Another Weapon In The War On Worms

This virus may hold the key to controlling codling moth.

By Jamie K. Hartshorn

LIKE something out of The Hot Zone, it quickly overcomes every cell, attacking the very building block of life — DNA. Within 36 hours, the body is little more than a liquefied shell.

No, this isn't Ebola or some other tropical disease, but codling moth granulosis virus (CMGV), a weapon in the war against codling moth. The virus only affects codling moths and closely related species. After 14 years of testing, CMGV received U.S. EPA registration in 1995 and is currently available for use in Washington state, Oregon, and other states on apples, pears, walnuts, and plums. The virus is also used in Europe and New Zealand.

One grower who wants to see its speedy California registration is Bill Denevan of Denevan Apples, a Watsonville, CA, organic apple and pear grower who has used the virus experimentally and under its conditional use permit. In combination with other control measures, the virus has "taken orchards with 50% to 60% codling moth damage to less than 1% damage."

CMGV was developed by Louis A. Falcon, a UC-Berkeley professor emeritus who worked with it for 30 years and decided it warranted registration and commercialization.

Sandoz and Microgenesys, Inc. explored the commercial potential of the virus but did not pursue it, says Howard Kaplan, operating officer for the Association for Sensible Pest Control (ASPC), a group subsequently incorporated in 1988 to develop and produce the virus.

The virus is produced in the insect itself, Kaplan explains. "Codling moths are reared and infected, and their bodies multiply the virus — they become little manufacturing factories. The virus is very target-specific, so it doesn't have the broad market reach of organophosphates. It's also naturally occurring, so it can't have patent protection, unless a superior strain were developed through genetic engineering, and that would mean far more registration hoops," says Kaplan. He points out that Bt was worked on for years but didn't catch on until recently.

The virus must be ingested by the pest, which makes it more difficult to use than Guthion (Bayer), the primary chemical control for codling moth. The virus enters through the gut wall, "so you're going to have a sting, but the pest will die and not reproduce, and treatment in one generation will help limit damage in the next," says Kaplan.

Virus Combines Well With Pheromones And Sanitation

"The use of pheromones for mating disruption is the cornerstone of the program," agrees Denevan, "followed by CMGV and orchard sanitation — burying the wormy fruit in the first generation."

Coupled with mating disruption, "the virus can help control borders, blow-ins, hot spots, and larvae resulting from random matings. Both methods do work better in low-population areas; putting them together has an additive effect," says Kaplan.

Phillip Unterschuetz, owner/operator of IFM, a Wenatchee, WA-based supplier of materials and information for certified organic production, has distributed the virus since its development in the 1980s. He sees the disappearance from the marketplace of ryania, a botanical poison used in organic production, as a boost to CMGV, since effectiveness of the two products is "very comparable."

Summer oils are another hot item among organic growers, but they are incompatible with the organic methods for scab and mildew control. "They're indiscriminate — they'll smother beneficials, too." Unterschuetz continues.

Cultural Considerations

CMGV has a short residual activity period, breaking down in sunlight, although ASPC is working on formulations to help combat this problem.

"Timing, coverage, and pest management are more critical with a virus than they are with Guthion," says Kaplan. "Put fresh material on during egg hatch." He advises timing sprays by the degree model, supported by traps, and also the use of sugar and NuFilm 17 or NuFilm P as a UV inhibitor/sticker-spreader.

Denevan, an ASPC shareholder, says that at $20 per acre, CMGV can be "pretty expensive if you use eight sprays, but I've been able to control codling moth with three virus sprays on the first generation and one oil spray, then maybe a single virus spray and one or two oil sprays on the second generation. Keep oil down to two or three sprays a year, and keep virus to three or four. The first year or two are not going to be money savers, but by the third year, you'll see a major cost savings."

Kaplan believes use of CMGV and mating disruption (or even CMGV and Guthion) will help slow the rate of growth of insect resistance to chemicals such as Guthion, and will aid in the build-up of beneficials.

Although a few companies plan to conduct trials, CMGV lacks registration and most likely will not be commercially viable for this season. He concludes that none of the measures — CMGV, mating disruption, and sanitation — "work by themselves, but put all three together, and you have a dynamite combination." ■

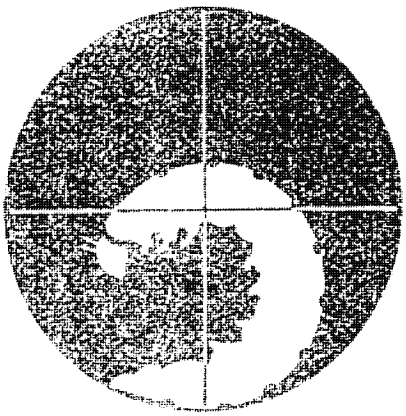
Hartshorn is a freelance writer based in Oakland, CA.

ATTACHMENT 15

Recommend Nu Film 17 for Preventing Washoff by Rainfall and for Use as a Sticker.

See "Instructions for the Use of THURICIDE to Control Gypsy Moth, Oak Moth, and other Leaf Eating Worms."

International Minerals & Chemical Corporation.



Instructions for the Use of THURICIDE to Control Gypsy Moth, Oak Moth, and other Leaf Eating Worms

1. Gypsy Moth eggs normally begin hatching soon after leaves begin expanding in early May. A good guideline for spraying THURICIDE HPC is when leaf expansion reaches 50%.

2. Foliage should be thoroughly wetted by the spray, but not to the point of excessive run-off. Thorough coverage of all foliage subject to worm attack is essential.

3. THURICIDE must remain on the leaves (and be eaten by worms) to be effective. To prevent washoff by rainfall, use of a sticker such as Nu Film 17, or Plyac is recommended.

4. If reinfestation occurs, or if egg hatch occurs over a long period (as can happen with Gypsy Moth), a second application should be made about 14 days after the first.

5. Shake the THURICIDE container thoroughly to make sure the active ingredient is well mixed, before adding to spray tank.

6. Pour the recommended amount of THURICIDE into a nearly filled spray tank. Be sure mixing is well agitated before spraying.

7. Combinations with fungicides and chemical insecticides in the tank are generally not deleterious to THURICIDE if the finished spray is used promptly.

8. Follow label instructions for storage and handling.

RECOMMENDED RATES

For control of Gypsy Moth, the following rates of THURICIDE HPC will be effective.

HIGH-PRESSURE, HIGH-GALLONAGE HYDRAULIC SPRAYERS

2 quarts THURICIDE HPC per 100 gallons of water. Apply 4 to 8 gallons of finished spray per tree, depending upon size of tree and quantity of vegetation.

MIST BLOWERS

2 quarts THURICIDE HPC per 10 gallons of water. Apply ½ to 1 gallon of finished spray per tree. Thorough coverage of all leaves is essential.

OTHER INSECTS

For control of Spring and Fall Cankerworm, Oak Moth Larvae, Tent Caterpillars, Fall Webworm, and Red-humped Caterpillar, the above rates may be reduced by one half.

IMC CROP AID PRODUCTS
INTERNATIONAL MINERALS & CHEMICAL CORPORATION,
P.O. Box 192, Libertyville, Illinois 60048

8105/3-72

ATTACHMENT 16

Nu-Film Bt has excellent sticker properties and has been recommended for use with THURICIDE

Nu-Film-Bt was replaced by Nu-Film-17 which is more efficient.

Adhesives and Surfactants

THURICIDE formulations contain a wetting agent that will spread THURICIDE evenly over many types of foliage when diluted at the rate of 2 quarts in up to 100 gallons. Additional wetting agent may be added to the spray tank mix if needed. However, it is better to add too little wetting agent than too much. Excess wetting agent will tend to run THURICIDE off foliage especially if the application is followed by heavy dew or rains.

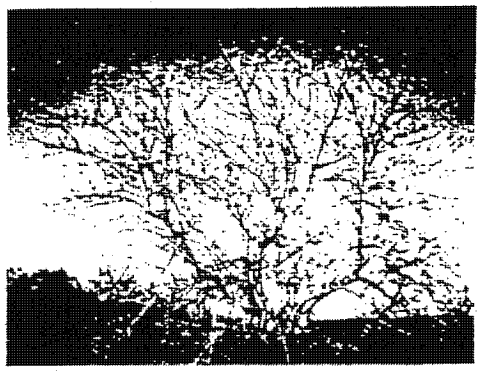
If THURICIDE is allowed to dry thoroughly on foliage after application, up to 25% of the deposit may remain on the upper surfaces after ¼ inch rainfall. It is likely that larger amounts will be retained on undersurfaces or protected areas. Reapplication should be considered following heavy rainfall or overhead irrigation.

Some adhesive materials have the ability to retain an effective THURICIDE deposit following ¼ inch rainfall or heavy dew. Plyac (Allied Chemical Corp.) is representative of adhesives that enhance THURICIDE insect control in damp and rainy situations. Such an adhesive tends to give uniform, well dispersed coverage under all conditions. A newer product, Nu-Film BT (Miller Chemical and Fertilizer Corp.) has also shown excellent sticker properties. However, some of the commercial spreader-sticker products have too much wetting agent. Products composed primarily of resinous substance will usually provide better adhesive properties.

Most commercially available adhesives have been lab checked and found to be compatible with THURICIDE. Highly alkaline adhesives such as Hi-Spread may be used with caution and holding time in the spray tank should be kept short.

In areas where heavy dew is regularly encountered, the addition of an adhesive is essential. Dew apparently tends to form underneath the deposit and lift it off the leaf. Since dew can accumulate in quantities of several thousand gallons of water per acre, deposit runoff may be expected. Whenever practical, application should be delayed until dew has dried.

THURICIDE products are used world-wide to prevent devastating defoliation by a broad range of Lepidopterous larvae. Treated versus untreated apple trees and broccoli are examples of the many crops protected by THURICIDE.



ATTACHMENT 17

**Letter from North Carolina State University
Recommending the Registration of
Terpene Resin Products Like Nu-Film 17
for Organic Production.**

NC STATE UNIVERSITY

Henderson County Center
740 Glover Street
Hendersonville, N.C. 28792
828-697-4891
www.ces.ncsu.edu/henderson/

May 5, 2005

To Whom It May Concern,

My name is Marvin Owings, Jr. I am an Extension Agent in Henderson County, North Carolina.

This letter is to help Miller Chemical with their registration of Terplene Resin products like Nu-Film-17 for organic production.

In 2002, I began working with local growers on organic apple production. Over the past three years, this process has lead to one grower achieving USDA organic certification.

Through this on-farm research project Nu-Film-17 has played a major role in the fruit growing process. Nu-Film 17 has allowed the organic growers extra benefit in disease prevention like scab disease and has helped with sunburn protection.

Without a doubt, their products has been one of the best tools we have to work as a spreader-sticker. I believe Nu-Film-17 type products add to our inventory of tools to help grow the highest quality organic fruit possible. It is my sincerest hope that you will consider registering these products again for organic production.

Sincerely,

Marvin A. Owings, Jr.
Extension Agent
Agriculture

MAO:smp

ATTACHMENT 18

Nu-Film 17 Uses -

Field Sprays of Bacillus subtilis and Fungicides for Control of Preharvest Fruit Diseases of Avacado in South Africa,

PLANT DISEASE, May 1997. Pages 455-459

Field Sprays of *Bacillus subtilis* and Fungicides for Control of Preharvest Fruit Diseases of Avocado in South Africa

L. Korsten, E. E. De Villiers, F. C. Wehner, and **J. M. Kotzé,** Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria 0002, Republic of South Africa

ABSTRACT

Korsten, L., De Villiers, E. E., Wehner, F. C., and Kotzé, J. M. 1997. Field sprays of *Bacillus subtilis* and fungicides for control of preharvest fruit diseases of avocado in South Africa. Plant Dis. 81:455-459.

In 3 consecutive years, preharvest applications of *Bacillus subtilis* field sprays integrated with copper oxychloride or benomyl consistently reduced severity of avocado black spot (BS), caused by *Pseudocercospora purpurea* at Omega, Republic of South Africa. Control was equal to that obtained with copper oxychloride or benomyl–copper oxychloride in the first and third years of spraying at Omega. In the second year, only the integrated treatment controlled BS, while copper oxychloride proved ineffective. The antagonist was applied on its own or integrated with copper oxychloride sprays at two other geographically distinct locations, Westfalia Estate and Waterval. The integrated and biological treatments at these localities were less effective than copper oxychloride sprays in controlling BS disease. Integrated control was more effective than *B. subtilis* sprays at Westfalia. On continuation of the biological spray program at Waterval for an additional three seasons, control was as effective as copper oxychloride in the last 2 years of spraying. Sooty blotch (SB), caused by an *Akaropeltopsis* sp., was reduced by the integrated treatment at Omega during the second season and at Westfalia during the first season. Although the two fungicide treatments reduced SB at Omega in the first season, copper oxychloride increased it above that of the control in the third season. Only the copper oxychloride treatment reduced SB at Waterval in the third season, while the *B. subtilis* treatment increased disease above that of the control in the fourth season.

Additional keywords: biocontrol, Cercospora spot

Black spot (BS) and sooty blotch (SB), caused by *Pseudocercospora purpurea* (Cooke) Deighton (8) and an *Akaropeltopsis* sp. (36), respectively, are the two most important preharvest fruit diseases of avocado (*Persea americana* Mill.) in South Africa (27). Losses from BS of up to 69% have been recorded in untreated orchards on the susceptible cultivar Fuerte (7), while SB results in lower market value of fruit due to unsightly discoloration of the skin (35,36). Traditionally, control of BS was based on preharvest benomyl, copper oxychloride, or cupric hydroxide sprays (6,30,31,41). In South Africa, chlorine is occasionally used postharvest to remove the black epiphytic growth of *Akaropeltopsis* (2). Several disadvantages associated with the repeated use of fungicides on avocados have been noted. These include costs of removing copper spray residues on fruit in the packhouse (10) and buildup of pathogen resistance to benomyl (8). Possible adverse effects of agrochemicals on human health and the environment necessitate alternative disease control measures such as biological and integrated control.

Biological control can be effective when antagonists are applied as preharvest treatments to control leaf and fruit diseases such as Cercospora leaf spot on groundnuts caused by *Cercospora arachidicola* (19), powdery mildew and anthracnose of mango caused by *Oidium mangiferae* and *Colletotrichum gloeosporioides* (25), rust on beans caused by *Uromyces phaseoli* (1), and charcoal rot on potato caused by *Macrophomina phaseolina* and *Botryodiplodia solanituberosi* (38). In these examples, *Bacillus* spp. were used as biocontrol agents (38). Preharvest antagonist sprays have also been effective in controlling postharvest fruit diseases. Korsten (20) and Korsten et al. (22) reported effective control of postharvest decay from anthracnose, stem-end rot (SE), and *Dothiorella–Colletotrichum* fruit rot complex (DCC) when using preharvest *B. subtilis* field sprays. Postharvest application of *B. subtilis* was similarly effective for control of these postharvest problems (20,21).

Evaluation of *B. subtilis* for control of the preharvest diseases BS and SB on avocado was therefore an obvious sequel to the successful evaluation of the antagonist as a biocontrol agent of postharvest avocado fruit diseases (22,25). This paper reports on field experiments in which *B. subtilis*, singly or integrated with copper oxychloride or benomyl–copper oxychloride, was compared with commercial chemical control field spray programs for control of BS and SB.

MATERIALS AND METHODS

Experiments were initiated in the 1990 to 1991 growing season at three geographically distinct sites: Omega, situated near Burgershall (Mpumalanga) (3 years); Westfalia Estate (block 34B) (1 year); and Waterval (block 4) (Northern Province) (4 years). At each site, 15- to 20-year-old Fuerte cultivar avocado trees of the same size, canopy density, and fruit set were selected and marked in a randomized block design. Treatments included commercial benomyl or copper oxychloride sprays, alone or integrated with *B. subtilis* spraying, and *B. subtilis* sprays alone (Table 1). Five double-tree replicates were used per treatment throughout the duration of the experiment. Unsprayed trees were included as controls in the first year, but the other 2 years at Omega they received one spray of copper oxychloride at the farmer's insistence to prevent inoculum buildup. Prior to initiation of these experiments, trees from all three sites were commercially sprayed with copper oxychloride. Commercial spraying was done during fruit development at the onset of the rainy season (usually the end of October), followed by a November and a January application. At Omega and Waterval, a history of heavy pressure of BS occurred. *B. subtilis* (B246) isolated from the avocado phylloplane, and which effectively inhibited in vitro and in vivo growth of avocado postharvest pathogens *C. gloeosporioides*, *Dothiorella aromatica*, *Thyronectria pseudotrichia*, *Phomopsis perseae*, *Pestalotiopsis versicolor*, and *Fusarium solani* (21), was selected for field sprays. This antagonist effectively controlled anthracnose, SE, and DCC when sprayed in avocado orchards (20) or when used in postharvest wax (21) or dip (24) applications.

B. subtilis (B246) was mass produced for field application as described in a previous study (21). The antagonist formulation was mixed into 500 liters of water in a spray tank to obtain a final concentration of 10^7 cells ml^{-1}. Copper oxychloride 85% WP (Demildex, Delta Chem, Meyerton, RSA) and benomyl 50% WP (Benlate, Agrihold, Pretoria, RSA) were prepared in

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separate spray tanks at registered rates (2.5 g a.i. liter^{-1} and 0.4 g a.i. liter^{-1}, respectively). Nu-Film 17 (Hygrotech Seed, Pretoria, RSA) was added at the registered rate of 0.02% (vol/vol) to all spray treatments. Each tree was sprayed using high-volume ground sprayers (100 liter) till runoff to obtain full coverage of leaves, fruit, stems, and branches. Application dates of the various treatment programs are shown in Table 1. The fungicide spray dates corresponded with commercial spray dates for each area. Discrepancies in the spray schedules between the different locations were mainly due to differences in rainfall patterns. The integrated management treatment using copper oxychloride and *B. subtilis* also was evaluated at Westfalia and Waterval for 1 year. At the latter two farms, an antagonist-only treatment was also included in the first season (1990 to 1991). Due to a severe drought and subsequent poor fruit set, experiments were discontinued at Westfalia. Thereafter, experiments using the antagonist alone were continued only at Waterval for 3 more consecutive years. During this period, the integrated treatment was discontinued due to the loss of a tree.

During commercial harvest (April), at least 30 fruit were picked randomly from each tree and evaluated on a 0 to 3 scale for BS and a 0 to 4 scale for SB (30) (Fig. 1). Data were analyzed using the Statistical Analysis System (SAS/STAT Mainframe Version 6 ed. 1987, SAS Institute, Cary, NC). A Kruskal-Wallis transformation of data and analysis of variance was performed, and significant differences were determined using Duncan's multiple range test at $P \leq 0.05$.

RESULTS

In the first season (1990 to 1991), BS was significantly reduced at all three sites by all spray treatments (Table 2). At Omega, the spray regimes were equally effective; whereas at Westfalia and Waterval, the fungicide treatment was significantly better than the *B. subtilis* or integrated treatment. *B. subtilis* was less effective than integrated and fungicide programs at Westfalia. At Waterval, *B. subtilis* sprays and the integrated management treatments were statistically equivalent, but both were less effective than the fungicide sprays. No control of SB was recorded for any of the treatments at Waterval, but both fungicide and integrated treatments significantly reduced this disease at Westfalia (Table 2). Only the fungicide sprays significantly reduced SB at Omega.

The following year (1991 to 1992), significant reductions of BS and SB were found only for the integrated treatment at Omega (Table 2). In the 1992 to 1993 season at the same location, the fungicide and integrated treatments were effective against BS. However, significantly higher levels of SB were observed where fungicides alone were used (Table 2). Fungicides alone were effective at Waterval in 1992 to 1993 (Table 2). During the following 2 years (1993 to 1994 and 1994 to 1995), the efficacy of *B. subtilis* was equal to that obtained with the fungicide treatment (Table 2), but only the fungicide sprays controlled SB during 1993 to 1994. In the 1994 to 1995 experiment, SB levels were higher in the *B. subtilis* treatment than in the control. Fungicides again were ineffective for SB control.

Table 1. Treatment programs and preharvest spray dates of fungicides, *Bacillus subtilis* (B246) alone and *B. subtilis* integrated with fungicide sprays for control of preharvest avocado fruit diseases

Location / Treatment	Concentration	Month of application				
		Oct.	Nov.	Dec.	Jan.	March
Omega 1990 to 1991						
Control	Unsprayed	–	–	–	–	–
Copper oxychloride	2.5 g a.i. liter^{-1}	+	+	–	–	–
Benomyl +	0.4 g a.i. liter^{-1}					
Copper oxychloride	2.5 g a.i. liter^{-1}	–	–	–	–	–
Benomyl +	0.4 g a.i. liter^{-1}					
B. subtilis	10^7 cells ml^{-1}	–	–	+	–	+
Omega 1991 to 1992						
Control[z]	2.5 g a.i. liter^{-1}	–	–	–	–	–
Copper oxychloride	2.5 g a.i. liter^{-1}	–	+	–	–	–
Copper oxychloride +	2.5 g a.i. liter^{-1}	–	–	–	–	–
B. subtilis	10^7 cells ml^{-1}	–	+	+	–	+
Omega 1992 to 1993						
Control[z]	2.5 g a.i. liter^{-1}	–	–	–	–	–
Copper oxychloride	2.5 g a.i. liter^{-1}	–	+	–	–	–
Copper oxychloride +	2.5 g a.i. liter^{-1}	–	–	–	–	–
B. subtilis	10^7 cells ml^{-1}	–	+	+	–	+
Westfalia 1990 to 1991						
Control	Unsprayed	–	–	–	–	–
Copper oxychloride	2.5 g a.i. liter^{-1}	–	+	–	–	–
Copper oxychloride +	2.5 g a.i. liter^{-1}	–	+	–	–	–
B. subtilis	10^7 cells ml^{-1}	–	–	+	–	+
B. subtilis	10^7 cells ml^{-1}	–	–	–	–	+
Waterval 1990 to 1991						
Control	Unsprayed	–	–	–	–	–
Copper oxychloride	2.5 g a.i. liter^{-1}	+	+	–	–	–
Copper oxychloride +	2.5 g a.i. liter^{-1}	+	–	–	–	–
B. subtilis	10^7 cells ml^{-1}	–	–	–	–	–
B. subtilis	10^7 cells ml^{-1}	–	+	–	–	–
Waterval 1992 to 1993						
Control	Unsprayed	–	–	–	–	–
Copper oxychloride	2.5 g a.i. liter^{-1}	+	+	–	–	–
B. subtilis	10^7 cells ml^{-1}	+	+	–	–	–
Waterval 1993 to 1994						
Control	Unsprayed	–	–	–	–	–
Copper oxychloride	2.5 g a.i. liter^{-1}	+	+	+	–	–
B. subtilis	10^7 cells ml^{-1}	+	+	+	–	–
Waterval 1994 to 1995						
Control	Unsprayed	–	–	–	–	–
Copper oxychloride	2.5 g a.i. liter^{-1}	+	+	–	–	–
B. subtilis	10^7 cells ml^{-1}	+	+	–	–	–

[z] One spray of copper oxychloride.

DISCUSSION

For biocontrol agents to be accepted commercially, it is essential to show consistent control over several years at more than one location (4). In our study, the most consistently effective treatment for BS of avocado was achieved with an integrated program of fungicide and *B. subtilis* sprays. A reduced number of chemical sprays coupled with promising levels of control makes an integrated approach to managing BS disease attractive to the avocado industry in South Africa. The single chemical application in the integrated program may act as a safeguard in years when the weather conditions do not favor antagonist activity (9). Similarly, the biological treatment proved effective at the two locations where it was tested in 1990 to 1991 (Waterval and Westfalia) and also during the last 2 of the 3 years where it was sprayed consecutively at Waterval. During the last 2 years of spraying with *B. subtilis*, control was equal to that achieved with fungicides. Tronsmo and Dennis (39) reported on biocontrol with *Trichoderma* spp. equivalent to commercial fungicides. Integrated control was more effective than *B. subtilis* spraying in one of the two experiments where it was evaluated. Similarly, *Trichoderma harzianum* sprayed alone or in combination with dichlofluanid was reported to control natural infections of *Botrytis cinerea* on apple (40) and grapevine (11).

Appendix 2. Field results from Coke Ranch releases.

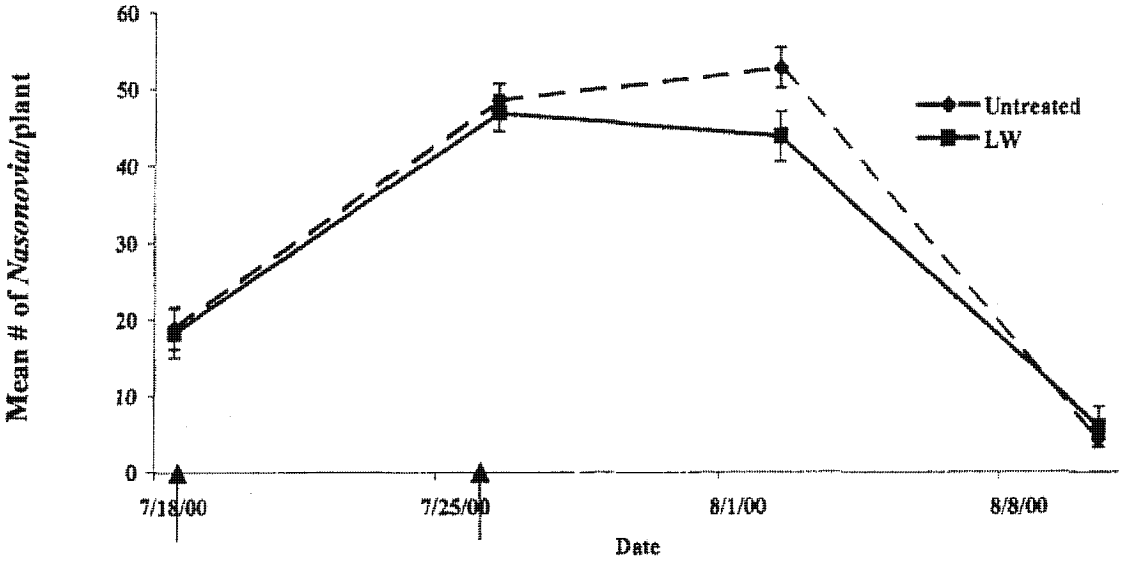

Figure 2.1 Mean number of *Nasonovia*/plant in untreated and lacewing egg release plots at Coke Ranch. Bars indicate standard errors. Arrows indicate release dates.

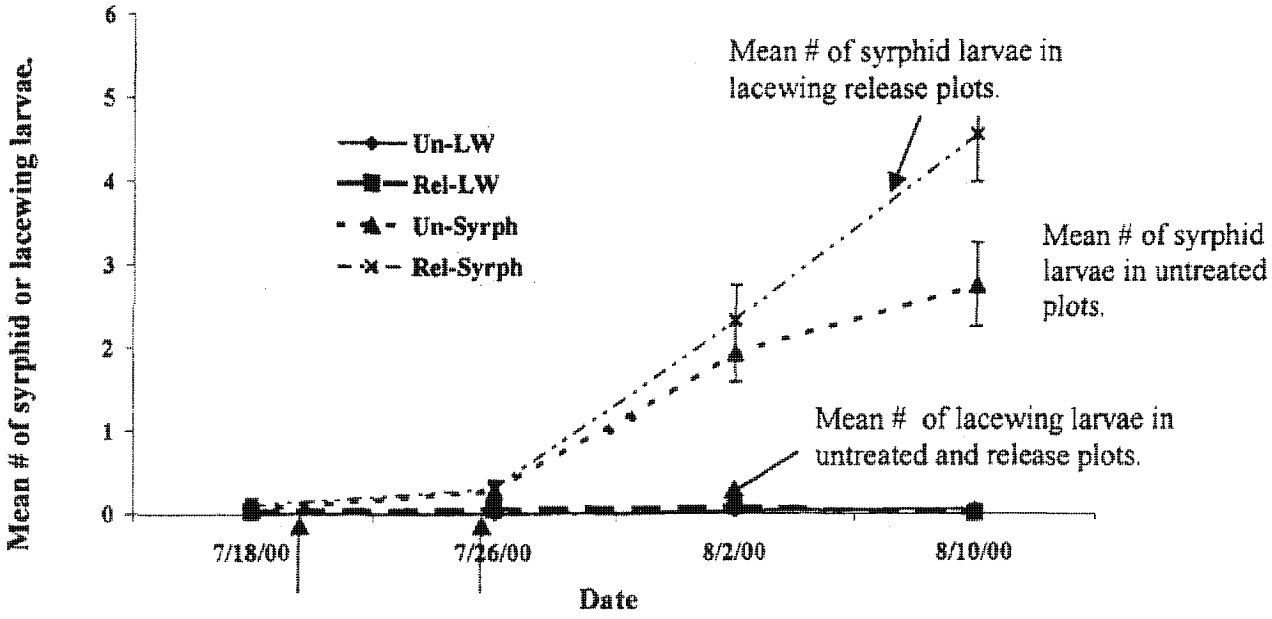

Figure 2.2 Mean number of syrphid and lacewing larvae in untreated and lacewing release plots at Coke Ranch. Bars indicate standard errors. Arrows indicate release dates.

Although field applications of biocontrol agents to reduce preharvest diseases have not often met with much success (18,34), we showed that *B. subtilis*, alone or integrated with fungicides, effectively reduced natural infections of *P. purpurea*, and to a limited extent, *Akaropeltopsis* sp. (integrated program only). The control obtained here is also unique in the sense that no in vitro evaluations were carried out prior to field evaluations since both pathogens are difficult to isolate, and they grow extremely slowly on agar media. The *B. subtilis* isolate used here (previously shown to be effective against avocado postharvest pathogens [20]) was therefore used directly in field sprays and found to be effective.

According to Swinburne (37), commercial acceptance of biocontrol agents depends on their ability to perform as well as or better than commercial fungicides. During the first season of spraying at Westfalia and Waterval, less effective control of BS was found with the biological and integrated treatments than with the fungicide treatment. This is in agreement with Knudsen and Spurr (19), Cullen et al. (5), and Korsten et al. (25). Acceptable control of plant diseases through biological or integrated treatments is not always evident the first season and therefore requires patience on the part of the grower. In follow-up experiments, the integrated (Omega) and biological (Waterval) treatments proved as effective as the fungicide program. It was also shown that the integrated treatment is consistently more effective over time and location compared with commercial fungicides and therefore has the greatest potential for acceptance by growers. Comparative cost of future commercial *B. subtilis* spray applications will be similar to that of benomyl but higher than that of copper fungicides (L. Korsten, *unpublished*). The financial aspect would further support an integrated approach.

Fungicide sprays most effectively controlled BS during the first year of evaluation at each of the locations. It is noteworthy to mention that during the second season at Omega, a very high incidence of BS was recorded on leaves of both control and fungicide-treated trees but not on trees receiving the integrated treatment (L. Korsten, *unpublished*). During the second season, BS symptoms were also abundant on young green leaves, contrary to the usual association of the disease with older, yellowing leaves (6). Variability in the degree of chemical control has been reported (12,28,37). Factors that may be related to less-than-desirable chemical control include declining levels of fungicide at the targeted site, rendering it ineffective (12), varying conditions under which tests were run (16), and high disease pressure (31,40). An unusually high incidence of BS on leaves at Omega during the second season may account for the relatively poor performance of copper sprays.

Timing of application is of crucial importance in biological control programs (3). In preliminary experiments at Omega in 1989 to 1990 (data not shown), application dates were based on epidemiological data for *P. purpurea* obtained from Westfalia (6). Both fungicide and integrated treatments were initiated in November, and fruit harvested early in the season (March) showed that neither treatment was effective in controlling BS. However, when fruit were harvested late in the season (May), only the fungicide treatment provided significant control. Subsequently, Lonsdale and Scott (32) showed that spore release by the pathogen at Omega occurs earlier than at Westfalia. Applications of *B. subtilis* aimed at establishing the antagonistic bacteria prior to arrival of *P. purpurea* inoculum resulted in sustained control thereafter.

Effective control of SB using a biocontrol agent has not previously been reported. Control of SB of avocado is difficult to achieve (2); and in practice, farmers depend on copper oxychloride sprays or postharvest treatments in the packhouse (2). However, effective control of SB is essential for commercial production (17) since the epiphytic growth of the fungus leaves unacceptable black blotches on fruit. SB was effectively controlled with fungicide sprays at Omega and Westfalia during the first year and at Waterval during the 1993 to 1994 season. In the third season at Omega, fungicide sprays were associated with an increase in disease above that of the control, while a similar tendency was observed at Waterval in the final year of spraying the biological treatment. Such observations are most often ascribed to the changed balance of naturally occurring microorganisms (13–15). In contrast with its effect on BS, *B. subtilis* sprayed alone appears to be ineffective against SB. Some control of SB was obtained with the integrated treatment. The inability of *B. subtilis* to reduce SB corresponds with the report by Leben (29), who effectively controlled anthracnose but not powdery mildew when unidentified bacteria were sprayed onto cucumber seedlings in the greenhouse.

Apart from preliminary progress reports for the South African avocado industry (22,26), this is the first report of biological control of avocado preharvest fruit diseases over an extended period of time. To our knowledge, this is also the first study in which control of the black epiphytic growth of *Akaropeltopsis* sp. using natural antagonists has been investigated. The consistent control of BS shown in this investigation together with its potential for control of SB (20,23,26) makes integrated and, to a lesser extent, biological control a feasible alternative to chemical control in the field. In addition, *B. subtilis* provided effective control of postharvest diseases of avocado (20,21,23,24), and the merits of combining a pre- and postharvest biocontrol program should be investigated.

The use of *B. subtilis* in a postharvest environment has been controversial, mainly because of its known ability to produce antibiotics under laboratory conditions. However, *B. subtilis* has been commercialized as a biocontrol agent for field applications, i.e., Bactophyte (33) for use on several vegetables, Epic and Kodak (Gustafson, Inc., Dallas, TX) to control root diseases on cotton and legumes, Sys-

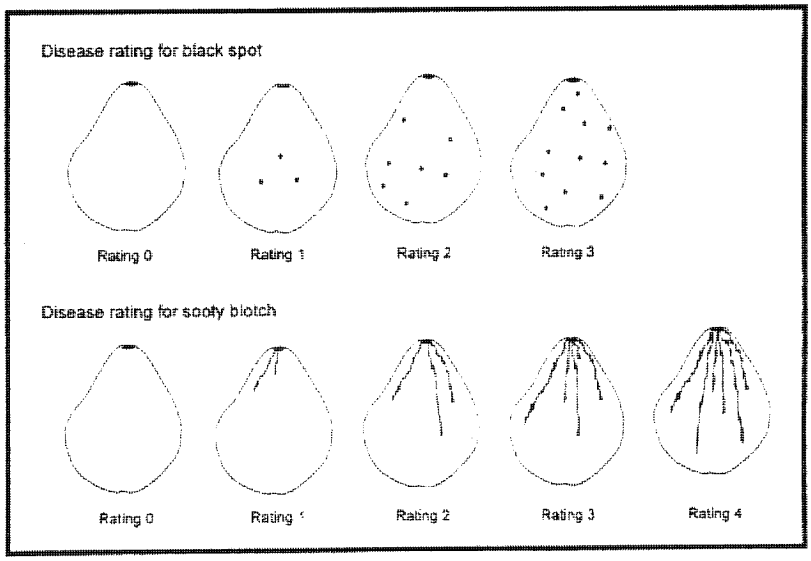

Fig. 1. Avocado fruit disease ratings for black spot, where 0 represents clean fruit, 1 = 1 to 5 spots, 2 = 6 to 10 spots, and 3 = >10 spots; and for sooty blotch, where 0 represents clean fruit, 1 = 1 to 10% of fruit surface area covered, 2 = 11 to 25%, 3 = 26 to 50%, and 4 = >50% of fruit covered.

tem 3 (Helena Chemical Co., Memphis, TN) for control of various seedling pathogens, and Biostart (Advanced Microbial Systems Inc., Shakopee, MN) for control of various fungal pathogens. The potential commercial use of *B. subtilis* in preharvest field applications for control of avocado fruit diseases is therefore a viable option, and future studies should concentrate on elucidating the mode of action of this common natural avocado phylloplane inhabitant (21).

ACKNOWLEDGMENTS

We thank K. Kockemoer, Omega, Burgershall, and the research staff of Westfalia Estate for making available their avocado orchards for experimental purposes. We also thank James Lonsdale, Constantia Estate, and Riaan Duvenhage, Merensky Technical Services, for their help during field trials and evaluations; Erna Maas for editorial comments; Amanda Lourens, Department of Statistics, University of Pretoria, for statistical analyses; and Daleen Muller for typing. Without the financial support of the South African Avocado Growers' Association, this study would not have been possible.

Table 2. Effect of preharvest field sprays with fungicides, *Bacillus subtilis* (B246) alone, and *B. subtilis* integrated with fungicides on severity of avocado black spot and sooty blotch

Location / Treatment	Disease severity	
	Black spot	Sooty blotch
Omega 1990 to 1991		
Control	1.89 a[y]	1.27 a
Copper oxychloride	0.36 b	0.42 b
Benomyl + Copper oxychloride	0.44 b	0.58 b
Benomyl + *B. subtilis*	0.72 b	1.85 a
F value	104.56	63.31
PR > *F*	0.0001	0.0001
Omega 1991 to 1992		
Control	1.34 a	1.25 a
Copper oxychloride	1.15 a	1.18 a
Copper oxychloride + *B. subtilis*	0.77 b	0.80 b
F value	23.78	17.48
PR > *F*	0.0001	0.0001
Omega 1992 to 1993		
Control	1.73 a	0.70 b
Copper oxychloride	1.12 b	1.55 a
Copper oxychloride + *B. subtilis*	0.74 b	0.89 b
F value	6.26	4.34
PR > *F*	0.0092	0.0299
Westfalia 1990 to 1991		
Control	1.25 a	0.69 a
Copper oxychloride	0.18 d	0.44 b
Copper oxychloride + *B. subtilis*	0.64 c	0.51 b
B. subtilis	0.93 b	0.67 a
F value	36.39	5.03
PR > *F*	0.0001	0.0019
Waterval 1990 to 1991		
Control	1.77 a	1.01 a
Copper oxychloride	0.42 c	1.01 a
Copper oxychloride + *B. subtilis*	1.10 b	1.17 a
B. subtilis	1.08 b	1.21 a
F value	38.70	2.51
PR > *F*	0.0001	0.0578
Waterval 1992 to 1993		
Control	1.67 a	...[z]
Copper oxychloride	0.82 b	...
B. subtilis	1.88 a	...
F value	6.51	
PR > *F*	0.0044	
Waterval 1993 to 1994		
Control	2.01 a	1.03 a
Copper oxychloride	1.17 b	0.28 b
B. subtilis	1.48 b	1.45 a
F value	9.11	16.29
PR > *F*	0.0009	0.0001
Waterval 1994 to 1995		
Control	0.61 a	1.17 b
Copper oxychloride	0.27 b	1.02 b
B. subtilis	0.15 b	1.76 a
F value	9.59	4.74
PR > *F*	0.0007	0.0173

[y] Means within columns for each location and each year evaluated followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test. Values represent mean disease severity. Fruit was evaluated on a 0 to 3 scale for black spot and on a 0 to 4 scale for sooty blotch (Fig. 1).

[z] Not monitored due to low disease incidence.

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ATTACHMENT 19

Nu-Film 17 Uses –

Disease Management in Organic Cucurbit Crops.

2004 Research Project for the
Ohio Vegetable and Small Fruit Research
and Development Program

2004
Research Project Report for the Ohio Vegetable and Small Fruit Research and Development Program

Project Title
Disease Management in Organic Cucurbit Crops

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Objectives of the Research

The goal of this research project was to develop disease management strategies for cucurbit crops that can be used on certified organic vegetable farms. The principle diseases of cucurbits in this area are powdery mildew, bacterial wilt, Phytopthora blight, downy mildew, and gummy stem blight. **The specific objective of this project was to evaluate products that are or soon will be approved for use in organic systems, and can be used to control the principle cucurbit diseases.**

We focused on squash as a representative cucurbit crop. Products were tested in a transitional organic field at OARDC, in a replicated experiment.

Experimental Design and Methods

The experiment was conducted at the Ohio Agricultural Research and Development Center, Badger Farm near Wooster, OH in a transitional organic field with Wooster silt loam soil. Composted poultry manure (4-0-0, 15 123 lb/A, 75% moisture; Daylay Egg Farm Inc., West Mansfield, OH) was incorporated into the test field on 7 Jun. Certified organic squash seeds (cv. Buttercup) were sown on 19 May into 50-cell plug trays containing Paygro organic potting mix #423 (35% composted pine bark, 50% Canadian sphagnum peat, 15% perlite v/v/v; Paygro Co./Garrick Ind., South Charleston, OH). The field was cultivated, beds prepared and black plastic laid on 8 Jun. Squash seedlings were hand-transplanted on 9 Jun. Treatments were arranged in a randomized complete block design with two rows and four replications per treatment. Each row consisted of 15 plants spaced 2 ft apart on 5 ft centers. Treatment rows were alternated with untreated border rows. One of the two rows per treatment was covered with a floating row cover (Johnny's Selected Seed, Winslow, ME) on 10 Jun. Floating row covers were removed on 8 Jul. Pyrethrum (Diatect V (6 lb/A)) was applied to plots that previously had been

covered, on 8, 16 and 30 Jul, and 6 Aug using a CO_2 backpack sprayer (146 gal/A, 40 psi). Treatments were applied on 22 and 29 Jul and 5, 12, and 19 Aug, using a CO_2 backpack sprayer (143 gal/A, 40 psi) for a total of five applications. The surfactants BioLink (0.5 fl. oz/gal) and Nu-Film-17 (12 fl. oz/A) were added to Armicarb 100 and Serenade Max treatments respectively. Severity of powdery mildew was determined on 3 and 18 Aug using a modified Horsfall-Barratt rating scale. Disease ratings were converted to midpoints (% powdery mildew) prior to statistical analysis. Fruits were harvested from the entire row of each plot on 25 Aug and sorted into three categories: healthy, diseased culls and healthy culls. The number and weight of fruits in each category were determined. Data were analyzed by ANOVA using SAS statistical software. Means were separated using Fisher's protected least significant difference test. Average maximum temperatures for 9-30 Jun, Jul, and 1-25 Aug were 77.7, 81.4, and 78.5 °F; minimum temperatures were 56.5, 60.8, and 55.6 °F; and total rainfall was 6.3, 3.6, and 3.8 in., respectively.

Results

Heavy rains early in the season flooded one block. As a result transplants were killed and data were collected for three blocks only. Bacterial wilt pressure was low, and there were no significant differences in the number of plants killed between protected (with row covers followed by pyrethrum treatment) and non-protected plots. Powdery mildew pressure was moderate to high. No other diseases were observed. Except for the two concentrations of SoilSoup compost tea, all treatments significantly reduced powdery mildew on squash compared to the untreated control. Serenade Max plus Kocide 2000 and the sulfur treatment were most effective in reducing powdery mildew severity, irrespective of the presence of row covers and treatment with pyrethrum. However, the protected control plots had significantly less powdery mildew on 18 Aug than the non-protected control plots.

Protected plots treated with either rate of SoilSoup compost tea, the low rate of Armicarb 100 (2.5 lb/A), or the high rate of Serenade Max (2 lb/A) plus Kocide 2000, and non-protected plots treated with the high rate of Armicarb 100 (5.0 lb/A), sulfur or the low rate of Serenade Max (1 lb/A) plus Kocide 2000 produced significantly higher marketable yield than the non-protected, untreated control. The proportion of marketable fruit was significantly higher in the protected, untreated plots than in the non-protected, untreated plots. With the exception of Armicarb 100 (2.5 lb/A, non-protected; 5.0 lb/A, protected), SoilSoup compost tea (full strength, protected), and Serenade Max (1 lb/A + Kocide 2000, protected) all treatments resulted in significantly higher proportions of marketable fruit than in the untreated, non-protected control. Among the non-protected plots, those treated with the high rate of Armicarb 100, sulfur, both rates of Serenade Max plus Kocide 2000 or the low rate of SoilSoup compost tea produced more marketable fruits than the non-protected, untreated control. There were no significant differences among any of the treatments in the weight of marketable fruit produced (data not shown).

Conclusions

Although bacterial wilt pressure was too low to evaluate the effectiveness of row covers in preventing this disease, the use of row covers appeared to result in other benefits, including larger, healthier-appearing plants and a higher percentage of marketable fruit compared to the non-covered control. All of the products tested except compost tea were effective at some level in powdery mildew control, although sulfur and Serenade Max + Kocide treatments were most effective.

Treatment and rate/A	Row cover + pyrethrum	% powdery mildew[z]		Marketable yield (ton/A)	% marketable fruit
		3 Aug	18 Aug		
SoilSoup Compost Tea 1/3 strength	-	22.2 a[y]	89.5 ab	1.9 cd	63.3 a-e
Control	-	19.0 ab	96.0 a	1.5 d	42.1 f
SoilSoup Compost Tea full strength	-	12.7 abc	94.3 ab	2.1 bcd	55.6 def
Armicarb 100 2.5 lb	-	2.0 d	40.0 de	1.8 cd	52.2 ef
Armicarb 100 5.0 lb	-	3.0 cd	40.0 de	2.4 abc	67.6 a-e
Sulfur 16 lb	-	3.0 cd	15.8 ef	3.0 a	72.9 a-d
Serenade Max 2 lb +[x] Kocide 2000 2 lb	-	3.0 cd	15.8 ef	2.2 bcd	64.3 a-e
Serenade Max 1 lb + Kocide 2000 2 lb	-	3.0 cd	11.2 f	2.4 abc	67.6 a-e
SoilSoup Compost Tea 1/3 strength	+	19.0 ab	87.8 ab	2.4 abc	81.0 a
Control	+	11.2 bcd	70.2 bc	2.2 a-d	67.3 a-e
Sulfur 16 lb	+	5.5 cd	15.8 ef	2.3 a-d	79.1 ab
SoilSoup Compost Tea full strength	+	4.0 cd	87.8 ab	2.8 ab	66.8 a-e
Armicarb 100 2.5 lb	+	3.0 cd	48.3 cd	2.5 abc	77.7 abc
Armicarb 100 5.0 lb	+	2.0 d	46.2 cd	1.9 cd	58.3 c-f
Serenade Max 2 lb + Kocide 2000 2 lb	+	2.0 d	15.8 ef	2.4 a-d	69.8 a-e
Serenade Max 1 lb + Kocide 2000 2 lb	+	2.0 d	12.7 f	2.2 bcd	59.4 b-f

[z]Disease rating based on the midpoint values of a modified Horsfall-Barratt rating scale where 1=0%; 2= 1-3%; 3= 4-6%; 4=7-12%; 5= 13-25%; 6=26-50%; 7=51-75%; 8= 76-87%; 9=88-94%; 10= 95-97%; 11=98-99% and 12= 100% powdery mildew coverage of leaves (upper surface).

[y]Values are the means of three replicate plots; means followed by the same letter within a column are not significantly different at $p \leq 0.05$.

[x]Treatments tank mixed together.

ATTACHMENT 20

Nu-Film 17 with Insecticides for Horticultural Insects:

A paper from the 54th Conference Proceedings (2001) of The New Zealand Plant Protection Society Incorporated.

New Zealand Plant Protection. 54:10-14 (2001)

A paper from the
54th Conference Proceedings (2001) of
The New Zealand Plant Protection Society Incorporated

ALTERNATIVE STRATEGIES TO CONTROL NEW ZEALAND FLOWER THRIPS ON NECTARINES

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ABSTRACT

Experiments conducted in both spring and preharvest (summer) aimed to prevent damage to nectarines caused by New Zealand flower thrips *Thrips obscuratus* and to minimise quarantine problems caused by thrips on export fruit. Reflective mulch and three insecticide programmes were compared with the standard tau-fluvalinate/chlorpyrifos programme in spring. Abamectin, spinosad and Pyrethrum Plus® reduced spring damage but none was more effective than the existing standard. At harvest time, carbaryl and spinosad plus Nu-film-17 reduced thrips numbers 3 days after treatment, but carbaryl was the only effective insecticide after 5 days. Reflective mulch reduced thrips numbers in spring and preharvest, providing a non-chemical alternative for thrips control. However, supplementary control measures would be needed for this treatment to meet quarantine standards at harvest time. Alternative spring and preharvest programmes for both Integrated Fruit Production and organic systems are suggested for nectarines, but registration is necessary before some insecticides can be used on summerfruit.

Keywords: Thrips, nectarines, insecticides, reflective mulch, fruit damage.

INTRODUCTION

Eleven thrips species have been recorded on blossom and young fruit of nectarines (*Prunus persica* (L.) Batsch var. *nucipersica* (Suckow) C.K. Schneid) but adults of New Zealand flower thrips *Thrips obscuratus* (Crawford) (NZFT) are responsible for the most severe damage (McLaren 1992). NZFT damages nectarines in the spring and infests ripening fruit at harvest time. Spring damage by NZFT can cause fruit to be rejected at harvest, while the presence of thrips on ripe fruit can be responsible for their rejection for export (zero thrips allowed on 600 fruit). Thrips infestations on ripening fruit can cause white stippling and loss of colour on nectarines and peaches.

Control measures are required to prevent thrips infestation for up to 8 weeks in spring (flowering onwards) and just prior to harvest in summer (the month depends on the crop and cultivar). The current spring control programme uses tau-fluvalinate (non-toxic to honeybees) at flowering, and chlorpyrifos between petal and shuck fall. Under Integrated Fruit Production (IFP) these insecticides are applied when thrips numbers exceed monitored thresholds (McLaren & Fraser 2000) but under conventional systems three to four sprays are applied at 10-day intervals. Currently thrips are controlled prior to harvest, with carbaryl or maldison. Under IFP, alternative treatments are required to replace organophosphate and carbamate pesticides and to reduce the number of applications of synthetic pyrethroid because they are disruptive to predatory mites (McLaren & Fraser 1993). Organic regimes require the use of non-chemical or BioGro-acceptable products.

Several new pesticides and a reflective mulch ground cover were compared with a standard spray programme in five trials conducted between 1996 and 1998 (G.F. McLaren, unpubl. data). The most promising candidates were then compared with the standard

tau-fluvalinate/ chlorpyrifos programme in 1999-2000. The results of this work are presented in this contribution. In addition, alternatives to carbaryl for the control of thrips before harvest were also investigated.

METHODS

Spring

A trial was conducted on a 0.4 ha block of 14-year old nectarine cv. Fantasia trees growing at 5 x 2 m spacing at Clyde Research Centre, Central Otago. Treatments were applied with a handgun to 4-tree plots in a randomised block design with four replicates. Treatments were applied four times: 16 September (80% bloom), 27 September (petal fall), 7 October and 18 October 1999. Treatments were applied in the morning, with the exception of Pyrethrum Plus®, which was applied in the evening. The reflective mulch was installed on 16 September and removed in early November. Details of the treatments are given in Table 1.

TABLE 1: Pesticides applied to nectarine trees for thrips control.

Treatment	Active ingredient	Product rate/100 litre
Pyrethrum Plus®	Mineral oil + pyrethrum	250 ml
Agrimec®	1.8% abamectin	37 ml
Success Naturalyte™	12% spinosad	40 ml
Mavrik® (first application)	24% tau-fluvalinate	20 ml
Lorsban® 750WG (3 applications)	75% chlorpyrifos	33.3 g

Thrips were sampled from two trees in each plot twice weekly over seven weeks from flowering to shuck fall (29 October). Twenty branches per tree were tapped over a 250 mm diameter yellow plate and the number of thrips recorded (McLaren & Fraser 2000). At harvest, 50 fruit were collected from the two central trees in each plot and graded for thrips damage as follows: none (Grade 0), within export standards (Grade 1), within local market standards (Grade 2) and reject (Grade 3). Percentage export was estimated by combining Grades 0 and 1.

Preharvest – trial 1

On 17 February 1997, lengths of 1.2 m wide reflective mulch (Extenday®) were laid on either side of two rows of 10 insecticide-free nectarine cv. Fantasia trees (4.5 x 4 m spacing) at the Clyde Research Centre. Seven days later, at the normal harvest time, 20 fruit were picked from each tree in these two rows and thrips numbers recorded. In addition, 20 fruit/tree were picked from the trees in the two adjacent rows on either side of the mulched rows.

Preharvest – trial 2

In February 2001, a trial was conducted on insecticide-free nectarine cv. Fantasia trees using a replicated randomised block design with four replicates. Plots consisted of two trees (6 x 4.5 m spacing) with an untreated tree between each plot. A two-tree space separated this sprayed trial from a block of 28 trees in four rows with reflective mulch laid underneath (1.2 m wide Extenday®). The reflective mulch was installed eight days before the first harvest while the replicated pesticide treatments were applied three days before the first harvest using a handgun.

The following treatments were applied on 16 February 2001:

- Success Naturalyte™ 40 ml/100 litre
- Success Naturalyte™ 40 ml/100 litre plus Nu-Film-17™ 120 ml/100 litre
- Carbaryl 50F (carbaryl) 240ml/100 litre

Three and five days later (19 and 21 February 2001) 100 fruit per two-tree plot were sampled on each occasion and the number of thrips recorded.

Thrips/tree, thrips/fruit, and percentage fruit damage data were subjected to analysis of variance by Newman-Keuls test after percentages had been arcsine transformed to stabilise the variance. Data were back-transformed for presentation.

RESULTS

Spring

The tau-fluvalinate/chlorpyrifos programme had significantly fewer thrips on the flowers and small fruits, with just 13% of those found in the untreated population, but thrips numbers on the other four treatments were not significantly different from those of the untreated control trees (Table 2).

Table 2: Mean infestation of nectarine flowers and fruitlets by New Zealand flower thrips on 14 sampling occasions in spring, and damage to fruit at harvest.

	Spring thrips (thrips/tree)	Fruit quality at harvest % with no damage[2]	% export[2]
Pyrethrum Plus®	0.84 ab[1]	26.9 ab	68.1 b
Abamectin	0.73 ab	33.2 ab	84.3 a
Spinosad	1.01 ab	32.1 ab	71.4 ab
Reflective mulch	1.01 ab	37.7 ab	71.4 ab
Tau-fluvalinate/ chlorpyrifos	0.22 a	51.2 a	75.8 ab
Untreated	1.71 b	15.0 b	54.5 c

[1]Means followed by the same letter in a column are not significantly different (P<0.05).
[2]Back-transformed percentages

The tau-fluvalinate/chlorpyrifos programme produced more fruit without damage (Grade 0) than the untreated control trees (P<0.05), but was not significantly different to the other four treatments. All the treatments produced higher export packouts than from the untreated control trees (P<0.05). Abamectin, spinosad, reflective mulch and tau-fluvalinate/chlorpyrifos treatments produced 17-30% more export grade fruit than the untreated. Pyrethrum Plus® was less effective in terms of % export fruit than abamectin but not different to the other three treatments.

Preharvest – trial 1

In 1997, reflective mulch reduced thrips numbers per fruit on the two treated rows compared with the second row on the west side of the mulch, but the mulch had some effect on thrips numbers on the rows nearest to it (Fig. 1). Thrips infestations were reduced from 3/fruit on the outer west row to 0.4/fruit in the mulch rows, an 87% reduction in thrips numbers.

Preharvest – trial 2

In 2001, trees treated with either spinosad and Nu-Film-17 or carbaryl had lower thrips numbers per fruit than the untreated trees or those treated with spinosad or reflective mulch on day 3 (Fig. 2). Spinosad and reflective mulch reduced thrips numbers compared with the untreated control trees but had more thrips/fruit than the carbaryl or spinosad and Nu-Film-17. However, by day 5, only the carbaryl treatment had significantly lower numbers than all the other treatments. Spinosad (with or without Nu-Film-17) and reflective mulch were still significantly different from the untreated control trees on day 5 but were not as effective as carbaryl.

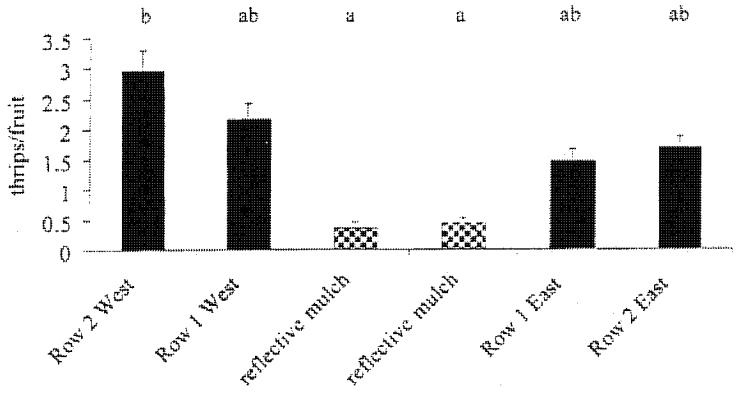

FIGURE 1: Number of New Zealand flower thrips per fruit on two rows of nectarine trees with reflective mulch beneath them compared to trees without mulch on either side of the mulched rows (n = 200 fruit/row). Bars with the same letter are not significantly different (P<0.05). The SEM is shown for each bar.

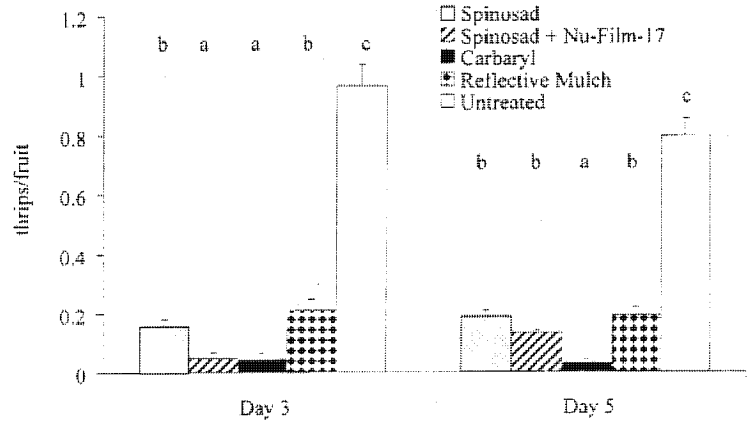

FIGURE 2: Effect of a single application of three treatments and under-tree reflective mulch just before harvest on thrips infestations on nectarine fruit 3 and 5 days after treatment (n=400 fruit/treatment). Bars with the same letter are not significantly different (P<0.05). The SEM is shown for each bar.

DISCUSSION AND CONCLUSION

The existing spring programme of tau-fluvalinate followed by chlorpyrifos was the most effective method of controlling thrips, but alternative insecticides are urgently needed for organic production and for parts of the IFP programme (SummerGreen™) in the near future. Both Pyrethrum Plus® and reflective mulch are accepted in Bio-Gro orchards and are useful options for organic systems. Reflective mulch has not been tested previously for thrips control within New Zealand but it presents a useful non-chemical option, provided the cost-benefit of its use is evaluated over several years. The mode of action of reflective mulch is unclear, but presumably it affects either host orientation by the adult thrips or it disturbs their habitat within the tree.

Two new products, abamectin and spinosad, showed promise for use in conventional and IFP orchards, but neither are currently registered for use on summerfruit. The preharvest trial in 2001 demonstrated how the efficacy of spinosad could be improved by the use of additives such as Nu-Film-17, bringing its performance close to that of the standard carbaryl treatment.

At harvest, carbaryl and spinosad with Nu-Film-17 were equally effective in reducing thrips numbers on fruit three days after treatment, but two days later only carbaryl remained fully effective. Alternative insecticides will be required if, or when, carbaryl is withdrawn from use on export fruit; to this effect spinosad shows promise for preharvest use. Organic growers may use reflective mulch for local market fruit but results presented here suggest that it would not reduce thrips numbers sufficiently to meet quarantine standards for export markets (a full tree canopy is likely to provide some protected sites for thrips). Organic export growers would need to combine reflective mulch with a treatment such as Pyrethrum Plus® to meet the quarantine standard of zero thrips in 600 fruit.

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McLaren, G.F.; Fraser, J.A. 2000: Development of thresholds for insecticidal control of New Zealand flower thrips on nectarines in spring. *N.Z. Plant Prot. 53*: 194-199.

ATTACHMENT 21

Nu-Film P

For Management of Lettuce Aphid, *Nasonovia ribisnigri* (Mosley) in Organic and Reduced-risk (IPM) leaf Lettuce.

Pest Management Grants- Applied Research.
Contract # CDPR-99-0224-CHANEY-03/01

PEST MANAGEMENT GRANTS-APPLIED RESEARCH FINAL REPORT
TITLE PAGE

Contract #: CDPR-99-0224-CHANEY-03/01

Contract Title: Evaluation of efficacy of green lacewings, *Chrysoperla rufilabris* (Burmeister), delivered using a liquid release technique, for management of lettuce aphid, *Nasonovia ribis-nigri* (Mosley), in organic and reduced-risk (IPM) leaf lettuce.

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Contractor Organization: University of California-Cooperative Extension.

Date: March 31, 2001.

Prepared for California Department of Pesticide Regulation.

Disclaimer

The statements and conclusions in this report are those of the contractor and not necessarily those of the California Department of Pesticide Regulation. The mention of commercial products, their source, or their use in connection with material reported herein is not to be construed as actual or implied endorsement of such products.

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Executive Summary

The lettuce aphid, *Nasonovia ribis-nigri*, has emerged as an extremely important new lettuce pest to California. To control lettuce aphid, growers have increased their use of highly toxic pesticides, including organophosphates and carbamates. Some organic growers have been driven to reduce their lettuce acreage due to this serious new pest. This project sought to utilize knowledge and experience gained during our first year of funding in 1999 to improve a liquid delivery system for green lacewing eggs in lettuce and evaluate the hatch of the released eggs and efficacy of subsequent lacewing larvae in decreasing lettuce aphid populations. Specific objectives of the project were: 1.) to improve the mechanical liquid distributor originally designed for grapes and modified for lettuce (row crop) production systems; 2.) to evaluate the effect of potential liquid sticker carriers on egg hatch in laboratory bioassays; 3.) to evaluate lacewing egg hatch in organic, unsprayed and Admire-treated lettuce fields distributed using the modified liquid distributor; and 4.) to evaluate the efficacy of lacewings in reducing lettuce aphid populations in the field.

The mechanical liquid distributor was modified to improve efficient delivery of lacewing eggs to lettuce. The vessel and tubing connections were made air and liquid-tight to prevent leaking, the number of emitter valves was increased from four valves to ten valves to enable coverage of more seed lines with one tractor pass, manifolds were constructed to enable flexibility in positioning the valves over the lettuce seed lines, and the electrical system and control box were made more robust to enable greater capacity.

Six liquid carriers, including two food-grade starches, three organically approved products and a dilute agar solution, were compared to water and an untreated control in laboratory bioassays for effects on egg hatch and adhesion. The carriers were evaluated after the eggs were submersed in the carriers for 15 minutes while gently stirring. Hatch of the treated eggs was compared relative to an untreated control. Although several of the carriers did not negatively effect egg hatch relative to the untreated controls, two carriers, a 0.05% solution of Nu-Film P™ and a 1% C DrySet™, were observed to give both good adhesion and good hatch, 86% and 95% mean relative egg hatch respectively, and were chosen for further field testing.

Low spring populations of the target pest, *Nasonovia ribis-nigri*, prevented early lacewing egg releases in Admire™-treated fields. Releases were conducted in two organic fields in late summer. At the first site, the releases failed due to problems with conditioning of the eggs, the grower's irrigation schedule, and equipment failure. In the second site, three releases using the modified distributor were conducted with four treatments: eggs distributed using water as the carrier, eggs distributed using Nu-Film P™ as the carrier, eggs distributed using C-DrySet™ as the carrier and an untreated control. Monitoring results showed the mean number of lacewing larvae was highest in the plants that received lacewing eggs using water as the carrier (0.5 lacewing larvae/plant on the last monitoring date), significantly higher, at $p = 0.05$, than either the plants that received eggs in C-DrySet™ (0.2 lacewing larvae/plant) or the untreated (0 lacewing larvae/plant) but not significantly different from those plants which received eggs in Nu-Film-P™ (0.27 lacewing larvae/plant). The mean number of apterous *Nasonovia* was highest in the untreated plants (44 *Nasonovia*/plant on the last monitoring date) and plants which received eggs in C-DrySet™ (43 *Nasonovia*/plant), significantly higher, at $p = 0.1$, than either the plants that

received eggs in NuFilm-P™ (32 *Nasonovia*/plant) or the plants that received eggs in water (31 *Nasonovia*/plant). However, syrphid larvae were also observed to be good predators of *Nasonovia* and were highest in the plants that received lacewing eggs in water (2.1 syrphid/plant).

We conclude that lacewing egg releases using the modified liquid distributor can increase the number of subsequent lacewing larvae in a field and reduce the number of apterous *Nasonovia* in those plants that receive eggs over untreated plants, especially when water is used as the egg carrier. However, in our releases, we found that syrphid larvae were also higher in our release plots and therefore we cannot attribute the reduction in *Nasonovia* in those plots to the lacewing alone. The use of augmentative biological control will continue to be an important component of IPM and organic systems, as demonstrated by grower's interest, and there continues to be a need to improve the efficiency and effectiveness of this potential tool.

Report.

a. Introduction

Lettuce aphid, *Nasonovia ribis-nigri*, is a new pest to California that colonizes the center of the lettuce head where it is difficult to reach with pesticide sprays. Lettuce infested with lettuce aphid is unmarketable. To control lettuce aphid, growers have increased their use of highly toxic pesticides, including organophosphates and carbamates. Organic lettuce growers have few, if any, acceptable control options and many have reduced their lettuce acreage because of losses due to *Nasonovia* infestations. The overall goal of this project was to evaluate the efficacy of augmentative biological control, specifically green lacewing eggs, for lettuce aphid, *Nasonovia ribis-nigri*, in both organic and reduced-risk (IPM) lettuce production systems. The project addressed the following priority areas: alternatives to highly toxic pesticides, reduction of worker exposure to pesticides, and protection of surface and ground water quality.

This project sought to utilize knowledge and experience gained in our first year of funding in 1999 to improve a liquid delivery system for lacewing eggs in lettuce and evaluate the hatch of the released eggs and efficacy of subsequent lacewing larvae in decreasing lettuce aphid populations. During the 1999 season, green lacewing egg releases were conducted in the Salinas Valley using water as the liquid carrier. Results showed good insectary quality and showed that the delivery system did not harm the eggs. However, only one release resulted in lacewing larvae in the field as evaluated by both clip cage and in-field monitoring. Dislodging of eggs from the plant surface after distribution but prior to hatching appeared to be one key factor in poor recovery of lacewing larvae after our egg releases. We concluded that a need exists to identify liquid carriers other than water for distributing the eggs, carriers that improve adhesion of the eggs to the plant without decreasing egg hatch.

The specific objectives and associated tasks for this project were as follows:

Objective 1: Improve the mechanical liquid distributor system originally designed for grapes and modified for lettuce (row crop) production systems.

Tasks: 1.) modify the suspension reservoir and replace tubing and fittings to reduce leaks and improve reliability; 2.) resizing emitter valves so as to reduce clogging with egg suspension; 3.) design adjustable distributor valve tips (nozzles) and/or adjustable valve mountings to help direct eggs to the center of lettuce heads which are growing over time; 4.) increase the number of distributor valves from 4 valves to 8-10 valves in order to cover 4-40" lettuce beds each with 2 seed lines or 2-80" lettuce beds each with 5 seed lines in one pass; 5.) improve the electrical system and control box electronics to enable capacity of the 8-10 valves.

Objective 2: Evaluate the effect of potential liquid sticker carriers on lacewing egg hatch in laboratory bioassays.

Tasks: 1.) identify potential liquid sticker carriers appropriate for use in organic systems; 2.) mix eggs with the carriers in laboratory bioassays; 3.) plate eggs after submersion into individual cells on cell plates; 4.) evaluate the effect of carriers by observing adhesion of eggs to plates and counting hatched larvae.

Objective 3: Evaluate lacewing egg hatch in organic, unsprayed and Admire-treated lettuce fields for eggs distributed using the modified liquid distributor.
and
Objective 4: Evaluate the efficacy of released lacewings in reducing lettuce aphid populations in the field.

Tasks: 1.) communicate with growers and PCAs to identify field sites and arrange for equipment use and tractor driver assistance; 2.) flag fields for trial set-up, 3.) monitor for *Nasonovia* presence in lettuce; 4.) condition eggs and prepare carriers for releases; 5.) conduct releases; 6.) collect plate and clip cage data for released lacewings; 7.) collect field monitoring data for *Nasonovia*, lacewings and native natural enemies; 8.) enter data and conduct data analysis; 9.) supervise a field assistant.

b. Materials and methods.
Note: for all experiments reported here, laboratory and field, green lacewing eggs, *Chrysoperla rufilabris* (Burmeister), were obtained from a commercial insectary (Beneficial Insectary, Oak Run, CA.). Eggs were packed without carrier and shipped either overnight standard or overnight priority. Upon receipt, three "control plates" of eggs were prepared to determine egg viability in the absence of any experimental effects, as in Wunderlich and Giles, 1998. Since green lacewing larvae are generalist predators and cannibalistic, the control eggs were separated by placement into individual cells on cell plates and incubated until hatch. Each plate held a maximum of sixty eggs, with a total sample size of approximately 180 eggs/date.

Objective 1: Improve the mechanical liquid distributor system originally designed for grapes and modified for lettuce (row crop) production systems.

The mechanical liquid distributor that was used during the 1999 project season was delivered to Ken Giles, UC-Davis Biological and Agricultural Engineering Dept., for performance assessment and improvements. To reduce air leaks in the original suspension reservoirs, the top-ends of the reservoirs were replaced by machined grooves for a tighter o-ring fit. The four nylon-threaded rods of each vessel were replaced with cadmium-coated steel to enable a tighter screw down. The original tubing from vessel to each valve was replaced with smaller diameter tubing and all of the compression fittings for the tubing were replaced to provide leak-proof flow. The entire electrical system was improved for increased valve capacity and for ease of field use.

A ten-liter Nalgene™ liquid carboy was modified and tested as replacement suspension reservoir. The bottom of the carboy was drilled with an inlet hole for air, so the vessel could be pressurized, and an exit hole for the egg suspension. The cap of the carboy was drilled and a 24-volt motor installed and attached to turn an inserted paint stirrer at 60 RPM to provide agitation to the egg suspension. A pressure gauge was inserted in the cap to measure vessel pressure.

The prototype carboy vessel was evaluated in the laboratory for mortality of green lacewing eggs after submersion over time in the vessel agitated by the stirrer and for uniformity of the egg suspension after such agitation and discharge from the system. The carboy vessel was filled with

8 liters of water and 7.2 g. of green lacewing eggs were added to bring the suspension to a concentration of 10 eggs/ml based on our observations of approx. 11,155 eggs/g. The carboy vessel was pressurized to approx. 1-3 psi. Four distributor valves were attached to the outlet of the vessel and the valves were set for output at 4 Hz., 20% duty cycle. The egg suspension was allowed to stir for approx. 20 minutes and then the valves were turned on and egg suspensions were collected from the valves, counted for uniformity, and plated for egg mortality. The experiment was conducted on two dates: 6/1 and 6/22. Five ml. of eggs were collected every 5 min for 50 min. on 6/1; while 10 ml. of eggs were collected every 5 min. for 20 min. on 6/22.

New emitter valves were constructed to increase the size of the distributed droplets and to improve the flexibility in directing the droplets towards the center of the lettuce plants where the target pest, *Nasonovia ribis-nigri*, colonizes. The number of emitter valves was increased from 4 to 10 to allow for efficiency in covering a typical "pass" (4-40" lettuce beds each with 2 seed lines or 2-80" lettuce beds each with 5 seed lines). The steel manifold "tees" for mounting the valves on a standard tractor tool bar were also modified for flexibility: a slot was cut into the tee and the valves were fitted with mounting screws which could be slid along the slot and tightened at the appropriate position.

Objective 2: Evaluate the effect of potential liquid sticker carriers on lacewing egg hatch in laboratory bioassays.

We considered both biological and practical implications to narrow the list of candidate carriers for testing, including: ease of mixing, availability to growers, reported previous success (either anecdotal or scientific), and propensity to attract unwanted interfering predators (i.e. sugar solutions attracting ants). Later, during field-testing, the issue of acceptable certified organic materials became an extremely important factor.

Researchers from Colorado State University, (Mannix, et al.,1999), used a 1% solution of a modified food starch to deliver green lacewing eggs into trees with reported success. We contacted the source of this starch, Cerestar Inc. (Hammond, Indiana), and included two food-grade starch products (C-DrySet™ and C Gel Instant™) in our testing. Corn calcium (Peaceful Valley Farm Supply, Grass Valley, Ca.) was included because it was recommended by a local PCA (Israel Morales, pers. comm.). A 0.05% solution of agar was recommended by a colleague working in biological control (Pete Gothro, pers. comm.). We also included the following organically-accepted adjuvants, based on EPA List 4 and the approved OMRI (Organic Materials Review Institute) list of organic materials: Nu-Film P™ (Miller Chemical and Fertilizer Corp., Hanover, Penn.), and Therm-X70™ (Cellu-con, Inc., Strathmore, Ca.).

For each date, the eggs were received from Beneficial Insectary (Oak Run, Ca.) and were placed into control plates as described above. The control plates containing the untreated eggs and the remaining cup of eggs (containing the eggs to be submerged in the carriers) were placed into a plastic box with a lid and a cup of water for humidity. The box, containing the eggs, was then placed in a growth chamber at 28°C (+/- 2°C) for 24-48 hours, until eggs turned from bright green to grayish-brown in color, indicating the lacewing were close to hatch.

When eggs appeared gray-brown in color the submersion experiments were conducted. Since there was little information on the carriers tested, several dilutions of each carrier were screened. Except for the C-DrySet™ solutions, each carrier was prepared the day of the submersion test: 100 to 200ml. of carrier solution was prepared and placed into a glass beaker with a stir bar. The C-DrySet™ solution was usually prepared the day before by heating in a hot water bath for 15 minutes at 90°C to make the solution stickier by hydrolyzing the starch as recommended by the manufacturer. The solution was allowed to cool completely before submersion of eggs.

A mass of eggs equivalent to 10 eggs per ml. of carrier solution, calculated using the estimated average density of 11,155 eggs/gram, was weighed and added to the carrier solution in the beaker. The eggs in solution were then stirred slowly on a stir plate for 15 minutes. A water control in which the eggs were submerged into water alone and stirred for 15 minutes to assess any effects on the eggs due to stirring was also included for each date. After 15 minutes, the solution containing the eggs was poured onto three plates and the eggs were immediately singulated into the plate cells using a fine tipped paintbrush, as was done with the control eggs. For each date, three plates (with 60 cells containing an egg per plate or a maximum of 180 eggs/treatment) were prepared for each carrier tested, for the untreated control eggs, and for the water control. All of the plates were placed inside of plastic boxes with lids and a cup of water for humidity (one plate from each treatment per box) and placed into the growth chamber at 28°C (+/- 2°C) until hatch of the eggs. The number of eggs hatched was counted for each plate and normalized to the untreated control hatch for that date.

Observations were made on each carrier solution as it was tested: the ease of mixing and relative solubility of the carrier, the texture ("stickiness") of the solution by feel between the fingertips, and the apparent adhesion of the eggs was observed. The adhesion of each carrier was assessed by turning the plates containing the eggs upside down and observing the stick of the eggs to the organza layer of the plates.

Objective 3: Evaluate lacewing egg hatch in organic, unsprayed and Admire-treated lettuce fields for eggs distributed using the modified liquid distributor.

Objective 4: Evaluate the efficacy of released lacewings in reducing lettuce aphid populations in the field.

Spring commercial fields: Haceinda 502 and 610.

We met with several challenges in conducting our field releases in the 2000 season. The first was the unexpected low populations of the target pest, *Nasonovia ribis-nigri*, in spring and early summer lettuce fields. Two commercial romaine fields (neither organic) located in the southern Salinas Valley near King City were targeted for releases and monitored. The first field, Hacienda Ranch Lot 502, (approx. 10 acres) did not receive Admire™ under any part of the field and was divided into two sides: one side to receive lacewing eggs once *Nasonovia* were detected and the other to receive Standard conventional sprays for *Nasonovia* in leaf lettuce (Provado™, and/or Dimethoate™ and/or Diazinon™). Thirty plants were monitored weekly in each part of the field (60 plants total) for seven weeks, April 5 through May 23.

The second field, Hacienda 610, was treated pre-plant with Admire™ under most of the field. Forty-eight beds along the edge were left untreated (no Admire™) so that lacewings could be applied to that section of the field. We also planned on releasing lacewings onto the treated portion of the field to see if the Admire™-treated plants would have any effect on the lacewing hatch. We monitored 20 plants weekly on each part of the field (40 plants total) for six weeks, April 25 through June 5.

Summer organic field trial: Coke Ranch.

Since *Nasonovia* pressure was low in spring conventional lettuce fields, we contacted several organic growers who presumably would have a greater likelihood of having an aphid-infested field. We identified the Coke Ranch as an organic site with *Nasonovia* present and the grower agreed to provide a tractor and driver for the releases.

We originally planned on applying two treatments: lacewing eggs using water as the carrier and lacewing eggs using C-DrySet™ as the carrier. When we contacted the grower and his PCA regarding our planned use of the C-DrySet™, we were notified of the need to get approval from the organic certifier (California Certified Organic Farmers) for the use of the C-DrySet™ material. Although C-DrySet™ is a food grade product, it is not currently on the OMRI-approved list of organically acceptable materials. We contacted CCOF and discussed the possibility of a CCOF approved Experimental Use Policy. This, however, would have jeopardized the grower's organic certification of the crop, and possibly, the organic certification of the land. We therefore decided to withdraw the use of the C-DrySet™ in this field trial and instead focused on field-testing our improved distributor using water as the carrier for the eggs.

A 3.2 acre block of Romaine was divided and flagged into 4 replicated blocks of untreated plots and plots to receive lacewing eggs. Plots were 0.4 acres each, consisting of 4, 80-inch beds wide. Each 80-inch wide bed had five seed lines planted on it. Two releases were made to the field (on July 19 and July 27) using the newly modified equipment and water as the carrier. We planned to release approx. 125,000 eggs/acre or 2-3 eggs/plant at the estimated planting density of 49,000 plants/acre. For the first release, however, we applied a higher rate (approx. 300,000 lacewing eggs/acre), since we did not have the C-DrySet™ treatment and had extra eggs. For the second release on July 27, we attempted to apply approx. 125,000 eggs/acre.

For all of our releases, the electronically controlled liquid delivery system was transported to the field site and mounted on the tractor tool bar the morning of the release. The cooperating growers provided the tractor and the tractor driver for each release, which took up to five hours including mounting, troubleshooting and actual application time.

For all of the releases a mass of 2.7g of eggs was measured into container cups, based on 11,155 eggs/gram and a release concentration of 10 eggs/ml in three liters of water. Tap water was used for the carrier and transported to the site. Eggs were mixed into the water immediately before loading and distribution in the field. One cup, or approx. 30,000 eggs, was mixed with three liters of water in each of the two vessels.

To assess handling and application effects, eggs mixed in water were collected from the distributor valves after travel through the apparatus during the field release and plated for

comparison with the control plates. Three plates, or a maximum of 180 eggs, were evaluated. Evaluation of environmental effects, temperature and wind, on egg hatch in the field after distribution was measured by covering the eggs with clip cages immediately after release. Fifteen eggs in each plot (60 eggs total) were caged after the first release. Only 25 eggs total were caged during the second release due to problems with the equipment (see results). The eggs were evaluated for hatch the following day by unclipping the cage and noting whether the egg was present, missing, crushed, or if the egg had hatched, a lacewing larva was present.

To evaluate lacewing larvae survivorship, and efficacy against the aphid *Nasonovia*, each field was monitored before and after each release for the presence of *Nasonovia* and lacewing larvae. The day before the release and weekly thereafter, (July 14 to August 10) ten plants were monitored in each of the four replicated plots receiving eggs and in the untreated plots. Lettuce plants were inspected and the number of aphids as well as other insect pests and any natural enemies (including lacewings) present were counted.

Late Summer/Early Fall : Tanimura & Antle Experimental Field.
In order to evaluate the C-DrySet™ carrier and compare it to water and Nu-Film P™ in a randomized block design, we moved our last release site to the Tanimura & Antle experimental field located in Spreckels, Ca. This enabled us to do field releases on organically managed ground without jeopardizing the grower's organic certification.

A 0.4-acre block of Romaine was divided into a randomized complete block design with 4 replicates of 3 treatments and an untreated control. Plots were approximately .02 acres in size: 4, 40-inch beds wide and 67 feet long. Treatments consisted of lacewing eggs released in water as the carrier, lacewing eggs released in Nu-Film P™ as the carrier, and lacewing eggs released in C-DrySet™ as the carrier. Three releases were made: on August 30, September 7, and September 13. A flagger directed the tractor driver to each of the appropriate blocks for application of the treatments. Due to a lack of time, no clip cages were placed over the released eggs nor were eggs collected from the valves.

The field was monitored weekly from August 23 to September 20. Ten plants from each replicate, or forty plants per treatment, were monitored. Only plants from the middle two beds of each plot were inspected.

c. Results.

Objective 1. : Improve the mechanical liquid distributor system originally designed for grapes and modified for lettuce (row crop) production systems.

Improvements made to the vessel and fittings succeeded in making the distributor air and water tight, no leaking was observed during field releases.

Egg hatch from eggs which were suspended in water in the Nalgene carboy prototype vessel, stirred for 10, 20 or 40 minutes and then plated for hatch showed no difference from untreated controls. Therefore, the stirring agitation did not appear to effect egg viability. Uniformity of distribution was affected, however. On June 1, the eggs/ml. distributed varied from 0 eggs at

T=10 min. to 27 eggs at T=30 min. Likewise, during the second experiment on June 22, the eggs/ml. varied from 3 eggs at T=15 min. to 11 eggs at T=20 min. Therefore, the stirring method of agitation employed in the carboy prototype did not adequately keep the eggs uniformly suspended and effected the distribution of the discharged eggs. Because of these problems, we decided not to use the carboy vessel prototype, and instead used the improved original suspension reservoirs for our field experiments. The original reservoirs have demonstrated very uniform discharge of the eggs, with only a 0.4% decrease in egg concentration per minute of agitation (Wunderlich, 1997).

Several problems were noted while laboratory testing of the new emitter valves, including electrical problems with firing of the valves and apparent clogging of eggs in the newly reconfigured valves. The electrical problems were solved by inspection of the control box and repair. Because of the intermittent clogging of eggs in the new valves, the original valves were re-employed in the system. Later, during the first field release at Coke Ranch (see objective 3), we encountered egg clogging in the smaller diameter tubing, which led us to replace it with the original sized tubing.

Objective 2: Evaluate the effect of potential liquid sticker carriers on lacewing egg hatch in laboratory bioassays.

Six carrier solutions, in addition to water, were screened in laboratory assays. Table 1 in Appendix 1 lists the carriers, manufacturers, rate and observations from the carriers we screened. Figure 1 shows the percent mean egg hatch, relative to the control eggs, for each of the tested carriers. The (n) or number of plates replicated differs for each carrier since those carriers which did not show some promise in both adhesive qualities and egg viability in early tests were eliminated from further testing to facilitate the timeline of the experiment.

Some of the carriers were difficult to mix up and prepare, including the agar solution (which had to be autoclaved to dissolve the agar), and the corn calcium, which was a very viscous material and was difficult to accurately measure.

C-Gel™ at a 1% and 5% concentration decreased hatch of the eggs to a mean of 56-65% hatch, which was considered unacceptable, therefore it was not considered further. Likewise, Therm-X70™ (10x) and NuFilm-P™ (2x and 10x) also decreased hatch as compared to the untreated controls and were therefore omitted from further testing.

Although corn calcium (1x), Therm-X70 (1x), and agar solution (.05%) showed no detrimental effect on egg hatch as compared to the untreated control, none of these three carriers appeared to stick the eggs to the surface of the plate in our observations. Therefore, these carriers were tested on only one or two dates before they were discarded from the pool of candidates.

Of the six carriers tested, only C-DrySet™ (1% and 3%) appeared to give the best adhesion without effecting hatch of the lacewings. Since there was no significant difference in hatch of eggs after immersion in the 3% solution compared to the 1%, we dropped the 3% solution and continued testing with the 1% C-DrySet™ concentration. We repeated testing on 7 dates, for a total of 21 replicated plates. The mean relative hatch from eggs submerged in the 1% C-DrySet

solution across all dates was 95% (standard error of 3.6%). Although the mean hatch from 9 replicates of NuFilm-P™ (1x) was only 86%, we still considered it a good candidate since it appeared to give good adhesion of the eggs, was available to growers and relatively easy to mix, and was an organically approved material. At the conclusion of our laboratory testing of carriers, we prepared to go to the field with two candidate carriers: NuFilm-P™, and C-DrySet™.

Objective 3: Evaluate lacewing egg hatch in organic, unsprayed and Admire-treated lettuce fields for eggs distributed using the modified liquid distributor.

Objective 4: Evaluate the efficacy of released lacewings in reducing lettuce aphid populations in the field.

Spring commercial fields: Haceinda 502 and 610.
In Hacienda Ranch Lot 502, only 3 plants were found to have *Nasonovia*: one plant had eight apterous *Nasonovia* aphids on May 5, and two plants had one alate *Nasonovia* each on May 23. In Hacienda 610, no *Nasonovia* on either the Admire-treated or the untreated side; therefore, no lacewing releases were made into either of these fields.

Coke Ranch.
Several problems were encountered during the releases at Coke Ranch. For the first release, we received the eggs from the insectary on July 18, the day before the scheduled release, and placed them into the growth chamber @ 25°C as per our protocol. However, on the morning of the release the eggs were still greenish in color, indicating they were still not close to hatch. Because of the scheduling of both the tractor driver and the grower's irrigation schedule, we conducted the release with the eggs even though they were not as close to hatch as we would have preferred.

On July 20, the day after the first release, there was no hatch in either the control plates or the eggs collected from the distributor and plated. On July 21, two days after the release, the mean hatch from the control plates was 94.4%, and the relative mean hatch of the eggs collected from the distributor valves after travel through the apparatus during the field release was only 69.7%, much lower than observed in previous testing.

Unsurprisingly, there was no hatch from the eggs we had clip caged when we checked them at 3:20 p.m. on July 20, the day after the release. At that time, of the 60 eggs caged, 46 of them were not yet hatched, 11 eggs were missing, and 3 eggs had been crushed. The grower then irrigated the field using sprinklers the evening of July 20.

During our second release, on July 27, we encountered problems with plugging of the tubing during the release. After repeated clogging, we stopped the release and did not put eggs onto the fourth replicate. For the plating procedure, we only collected eggs from the two valves that were not clogging. Because of the time spent trying to unclog the tubing, we only were able to clip a total of 25 eggs in the first three replicates. After this release, we replaced the tubing with the larger-diameter tubing and encountered no further problems with clogging.

The mean control plate hatch from the second release was 92.7%, and the mean hatch from eggs collected after immersion in the water and travel through the distributor and the two valves that were not clogging was 95.4%. On July 28, the day after the second release, 5 of the 25 clip caged eggs had hatched and contained a lacewing larva, 2 of the 25 eggs were missing, and the remaining 18 eggs were unhatched.

In Appendix 2, Figure 2.1 and Figure 2.2 show the results from our field monitoring at Coke Ranch. Figure 2.1 shows the mean number of *Nasonovia*/plant, which reached a peak on August 2 with a mean of 53 aphids/plant in the untreated plots and 44 aphids/plant in the plots that received lacewing eggs. We do not attribute this difference to our releases, however.

Figure 2.2 shows the mean number of either lacewing or syrphid larvae in the field on the same dates. We did not find any lacewing larvae in the field the week following our first release. We believe this release failed to result in any larvae, due to the young age of the eggs and the grower's irrigation, even though we put out a rate of eggs equivalent to 300,000 per acre. On August 2, the week following our second release, we found a total of three lacewing larvae on three of the 40 plants we monitored. Given the problems with the clogging of the equipment, we were not surprised by this low number. Syrphids appeared to be the dominant natural enemy of the aphids in the field and reached a mean of 4.5 syrphid larvae per plant in the plants that received lacewing eggs and 2.75 syrphid larvae per plant in the untreated plants. We are unsure why there were greater numbers of syrphids in our release plots and we have no reason to believe our releases encouraged the presence of syrphids.

Tanimura and Antle Field.

In Appendix 3, Figures 3.1 and 3.2 show the results from our field monitoring after the three releases at the Tanimura and Antle field site. Figure 3.1 shows the mean number of apterous *Nasonovia*/plant found in each treatment compared to the untreated control. We had good aphid pressure in this field: the mean number of *Nasonovia*/plant grew steadily in all blocks, including our release plots, throughout the month. On Sept. 20, the last date we monitored the field, the mean number of *Nasonovia*/plant was highest in the untreated block (44 *Nasonovia*/plant) and also in the block which received lacewing eggs in the C-DrySet™ carrier (43 *Nasonovia*/plant) both significantly higher (at $p=0.1$) than the blocks which received lacewing eggs in either Nu Film P™ (32 *Nasonovia*/plant) or in water (31.3 *Nasonovia*/plant).

Figure 3.2 shows the mean number of lacewing larvae found in each treated block as compared to the untreated. We found the highest number of lacewing larvae in the block that received eggs using water as the carrier (mean of 0.5 lacewing larvae/plant on Sept. 20), significantly higher than either the plants that received eggs in C-DrySet™ (0.2 lacewing larvae/plant) or the untreated plants (0 lacewing larvae/plant) but not significantly different from those plants which received eggs in Nu-Film-P™ (0.27 lacewing larvae/plant). We found no lacewing larvae in the untreated plots at any time.

We also found native syrphid larvae in the plots. On Sept. 20 there were a significantly higher mean number of syrphid larvae in the plots which received lacewing eggs in water (2.1 syrphid larvae/plant) than either the C-DrySet™ (0.8 syrphid larvae plant) or the untreated (0.3 syrphid

larvae/plant). The Nu-Film P™ treatment had a mean of 1.5 syrphid larvae/plant on the same date.

d. Discussion

Results from our work demonstrate the importance of field-testing results from laboratory experiments designed for field application. Changes made in the laboratory to our distributor, new emitter valves and narrower tubing, did not bear out in our field tests and we had to replace the emitters and tubing with the originals. Likewise, the carriers C-DrySet™ and Nu-Film P™ demonstrated good egg adhesion and hatch after egg submersion in the carriers in beaker experiments in the laboratory. We expected the adhesion lent to the eggs by the carriers to result in better stick of the eggs to the plants and subsequently more lacewing larvae in the field. But when we conducted field releases using the mechanical distributor to dispense the lacewing eggs immersed in the C-DrySet™ or Nu-Film P™ compared to water as the carrier, neither of the carriers significantly improved the number of lacewing larvae found in the field over water. We are unsure why this was the case. It could be that the carriers somehow made the eggs more susceptible to damage during delivery or more susceptible to desiccation once distributed. Therefore, we were unable to identify a carrier for lacewing egg distribution that is better than water, and in fact, found the greatest number of lacewing larvae in the plots which distributed eggs using water as the carrier.

Furthermore, we cannot conclude that the decrease in the mean number of *Nasonovia* in the plots that received lacewing eggs was a result of the preying of the lacewing larvae on the *Nasonovia* alone. This is because other natural enemies, especially syrphid larvae, were present in the release plots (which were organic and did not receive any other sprays, not even organically-approved materials), and the syrphid larvae were also observed to prey on the *Nasonovia*. Interestingly, in both of our field trials we found more syrphid larvae in the plots that received lacewing eggs than in the untreated plots. We are unsure why this was the case.

Feedback from several growers and PCAs indicated a great interest in the mechanical release technology, but with a clear need to demonstrate lacewing survivorship in the field once egg distribution was made. For example, several of our cooperators were already purchasing lacewing eggs in large quantities and distributing them using their own equipment, but most PCAs complained they could not find lacewing larvae after their egg distribution. In our field test at Tanimura and Antle, we were able to show cooperating PCAs lacewing larvae in plants which received the egg treatments using our experimental equipment, which had been carefully tested not to harm the eggs during delivery (Wunderlich, 1997). This increased the PCAs confidence in the lacewing release strategy. Organic growers especially, some of whom made repeated applications for lettuce aphid with organically approved pesticides such as Neem without good results, have a need for continuing work on improving biological control methods. We should note, however, that it is extremely difficult to test new potential organic materials in commercial organic fields without risking grower's loss of certification.

e. Summary and Conclusions.

The overall goal of this project was to evaluate the efficacy of augmentative biological control, specifically green lacewing eggs, for lettuce aphid, *Nasonovia ribis-nigri*, in both organic and reduced-risk (IPM) lettuce production systems. We conclude that lacewing egg releases using

the modified liquid distributor can increase the number of subsequent lacewing larvae in a field and reduce the number of apterous *Nasonovia* in those plants that receive eggs over untreated plants, especially when water is used as the egg carrier. However, in our releases, we found that syrphid larvae were also higher in our release plots and therefore we cannot attribute the reduction in *Nasonovia* in those plots to the lacewing alone.

This work made the following accomplishments:

We improved the mechanical liquid delivery system originally designed for green lacewing egg releases in grapes and modified it for efficient lacewing egg releases in row-crops.

We tested six liquid carriers for effects on green lacewing egg adhesion and hatch in laboratory bioassays and identified two carriers, C-DrySet™ (Cerestar USA, Inc., Hammond, Indiana) and Nu-Film P™ (Miller Chemical and Fertilizer Corp., Hanover, Penn.) for further field testing.

We conducted five green lacewing egg releases in two field sites using the mechanical delivery system and compared egg hatch and survivorship after release in water, C-DrySet™ and Nu-Film P™ as carriers to an untreated control.

We concluded from the results of our monitoring after field releases that the carriers C-DrySet™ and Nu-Film P™ did not improve lacewing survivorship in the field over water as the egg carrier.

We found that, relative to an untreated control, *Nasonovia* populations decreased in plots that received lacewing eggs using water as the carrier and that lacewing larvae and syrphid larvae increased in those plots which received lacewing eggs. The syrphid larvae, with the lacewing larvae, most likely contributed to the *Nasonovia* population decline.

Appendix 1. Laboratory Results.

Table 1. Carriers screened in laboratory bioassays.

Carrier Name	Manufacturer	1x rate	Observed adhesion of eggs to plate.	Remarks
C DrySet	Cerestar USA, Inc. Hammond, Indiana.	1%	Good.	Converted corn dextrin. Derived by dry-heating unmodified starch in the presence of acid (HCl). GMO-free corn will be available in future. Need to heat while mixing for better stickiness.
C DryGel	Cerestar USA, Inc. Hammond, Indiana.	1%	Fair.	Dried glucose syrup obtained by acid conversion of common corn starch.
Corn calcium	Peaceful Valley Farm Supply Grass Valley, Ca.	0.2%	None.	According to Peaceful Valley, ingredients are: corn syrup, sugar cane molasses and barley malt extract. Corn syrup is obtained through a hydrolysis method where corn starch and water are put with HCl and enzymes and then neutralized with NaOH. No "calcium" per se. Difficult to mix.
Nu-film P	Miller Chemical and Fertilizer Corp. Hanover, Penn.	0.05%	Fair-good.	OMRI approved.
Therm X70	Cellu-con, Inc. Strathmore, Ca.	0.05%	None.	20% saponin from Yucca, 80% plant extract.
Agar solution		0.05%	None.	Needs to be autoclaved to dissolve.

Appendix 1. Laboratory Results.

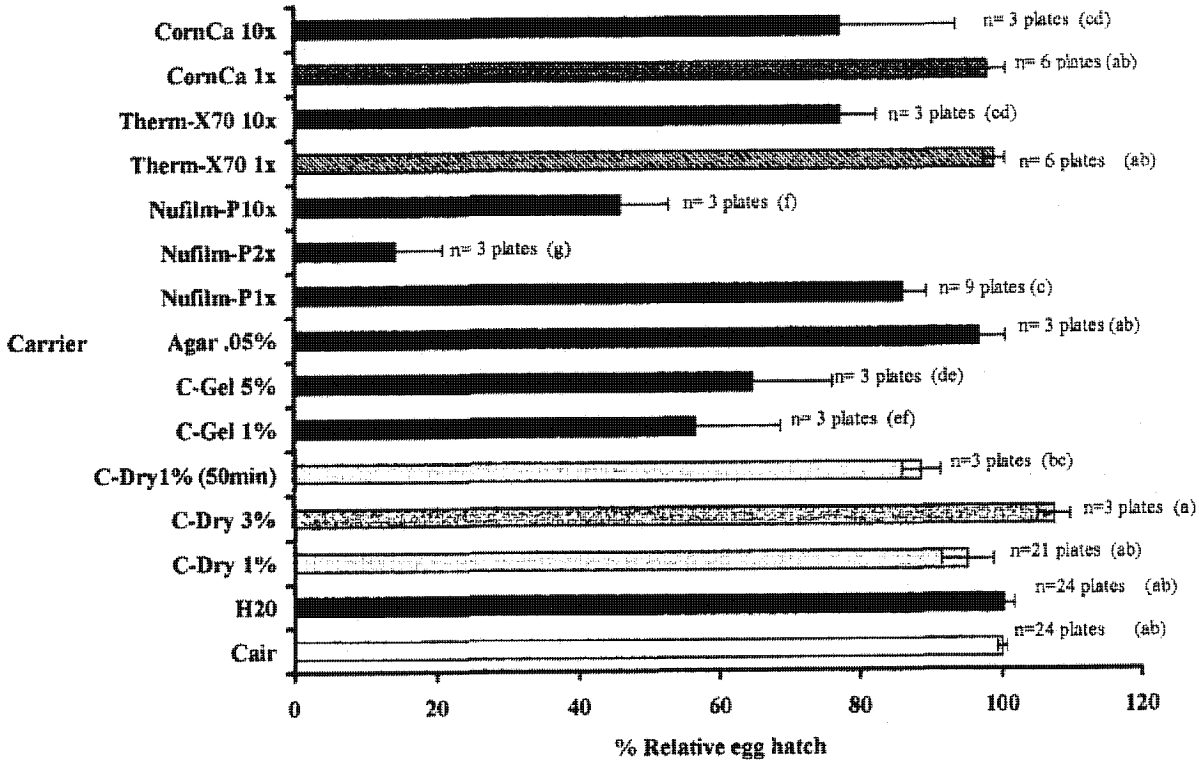

Figure 1. Percent relative lacewing egg hatch after immersion in various liquid carriers during laboratory bioassays. Bars indicate standard errors. The number of replicated plates is indicated by n. Different letters indicate significant differences in treatments based on Fisher's LSD at p = 0.05.

Appendix 2. Field results from Coke Ranch releases.

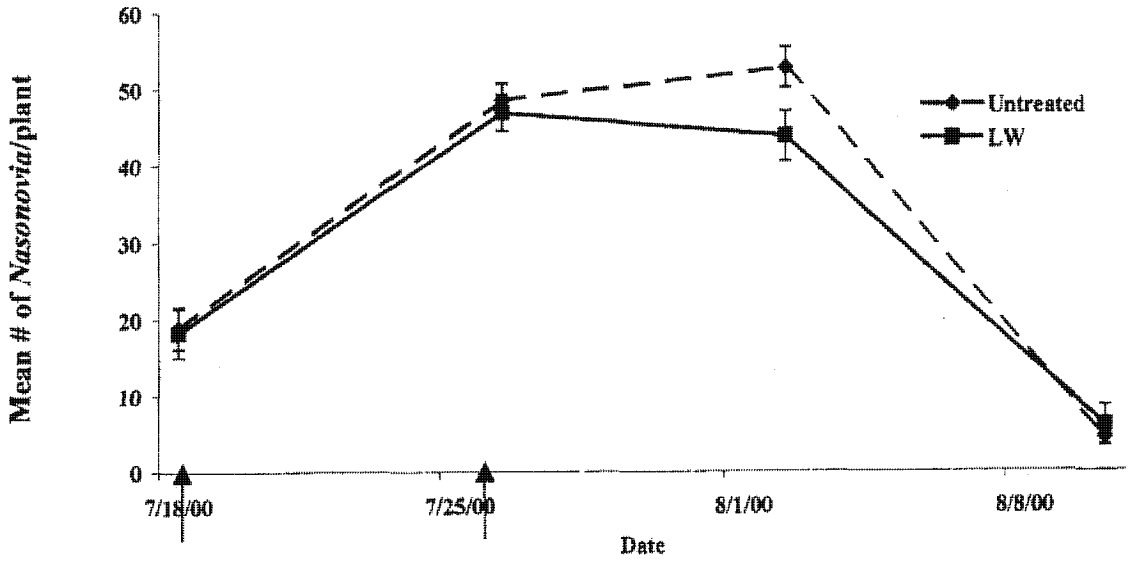

Figure 2.1 Mean number of *Nasonovia*/plant in untreated and lacewing egg release plots at Coke Ranch. Bars indicate standard errors. Arrows indicate release dates.

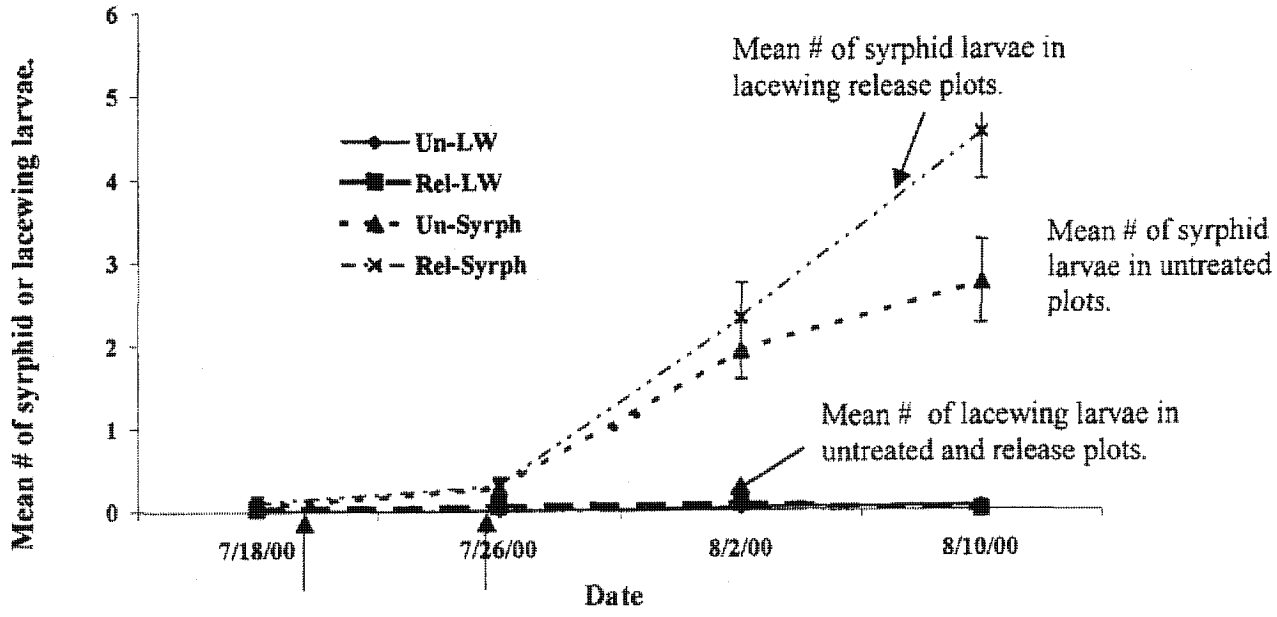

Figure 2.2 Mean number of syrphid and lacewing larvae in untreated and lacewing release plots at Coke Ranch. Bars indicate standard errors. Arrows indicate release dates.

Appendix 3. Field results from T&A releases.

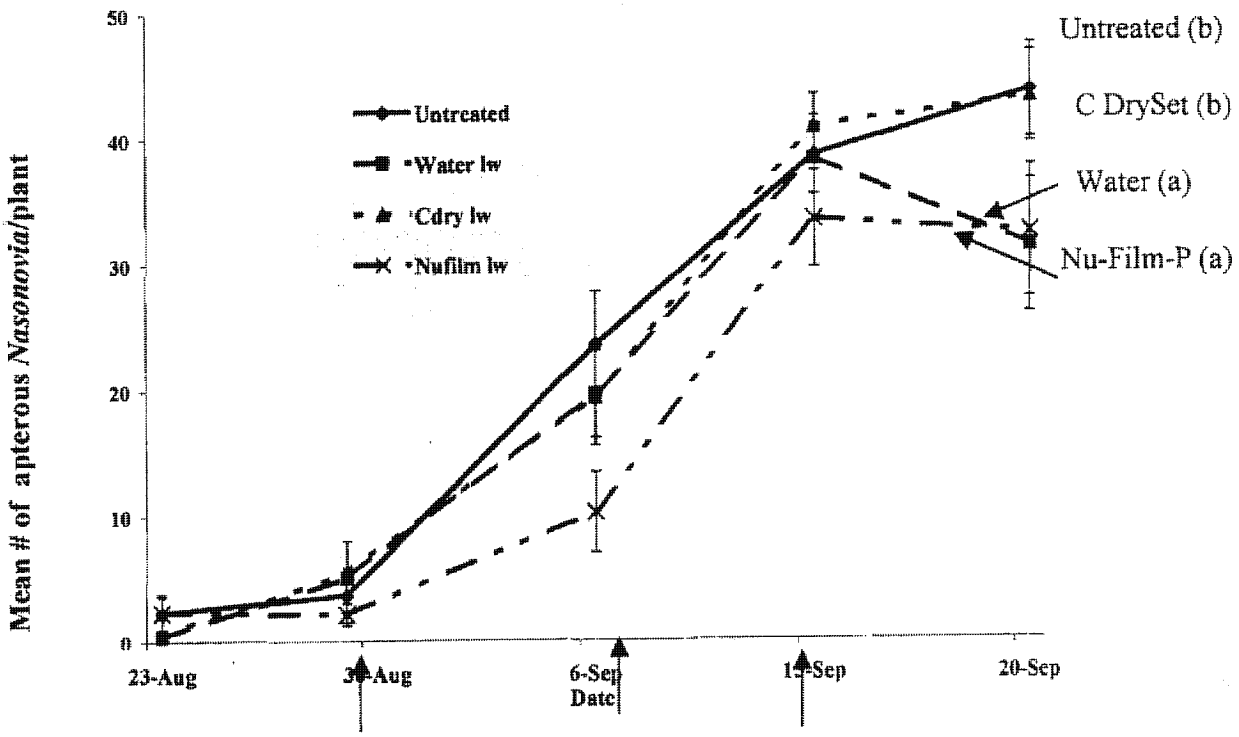

Figure 3.1 Mean number of apterous *Nasonovia* in untreated plots and plots that received lacewing eggs in various carriers. Bars indicate standard errors. Arrows indicate release dates. Different letters indicate significant differences in treatments based on Fisher's LSD at p = 0.1.

Appendix 3. Field results from T&A releases.

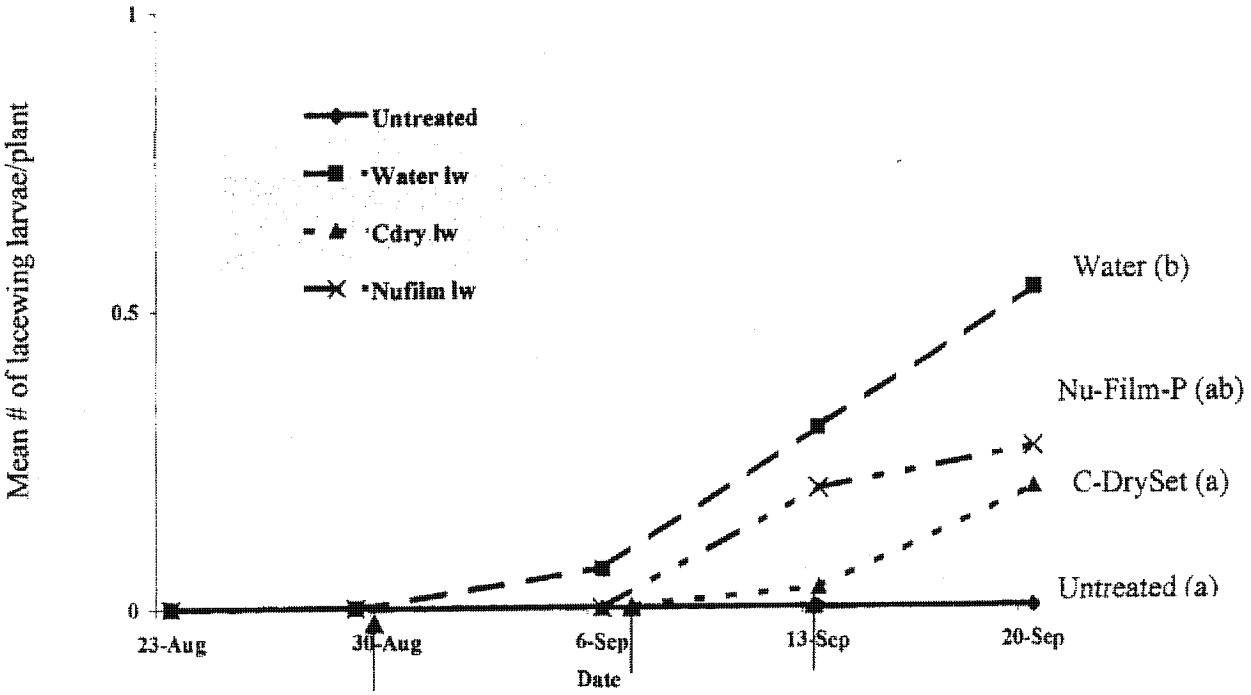

Figure 3.2 Mean number of lacewing larvae in untreated plots and plots which received lacewing eggs in various carriers. Bars indicate standard errors. Arrows indicate release dates. . Different letters indicate significant differences in treatments based on Fisher's LSD at p = 0.05.

Appendix 4: References Cited.

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ATTACHMENT 22

SUSTAIN®

Evaluation of Tank-Mix Combinations of Prefar 4-E Selective Herbicide in Melons (Gowan Trial Number: PRE-05-02-T1)

EVALUATION OF TANK-MIX COMBINATIONS OF PREFAR 4-E SELECTIVE HERBICIDE IN MELONS

GOWAN TRIAL NUMBER: PRE-05-02-T1

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EVALUATION OF TANK-MIX COMBINATIONS OF PREFAR 4-E SELECTIVE HERBICIDE IN MELONS

GOWAN TRIAL NUMBER: PRE-05-02-T1

Introduction:

In the low desert region of California, weed control in melons often requires extensive hand weeding. This trial was conducted to determine whether pre-emergent reduced rate tank-mix combinations provide weed control equal to, or better than high rates of Prefar alone.

Materials and Methods:

Site location:	Schaffner Farms Holtville, CA
Host Crop:	Cantaloupe
Variety:	Easy Rider
Developmental stage:	Pre-emergent
Planting date:	February 3, 2005
Soil type:	Silty clay loam
Cultural practices:	Furrow irrigated 80" raised beds with seed-line located on south facing side of mid-bed trench
Experimental Design:	Randomized complete block
Replication and Units:	4 replicates of 25' x 20" band over seed-line
Application:	
Equipment:	Pressurized CO_2 backpack sprayer (30 psi) 2 nozzles (Teejet 8002vs)
Rate:	50 gallons / acre
Date:	February 10, 2005 (10:00-10:30 am)

Treatments:

1. Prefar 4-E[1] @ 6 qts / acre
2. Prefar 4-E @ 3 qts / acre
3. Prefar 4-E @ 3 qts / acre + Agri-Dex[2] @ 32 oz / 50 gal
4. Prefar 4-E @ 3 qts / acre + Sustain[3] @ 8 oz / 50 gal
5. Prefar 4-E @ 3 qts / acre + Coax[4] @ 120 oz / 50 gal
6. Untreated

[1] Gowan Company
Bensulide S-(O,O-diisopropyl phosphorodithioate) ester of
N-(2-mercaptoethyl) benzenesulfonimide.....40.0%
EPA Reg. No. 10163-200 EPA Est. No. 66196-CA-1
Lot No. AFAK4007

[2] Helena Chemical Company
Paraffin base petroleum oil
Polyol fatty acid esters
Polyethoxylated derivatives thereof.....99.0%
CASN 09204 CA. Reg. No. 5905-50094-AA
Lot No. KC310775

[3] Miller Chemical & Fertilizer Corporation
Pinene (terpene) Polymers, petrolatum, a-(p-Dodecylphenyl) –
Omega-hydroxypoly (oxyethylene).....100%
EPA Reg. No. Exempt Epa Est. No. 72-PA-1
CA Reg. No. 72-50015-AA

[4] CCT Corporation
Specially prepared Pharmamedia® cottonseed flour, disaccharide and
vegetable lipid oil.....35.0%
CA Reg. No. 52338-50002-AA

Evaluations:

Evaluations were conducted at 5, 10, and 15 days post-emergence of melon crop. Within each plot, four 12" x 12" areas, centered on the seed-line, were examined (Figure 1). The number of germinated melon plants, and the number of weeds, by species, was recorded (Tables 1-3, Figures 2-3).

Statistical analysis:

Raw data from the final evaluation was analyzed according to the Least Significant Difference Test (P=0.05) using the MSTAT-C program (Table 4). In addition, the percent control was determined from the treatment means using the following formula:

$$100 \times \left(\frac{\text{\#weeds in untreated plot - \#weeds in treated plot}}{\text{\#weeds in untreated plot}}\right.$$

Results and Discussion:

The test products were applied on February 10, 2005. Due to immanent rainfall, the field was not immediately irrigated. Rainfall began within 2 hours of application, with 0.42" of rain occurring within 48 hours (see appendix 1 for complete weather data).

Evaluations were conducted on March 7, 12, and 17, 2005. Although melon germination was variable, both within the test plots and in the surrounding untreated area, no significant differences (p=0.05) were found between treatments.

Nettleleaf goosefoot (*Chenopodium murale*) was, by far, the most abundant weed, and found within each plot. Less common weeds, not occurring in numbers allowing analysis, were little mallow (*Malva parviflora*), silversheath knotweed (*Polygonum argyrocoleon*), barnyardgrass (*Echinochloa crus-galli*), London rocket (*Sisymbrium irio*), California burclover (*Medicago polymorpha*), and annual sowthistle (*Sonchus oleraceus*).

The 6 quart per acre rate of Prefar 4-E was the most effective treatment, providing 68.4% control, compared to the untreated plots. Prefar 4-E @ 3 qts / acre + Sustain @ 8 oz / 50 gal and Prefar 4-E @ 3 qts / acre + Agri-Dex @ 32 oz / 50 gal were similarly effective (p=0.05) with 46.8 and 38.3% control, respectively. The 3 qt / acre rate of Prefar 4-E provided 34% control. Prefar 4-E @ 3 qts / acre + Coax @ 120 oz / 50 gal gave 31.9% control, but was not statistically different (p=0.05) from the untreated plots.

In conclusion, the addition of Sustain and Agri-Dex improved the performance of the 3 quart per acre rate of Prefar 4-E to a level similar to that of the 6 quart per acre rate of Prefar 4-E alone, whereas the addition of Coax did not improve performance.

TABLES

Table 1. Number of Germinated Melon Plants and Weeds, by Species, on March 7, 2005

Trt	Rep	#melons	#goosefoot	#malva	#knotweed	#barnyardgrass	#rocket	#clover	#sowthistle	#total weeds
1	1	10	2	0	0	0	1	0	0	3
1	2	10	2	1	0	0	0	0	0	3
1	3	7	7	1	0	0	0	0	0	8
1	4	11	4	0	0	0	0	0	0	4
2	1	8	8	0	1	1	0	0	0	10
2	2	10	1	0	0	0	0	0	0	1
2	3	6	7	0	0	0	1	0	0	8
2	4	10	6	0	0	0	0	0	0	6
3	1	7	6	0	0	0	0	0	0	6
3	2	8	7	0	0	0	0	0	0	7
3	3	5	8	0	0	0	0	0	0	8
3	4	8	4	0	0	0	0	0	0	4
4	1	8	6	0	0	0	0	0	0	6
4	2	9	4	0	0	0	0	1	0	5
4	3	12	5	1	0	0	0	0	0	6
4	4	4	5	0	0	0	0	0	0	5
5	1	8	3	2	0	0	0	0	0	5
5	2	12	3	0	0	0	0	0	0	3
5	3	11	3	0	0	0	0	0	0	3
5	4	7	1	0	0	0	0	1	0	2
6	1	10	15	1	0	0	0	0	0	16
6	2	10	7	0	0	0	0	1	0	8
6	3	7	6	3	0	0	0	0	0	9
6	4	10	11	0	0	0	0	0	0	11

Table 2. Number of Germinated Melon Plants and Weeds, by Species, on March 12, 2005

Trt	Rep	#melons	#goosefoot	#malva	#knotweed	#barnyardgrass	#rocket	#clover	#sowthistle	#total weeds
1	1	7	5	1	0	0	0	0	0	6
1	2	7	2	0	0	0	0	0	0	2
1	3	7	7	1	0	0	1	0	0	9
1	4	8	7	0	0	0	0	0	0	7
2	1	6	4	0	0	1	0	1	0	6
2	2	6	2	1	0	0	0	0	0	3
2	3	6	6	0	0	0	1	0	0	7
2	4	8	5	0	0	0	0	0	0	5
3	1	8	7	0	0	0	1	0	0	8
3	2	7	10	0	0	0	0	0	0	10
3	3	4	10	0	0	0	0	0	0	10
3	4	6	3	0	0	0	0	0	0	3
4	1	7	6	0	0	0	0	0	0	6
4	2	6	4	1	0	0	0	0	0	5
4	3	9	8	1	0	0	0	0	0	9
4	4	3	5	0	0	0	0	0	0	5
5	1	7	3	1	0	0	1	0	0	5
5	2	10	5	0	0	0	0	2	0	7
5	3	9	3	2	0	0	0	0	0	5
5	4	5	4	0	0	0	0	0	1	5
6	1	8	14	1	0	0	0	0	1	16
6	2	8	10	0	0	0	0	0	0	10
6	3	5	13	2	0	0	1	0	0	16
6	4	6	14	0	0	0	0	0	0	14

Table 3. Number of Germinated Melon Plants and Weeds, by Species, on March 17, 2005

Trt	Rep	#melons	#goosefoot	#malva	#knotweed	#barnyardgrass	#rocket	#clover	#sowthistle	#total weeds
1	1	5	3	0	0	0	0	0	0	3
1	2	9	5	0	0	0	0	0	0	5
1	3	5	4	0	0	0	0	0	0	4
1	4	8	3	0	0	0	0	0	0	3
2	1	5	6	0	0	0	0	0	0	6
2	2	8	9	0	0	0	0	0	0	9
2	3	6	6	0	0	0	1	0	0	7
2	4	8	8	0	0	0	1	0	0	9
3	1	5	7	0	0	0	1	0	0	8
3	2	8	6	1	0	0	1	0	0	8
3	3	4	8	0	0	0	0	0	0	8
3	4	5	5	0	0	0	0	0	0	5
4	1	5	5	1	0	0	0	0	0	6
4	2	7	2	1	0	0	0	0	0	3
4	3	8	12	0	0	0	0	0	0	12
4	4	5	4	0	0	0	0	0	0	4
5	1	5	7	1	0	0	1	0	0	9
5	2	8	4	0	0	0	0	0	1	5
5	3	8	6	0	1	0	0	1	0	8
5	4	4	8	0	0	0	0	1	1	10
6	1	8	12	0	0	0	0	0	0	12
6	2	7	11	0	0	0	0	0	0	11
6	3	7	7	2	0	0	0	0	0	9
6	4	6	15	0	0	0	0	0	0	15

Table 4. Number of Germinated Melon Plants and Number of Weeds on March 17, 2005

Treatment	# Melons	# Weeds
Prefar 4-E @ 6 qts / acre	6.75 a[1]	3.75 c
Prefar 4-E @ 3 qts / acre	6.75 a	7.75 b
Prefar 4-E @ 3 qts / acre + Agri-Dex @ 32 oz / 50 gal	5.50 a	7.25 bc
Prefar 4-E @ 3 qts / acre + Sustain @ 8 oz / 50 gal	6.25 a	6.25 bc
Prefar 4-E @ 3 qts / acre + Coax @ 120 oz / 50 gal	6.25 a	8.00 ab
Untreated	7.00 a	11.75 a

[1] Means within the same column followed by the same letter do not differ as determined by the Least Significant Difference test ($P \leq 0.05$).

FIGURES

Figure 1. 12" x 12" sub-sample with weeds and germinated melon plants

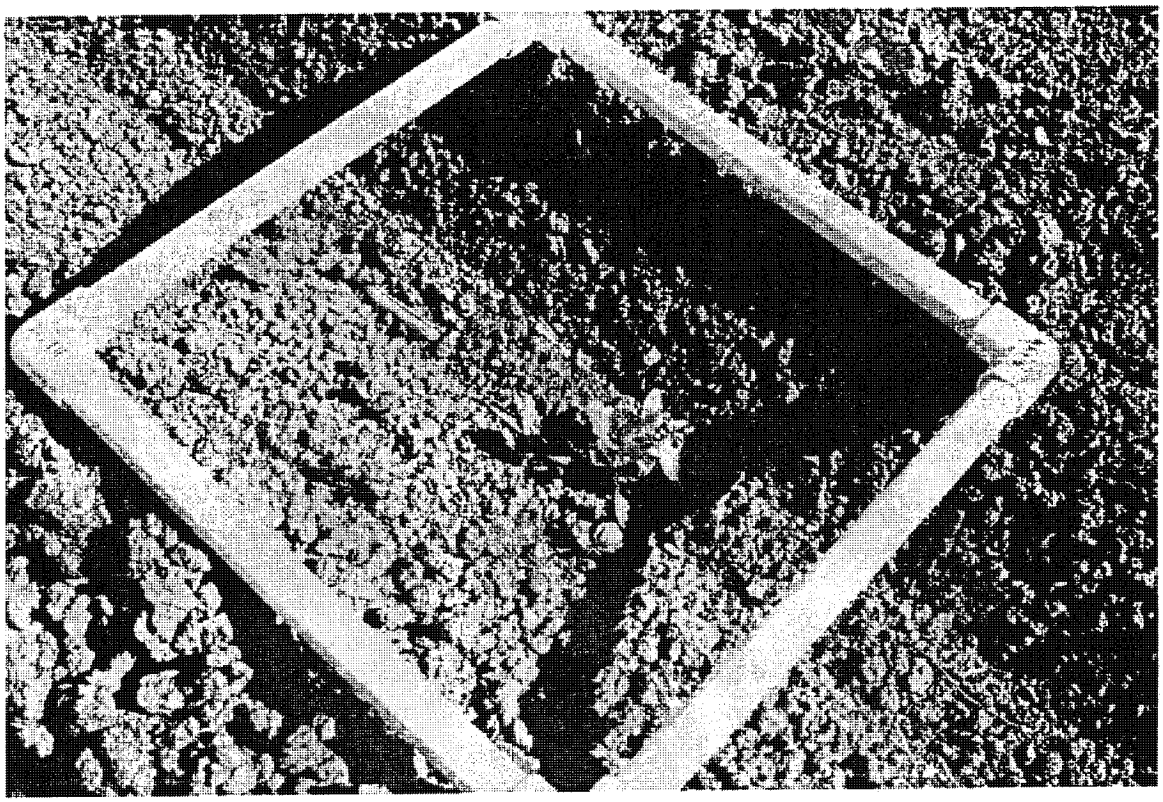


Figure 2. Total Number of Weeds on March 7, 12, and 17, 2005

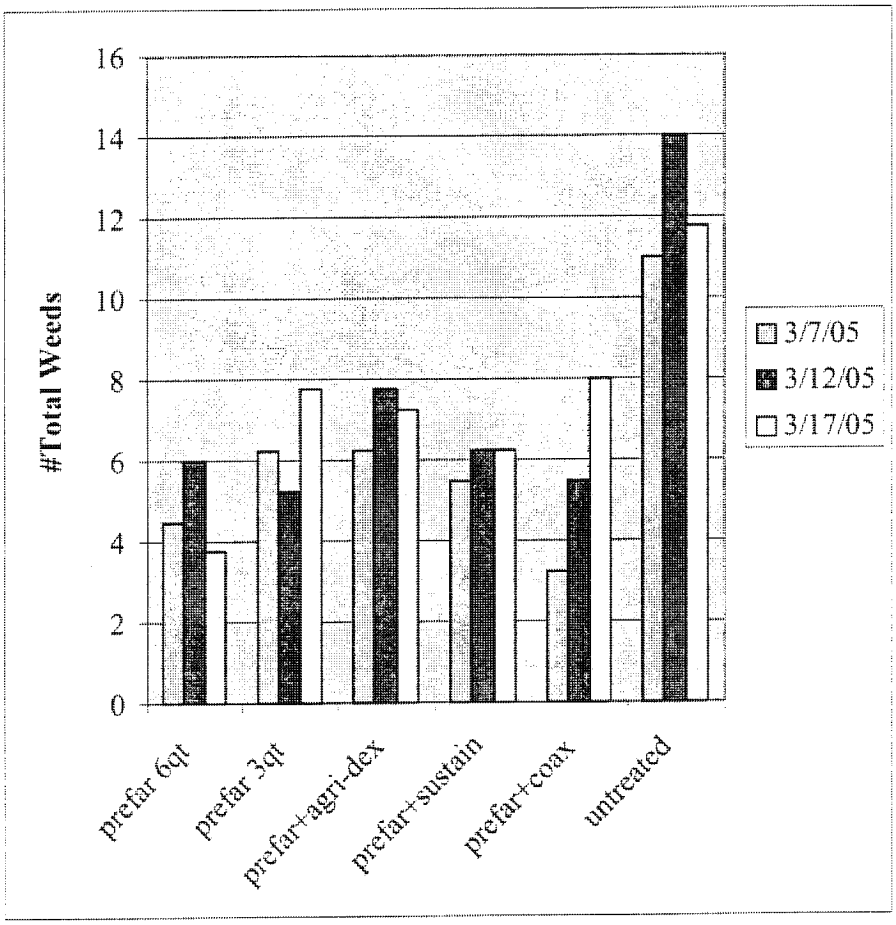
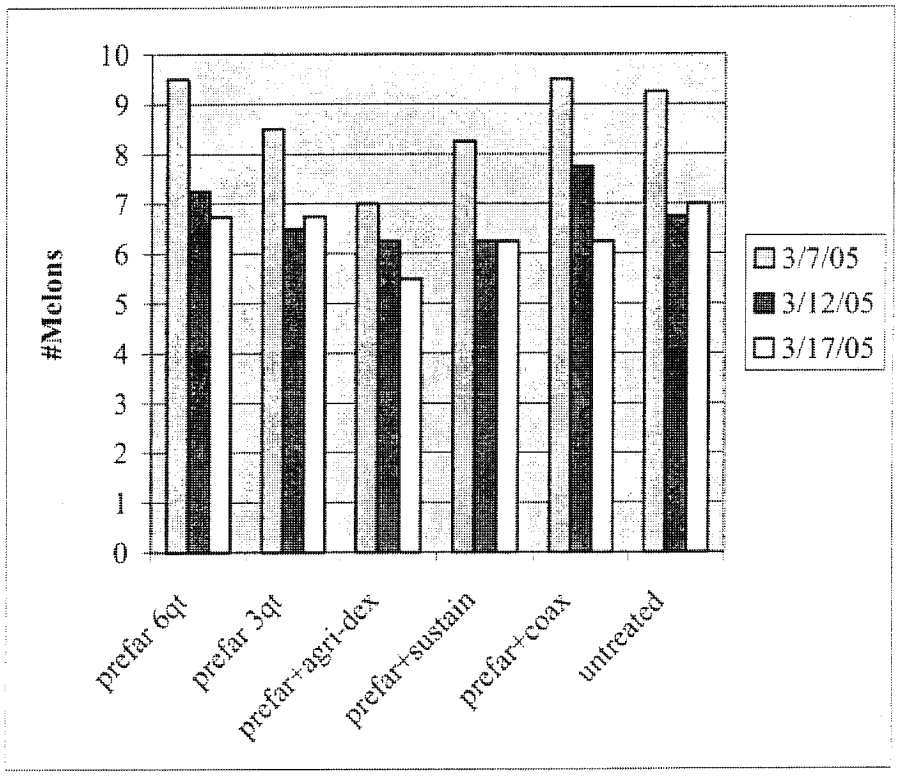


Figure 3. Melon Germination on March 7, 12, and 17, 2005



APPENDIX

Appendix 1. Weather Data Collected from CIMIS #87, Meloland in Holtville, CA (Approximately 2 Miles from the Test Site)

DATE	OBS TIME	PRECIP AMOUNT (IN)	AIR TEMP (F) MAX	AIR TEMP (F) MIN	WIND DD	WIND SS	SOIL TEMP (F) MAX	SOIL TEMP (F) MIN	RELATIVE HUMIDITY MAX	RELATIVE HUMIDITY MIN
02-10-2005	23:59	0.04	68	48	W	3	58	56	90.6	43.2
02-11-2005	23:59	0.32	60	54	SE	4	58	58	96.3	89.4
02-12-2005	23:59	0.06	70	52	SW	4	59	58	96.6	62.0
02-13-2005	23:59	0.01	72	47	SE	3	60	58	95.2	57.5
02-14-2005	23:59	0.00	69	52	SE	3	60	59	94.1	65.4
02-15-2005	23:59	0.00	64	51	NW	3	60	60	96.1	77.9
02-16-2005	23:59	0.00	71	45	S	3	60	58	96.5	54.2
02-17-2005	23:59	0.00	67	50	W	3	60	59	93.3	68.8
02-18-2005	23:59	0.26	64	50	SE	4	60	59	96.1	83.5
02-19-2005	23:59	0.67	71	50	SE	5	60	59	96.5	72.3
02-20-2005	23:59	0.00	70	51	SE	5	61	59	96.6	64.5
02-21-2005	23:59	0.00	66	49	SE	7	61	60	95.9	70.4
02-22-2005	23:59	0.00	68	47	E	3	61	59	96.6	59.5
02-23-2005	23:59	0.00	65	48	NW	5	60	60	95.7	69.0
02-24-2005	23:59	0.00	70	49	SE	3	60	59	95.8	49.0
02-25-2005	23:59	0.00	72	45	SW	3	60	58	89.5	44.0
02-26-2005	23:59	0.00	74	47	W	5	60	59	84.7	42.1
02-27-2005	23:59	0.00	75	48	S	3	61	59	83.5	38.0
02-28-2005	23:59	0.00	75	47	W	3	61	59	85.2	37.2
03-01-2005	23:59	0.00	77	48	W	3	62	59	83.3	37.2
03-02-2005	23:59	0.00	76	50	W	7	63	60	75.9	33.0
03-03-2005	23:59	0.00	76	52	W	6	64	60	78.5	31.4
03-04-2005	23:59	0.00	75	51	W	9	63	61	73.4	36.5
03-05-2005	23:59	0.17	71	49	NW	5	63	60	90.5	44.1
03-06-2005	23:59	0.00	74	49	SW	3	64	60	90.8	47.5
03-07-2005	23:59	0.00	79	51	S	3	65	61	88.9	33.4
03-08-2005	23:59	0.00	87	47	W	3	65	61	82.2	26.1
03-09-2005	23:59	0.00	88	51	S	3	66	62	74.5	18.2

Appendix 1, continued. Weather Data Collected from CIMIS #87, Meloland in Holtville, CA (Approximately 2 Miles from the Test Site)

DATE	OBS TIME	PRECIP AMOUNT (IN)	AIR TEMP (F) MAX	AIR TEMP (F) MIN	WIND DD	WIND SS	SOIL TEMP (F) MAX	SOIL TEMP (F) MIN	RELATIVE HUMIDITY MAX	RELATIVE HUMIDITY MIN
03-10-2005	23:59	0.00	89	52	SW	2	66	62	72.8	22.5
03-11-2005	23:59	0.00	91	55	SW	3	67	63	73.4	22.1
03-12-2005	23:59	0.00	91	56	W	5	68	63	67.2	23.9
03-13-2005	23:59	0.00	85	55	W	9	68	65	78.7	28.2
03-14-2005	23:59	0.00	78	49	N	8	66	64	72.0	13.5
03-15-2005	23:59	0.00	74	40	NW	4	65	61	55.7	9.1
03-16-2005	23:59	0.00	76	40	S	3	66	60	54.7	17.6
03-17-2005	23:59	0.00	82	46	SE	7	65	61	62.6	17.8
03-18-2005	23:59	0.01	73	55	W	7	64	61	75.8	25.6
03-19-2005	23:59	0.01	75	54	SW	6	64	60	83.9	47.6
03-20-2005	23:59	0.00	77	56	W	11	63	61	62.9	26.6
03-21-2005	23:59	0.00	79	53	W	7	64	60	63.1	19.7

ATTACHMENT 23

SUSTAIN®
Terpene Polymer

Rice Trial Ratings.
South Texas Ag Research Coastal.
(June 2004 Fax Transmission from Larry Emerson)

SOUTH TEXAS AG RESEARCH

Coastal

Brookshire, Texas
FAX: 281-934-2305
Phone: 281-934-2312
E-mail: mrson@txucom.net

FAX Transmission

Date: June 24, 2004
To: Mike Fiery
Company: **Miller Chemical Co.**

FAX No.: **717-632-4581**
From: Larry Emerson

RE: **rice trial ratings**

Mike,

Four pages with my ratings will follow this page. I plan to take one more rating this week if the rain will ever stop. The last ratings on June 11 seem to show that the signal grass control is holding up better in the "with polymer" treatments than without.

I don't feel like you got a real good shake on this trial and want to offer to repeat the trial or a similar trial next year for half price. The weather has been so unusual this year.

Miller Chemical Command Rice Herbicide Trial

Planted: 04/22/2004

Treatments Applied: 04/23/2004

Rating Date: 6/11/04

Treatment 01: UTC	Plots: 101, 204, 302, 403
Treatment 02: 0.3 # ai/ac Command	Plots: 102, 201, 305, 404
Treatment 03: 0.3 # ai/ac Command + Polymer	Plots: 103, 205, 304, 401
Treatment 04: 0.4 # ai/ac Command	Plots: 104, 202, 303, 405
Treatment 05: 0.3 # ai/ac Command + Polymer	Plots: 105, 203, 301, 402

Plot No.	Rice Injury	Barnyard Grass	Signal Grass	Sprangletop	Stand Reduction
101	0	0	0	0	0
102	5	100	50	100	0
103	10	100	80	100	10
104	30	100	95	100	50
105	20	100	95	100	50
201	20 ~~10~~ ~~15~~	100	50 ~~40~~	100	10
202	~~30~~ 35	100	75 ~~80~~	100	20
203	~~40~~ 45	100	95	100	20
204	0	0	0	0	0
205	15	100	60	100	10
301	40	95	95	100	30
302	0	0	0	0	0
303	50	100	90	100	50
304	15	95	85 ~~75~~	100	10
305	10	95	75 ~~65~~	100	5
401	15	100	70	100	5
402	55	100	80	100	40
403	0	0	0	0	0
404	10	100	60 ~~40~~	100	10
405	30	100	90	100	30

Miller Chemical Command Rice Herbicide Trial

Planted: 04/22/2004
Treatments Applied: 04/23/2004
Rating Date: 5/28/04

Treatment 01: UTC — Plots: 101, 204, 302, 403
Treatment 02: 0.3 # ai/ac Command — Plots: 102, 201, 305, 404
Treatment 03: 0.3 # ai/ac Command + Polymer — Plots: 103, 205, 304, 401
Treatment 04: 0.4 # ai/ac Command — Plots: 104, 202, 303, 405
Treatment 05: 0.3 # ai/ac Command + Polymer — Plots: 105, 203, 301, 402

Plot No.	Rice Injury	Barnyard Grass	Signal Grass	Sprangletop	Stand Reduction
101	0	0	0	0	0
102	25	100	80	100	0
103	30	100	90	100	5
104	50	100	100	100	40
105	50	100	100	100	40
201	30	100	85	100	5
202	50	100	95	100	20
203	55 60	100	100	100	20
204	0	0	0	0	0
205	40	100	80	100	5
301	50	100	100	100	15
302	0	0	0	0	0
303	60	100	95	100	50
304	50	100	85	100	15
305	50	100	80	100	15
401	35 30	100	90	100	10
402	60	100	95	100	50
403	0	0	0	0	0
404	30	100	85	100	10
405	50	100	100	100	40

Miller Chemical Command Rice Herbicide Trial

Planted: 04/22/2004
Treatments Applied: 04/23/2004
Rating Date: 5/15/04

Treatment 01: UTC — Plots: 101, 204, 302, 403
Treatment 02: 0.3 # ai/ac Command — Plots: 102, 201, 305, 404
Treatment 03: 0.3 # ai/ac Command + Polymer — Plots: 103, 205, 304, 401
Treatment 04: 0.4 # ai/ac Command — Plots: 104, 202, 303, 405
Treatment 05: 0.3 # ai/ac Command + Polymer — Plots: 105, 203, 301, 402

Plot No.	Rice Injury	Barnyard Grass	Signal Grass	Sprangletop	Redvine Sheath
101	0	0	0	0	0
102	40	100	100	100	0
103	50	100	100	100	5
104	60	100	100	100	25
105	70	100	100	100	20
201	40	100	100	100	0
202	70	100	100	100	25
203	80	100	100	100	30
204	0	50	40	100	0
205	50	100	100	100	10
301	50	100	100	100	20
302	0	30	30	100	0
303	75	100	100	100	40
304	50	100	100	100	10
305	60	100	100	100	20
401	40	100	100	100	15
402	60	100	100	100	50
403	0	60	50	100	0
404	40	100	100	100	10
405	80	100	100	100	60

Miller Chemical Command Rice Herbicide Trial

Planted: 04/22/2004
Treatments Applied: 04/23/2004
Rating Date: 5/05/04

Treatment 01: UTC — Plots: 101, 204, 302, 403
Treatment 02: 0.3 # ai/ac Command — Plots: 102, 201, 305, 404
Treatment 03: 0.3 # ai/ac Command + Polymer — Plots: 103, 205, 304, 401
Treatment 04: 0.4 # ai/ac Command — Plots: 104, 202, 303, 405
Treatment 05: 0.3 # ai/ac Command + Polymer — Plots: 105, 203, 301, 402

Plot No.	Chlorosis Rice Injury	Barnyard Grass	Signal Grass	Sprangletop	Crab
101	0	0	0	0	0
102	80	100	100	100	100
103	80	100	100	100	100
104	98	100	100	100	100
105	98	100	100	100	100
201	85	100	100	100	100
202	90	100	100	100	100
203	95	100	100	100	100
204	10	95	90	95	95
205	98	100	100	100	100
301	90	100	100	100	100
302	5	95	90	95	95
303	95	100	100	100	100
304	80	100	100	100	100
305	85	100	100	100	100
401	85	100	100	100	100
402	90	100	100	100	100
403	15	90	80	100	100
404	85	100	100	100	100
405	98	100	100	100	100

Rice: 2 leaf
BYG: 2 leaf
SG: 3 leaf
Crab: 3 leaf

Weed control & rice injury in the UTC plots was due to heavy rains moving the Command from the treated plots to the UTC plots. Plot 101 was uphill from any treated plots.

ATTACHMENT 24

SUSTAIN® Trial on Fall Lettuce.
Report from Soil Serve, Yuma, Az,
November 23, 2003

Martha King

From: Tommy Wildermuth [wild.one@mindspring.com]
Sent: Tuesday, December 23, 2003 6:00 PM
To: Mike Fiery; Martha King
Subject: FW: sustain/kerb

This is the Sustain trial I did with Soil Serve here in Yuma. The only thing added by the P.C. A. was that we don't publish the growers name on any works outside of Miller Chemical. So, with that said; I hope this gives some data for other sales.

----- Original Message -----
From: Tommy Wildermuth
To: Mike Williams
Sent: 11/25/03 9:55:01 PM
Subject: sustain/kerb

--- Tommy Wildermuth
--- wild.one@mindspring.com
--- EarthLink: The #1 provider of the Real Internet.

--- Tommy Wildermuth
--- wild.one@mindspring.com
--- EarthLink: The #1 provider of the Real Internet.

11/23/2003

Grower: Salyer Yuma Farm - Dickson Ranch, Bard, CA
Cooperator: Soil Serve - Yuma, AZ
Ground rig, soil applied Kerb on Lettuce

To evaluate if Sustain, soil applied adjuvant will increase efficacy of Kerb pre-emergent herbicide on fall lettuce.

Block #4 - 17 acres
First 19 beds sprayed without any adjuvant (west end of field)
Second 12 beds sprayed with adjuvant, Sustain
Rest of field sprayed without adjuvant (east side of field)
Applied on 10/22/03 at 8:00p.m. Applicator: Soil Serve
First weed counts taken on 11/19/03 by Tommy Wildermuth and Jim Tribby
Weeds present: *London rocket, Nettleleaf goosefoot, Shepherdspurse, Mallow, Foxtail, Sowthistle.*

Results:		**West side**	**Sustain**	**East side**
	#1	5	2	4
	#2	3	2	4
	#3	4	3	4
	#4	4	3	4
	#5	2	4	1
% Higher		**29%**	0	**22%**
Based on 10' samples (.0008034 acres).				
Weeds per acre		**22,402.30**	**17,424**	**21,157.70**

Five random counts were conducted in each of the trial areas. 10' was the standard measure decided upon Lettuce had recently been thinned, and cultivated in the furrows and bed tops. The weeds counted were those emerged since thinning, and were not abated during cultivation. Sizes of weeds ranged from codoled size up to 3-4 true leaves.

11/23/2003

Grower: Salyer Yuma Farm - Dickson Ranch, Bard, CA
Cooperator: Soil Serve - Yuma, AZ
Ground rig, soil applied Kerb on Lettuce

To evaluate if Sustain, soil applied adjuvant will increase efficacy of Kerb pre-emergent herbicide on fall lettuce.

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First weed counts taken on 11/19/03 by Tommy Wildermuth and Jim Tribby
Weeds present: *London rocket, Nettleleaf goosefoot, Shepherdspurse, Mallow, Foxtail, Sowthistle.*

Results:		**West side**	**Sustain**	**East side**
	#1	5	2	4
	#2	3	2	4
	#3	4	3	4
	#4	4	3	4
	#5	2	4	1
% Higher		**29%**	0	**22%**
Based on 10' samples (.0008034 acres).				
Weeds per acre		**22,402.30**	**17,424**	**21,157.70**

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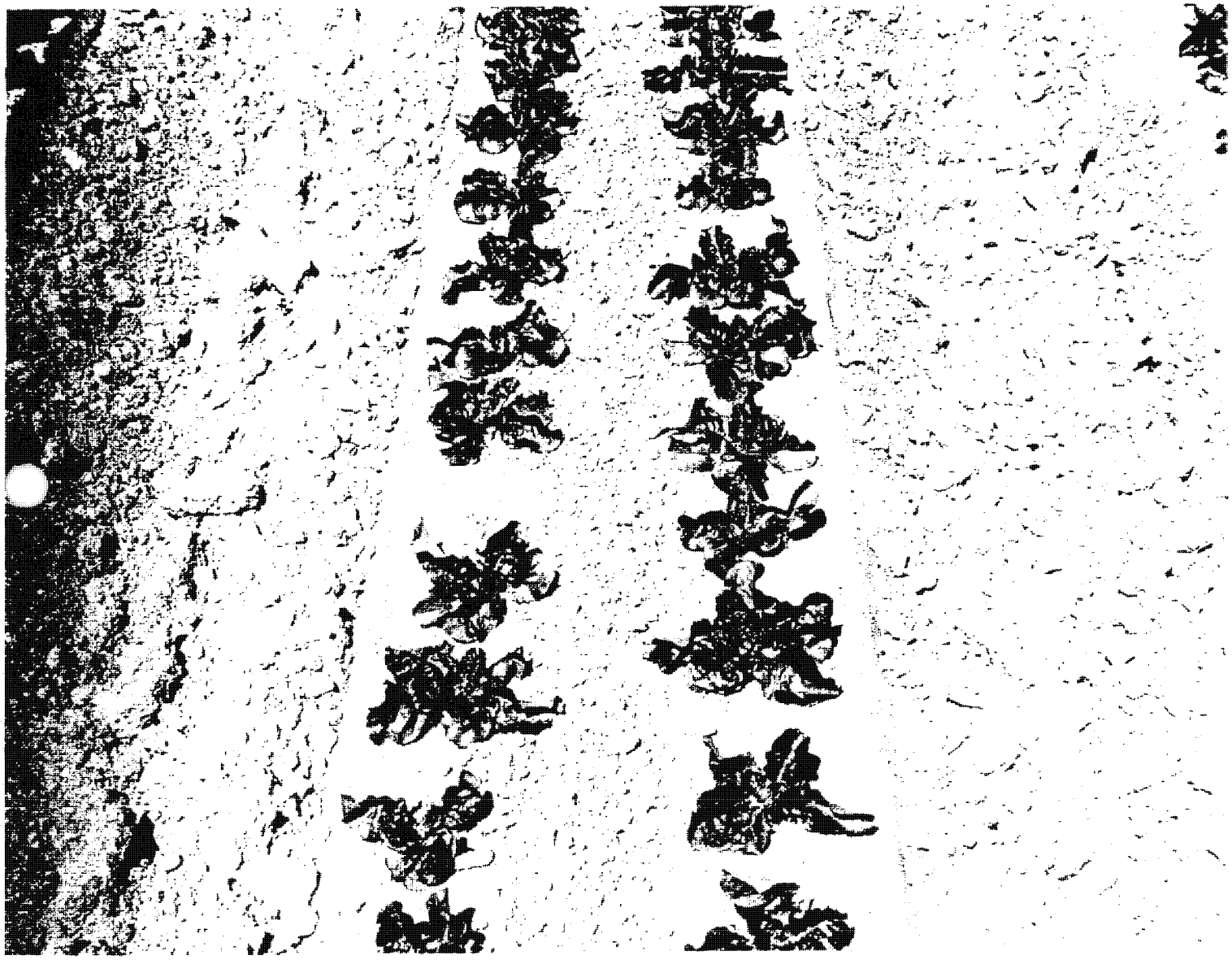

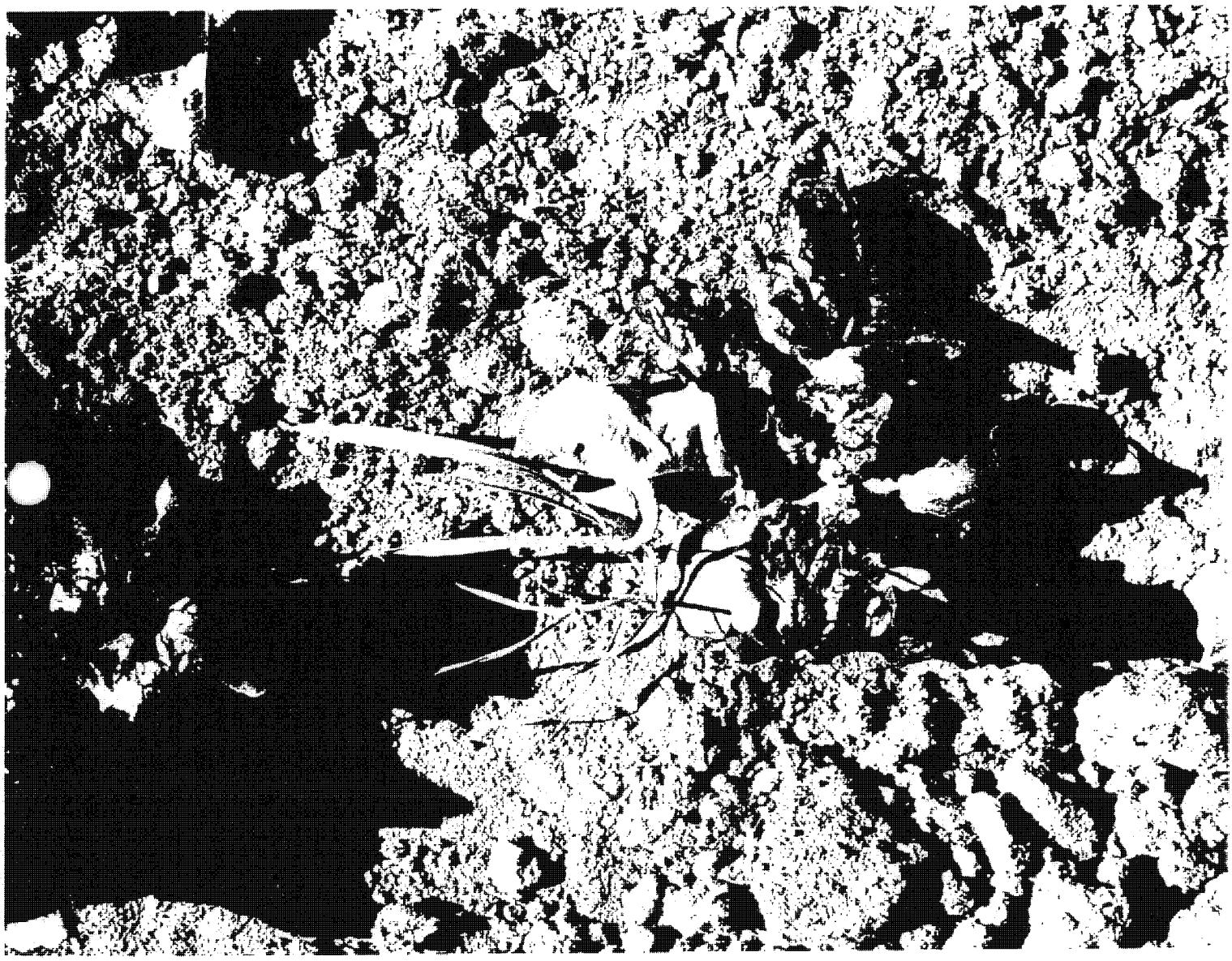

ATTACHMENT 25

SUSTAIN® in Carrots 2003

FMG Ag Products

SUSTAIN IN CARROTS 2003

GROWER: GREG LEE, MOSEL LAKE, WA

CO-OPERATOR: BARRY KIRKWOOD
WILBUR ELLIS, MOSES LAKE

CROP: CARROTS

APPLICATION:

LOROX 2#/AC.
SUSTAIN 1PT./AC
HASTEN 1PT./AC

SOIL WAS VERY SANDY AND IRRIGATION WAS VERY HEAVY.
GROWER NOTED BETTER WEED CONTROL ON TOUGHER SPECIES AND LONGER CONTROL AS WELL.

08/16/2004 (2004WDM02) Standardized Summary Page 1 of

FMC Ag Products Group

Spartan/Sustain - Tobacco

Project Code: Master Prt #: Discipline: H
Trial Number: 2004WDM02 Location: Nathalie, VA
Cooperator: Carr By: W.D. Martin

Trt No	Treatment Name	Form Amt	Fm Ds	Rate	Rate Unit	Appl Code	Method/ Timing	NIOTA 05/21/2004 P NE PT 7 A TRAN	NIOTA 05/21/2004 P ST PT 7 A TRAN	NIOTA 05/21/2004 P SR PT 7 A TRAN	NIOTA 05/28/2004 P NE PT 14 A TRAN
Parameter Code											
Date											
Flag											
Pest Stage											
Eval											
Size/Eval Type											
AN											
Day/Before After											
Activity											
Trt-Eval Interval											
PRM Data Type											
# Subsamples, Dec.											
1	Spartan	4	F	0.1875	lb ai/A	A	PPBCPR	0.0	0.0	0.0	0.0
2	Spartan	4	F	0.1875	lb ai/A	B	PPBCIC	0.0	0.0	0.0	0.0
3	Spartan	4	F	0.1875	lb ai/A	A	PPBCPR	0.0	0.0	0.0	0.0
3	Sustain			1	qt pr/A	A	PPBCPR				
4	Spartan	4	F	0.1875	lb ai/A	B	PPBCIC	0.0	0.0	0.0	0.0
4	Sustain			1	qt pr/A	B	PPBCIC				
5	Spartan	4	F	0.25	lb ai/A	A	PPBCPR	0.0	0.0	0.0	0.0
6	Spartan	4	F	0.25	lb ai/A	B	PPBCIC	0.0	0.0	0.0	0.0
7	Spartan	4	F	0.25	lb ai/A	A	PPBCPR	0.0	0.0	0.0	0.0
7	Sustain			1	qt pr/A	A	PPBCPR				
8	Spartan	4	F	0.25	lb ai/A	B	PPBCIC	0.0	0.0	0.0	0.0
8	Sustain			1	qt pr/A	B	PPBCIC				
9	Spartan	4	F	0.3125	lb ai/A	A	PPBCPR	0.0	0.0	0.0	0.0
10	Spartan	4	F	0.3125	lb ai/A	B	PPBCIC	0.0	0.0	0.0	0.0
11	Spartan	4	F	0.3125	lb ai/A	A	PPBCPR	0.0	0.0	0.0	0.0
11	Sustain			1	qt pr/A	A	PPBCPR				
12	Spartan	4	F	0.3125	lb ai/A	B	PPBCIC	0.0	0.0	0.0	0.0
12	Sustain			1	qt pr/A	B	PPBCIC				
13	Untreated							0.0	0.0	0.0	0.0
SD (P=.05)								0.00	0.00	0.00	0.00
tandard Deviation								0.00	0.00	0.00	0.00
CV								0.0	0.0	0.0	0.0

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Parameter Code							NIOTA	NIOTA	NIOTA	NIOTA
Date							05/21/2004	05/21/2004	05/21/2004	05/28/2004
Flag							P	P	P	P
Pest Stage										
Eval							NE	ST	SR	NE
Size/Eval Type							PT	PT	PT	PT
AN										
Day/Before After							7 A	7 A	7 A	14 A
Activity							TRAN	TRAN	TRAN	TRAN
Trt-Eval Interval										
PRM Data Type										
# Subsamples, Dec.										
Trt No	Treatment Name	Amt	Form Fm Ds	Rate	Rate Unit	Appl Code	Method/ Timing			
Replicate F							0.000	0.000	0.000	0.000
Replicate Prob(F)							1.0000	1.0000	1.0000	1.0000
Treatment F							0.000	0.000	0.000	0.000
Treatment Prob(F)							1.0000	1.0000	1.0000	1.0000

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Pigweed

Trt No	Treatment Name	Form Amt	Fm Ds	Rate	Rate Unit	Appl Code	Method/ Timing	NIOTA 05/28/2004 P	NIOTA 05/28/2004 P	AMARE 05/28/2004 E	NIOTA 06/04/2004 P
	Parameter Code							NIOTA	NIOTA	AMARE	NIOTA
	Date							05/28/2004	05/28/2004	05/28/2004	06/04/2004
	Flag							P	P	E	P
	Pest Stage										
	Eval							ST	SR	CO	ST
	Size/Eval Type							PT	PT	PT	PT
	AN										
	Day/Before After							14 A	14 A	14 A	21 A
	Activity							TRAN	TRAN	TRAN	TRAN
	Trt-Eval Interval										
	PRM Data Type										
	# Subsamples, Dec.										
1	Spartan	4	F	0.1875	lb ai/A	A	PPBCPR	0.0	0.0	82.5	0.0
2	Spartan	4	F	0.1875	lb ai/A	B	PPBCIC	0.0	0.0	83.8	0.0
3	Spartan	4	F	0.1875	lb ai/A	A	PPBCPR	0.0	0.0	88.8	0.0
3	Sustain			1	qt pr/A	A	PPBCPR				
4	Spartan	4	F	0.1875	lb ai/A	B	PPBCIC	0.0	0.0	90.0	0.0
4	Sustain			1	qt pr/A	B	PPBCIC				
5	Spartan	4	F	0.25	lb ai/A	A	PPBCPR	0.0	0.0	90.0	0.0
6	Spartan	4	F	0.25	lb ai/A	B	PPBCIC	0.0	0.0	90.0	0.0
7	Spartan	4	F	0.25	lb ai/A	A	PPBCPR	0.0	0.0	95.0	0.0
7	Sustain			1	qt pr/A	A	PPBCPR				
8	Spartan	4	F	0.25	lb ai/A	B	PPBCIC	0.0	0.0	97.5	0.0
8	Sustain			1	qt pr/A	B	PPBCIC				
9	Spartan	4	F	0.3125	lb ai/A	A	PPBCPR	0.0	0.0	98.8	0.0
10	Spartan	4	F	0.3125	lb ai/A	B	PPBCIC	0.0	0.0	98.8	0.0
11	Spartan	4	F	0.3125	lb ai/A	A	PPBCPR	0.0	0.0	100.0	0.0
11	Sustain			1	qt pr/A	A	PPBCPR				
12	Spartan	4	F	0.3125	lb ai/A	B	PPBCIC	0.0	0.0	100.0	0.0
12	Sustain			1	qt pr/A	B	PPBCIC				
13	Untreated							0.0	0.0	0.0	0.0
	LSD (P=.05)							0.00	0.00	2.45	0.00
	Standard Deviation							0.00	0.00	1.71	0.00
	CV							0.0	0.0	2.0	0.0
	Replicate F							0.000	0.000	2.182	0.000
	Replicate Prob(F)							1.0000	1.0000	0.1071	1.0000
	Treatment F							0.000	0.000	952.855	0.000
	Treatment Prob(F)							1.0000	1.0000	0.0001	1.0000

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Trt No	Treatment Name	Form Amt	Fm Ds	Rate	Rate Unit	Appl Code	Method/ Timing	NIOTA 06/04/2004 P SR PT 21 A TRAN	AMARE 06/04/2004 E CO PT 45 A TRAN	NIOTA 06/28/2004 P ST PT 45 A TRAN	NIOTA 06/28/2004 P SR PT 45 A TRAN
	Parameter Code										
	Date										
	Flag										
	Pest Stage										
	Eval										
	Size/Eval Type										
	AN										
	Day/Before After										
	Activity										
	Trt-Eval Interval										
	PRM Data Type										
	# Subsamples, Dec.										
1	Spartan	4	F	0.1875	lb ai/A	A	PPBCPR	0.0	85.0	0.0	0.0
2	Spartan	4	F	0.1875	lb ai/A	B	PPBCIC	0.0	85.0	0.0	0.0
3	Spartan	4	F	0.1875	lb ai/A	A	PPBCPR	0.0	90.0	0.0	0.0
3	Sustain			1	qt pr/A	A	PPBCPR				
4	Spartan	4	F	0.1875	lb ai/A	B	PPBCIC	0.0	90.0	0.0	0.0
4	Sustain			1	qt pr/A	B	PPBCIC				
5	Spartan	4	F	0.25	lb ai/A	A	PPBCPR	0.0	90.0	0.0	0.0
6	Spartan	4	F	0.25	lb ai/A	B	PPBCIC	0.0	93.8	0.0	0.0
7	Spartan	4	F	0.25	lb ai/A	A	PPBCPR	0.0	95.0	0.0	0.0
7	Sustain			1	qt pr/A	A	PPBCPR				
8	Spartan	4	F	0.25	lb ai/A	B	PPBCIC	0.0	98.8	0.0	0.0
8	Sustain			1	qt pr/A	B	PPBCIC				
9	Spartan	4	F	0.3125	lb ai/A	A	PPBCPR	0.0	98.8	0.0	0.0
10	Spartan	4	F	0.3125	lb ai/A	B	PPBCIC	0.0	100.0	0.0	0.0
11	Spartan	4	F	0.3125	lb ai/A	A	PPBCPR	0.0	100.0	0.0	0.0
11	Sustain			1	qt pr/A	A	PPBCPR				
12	Spartan	4	F	0.3125	lb ai/A	B	PPBCIC	0.0	100.0	0.0	0.0
12	Sustain			1	qt pr/A	B	PPBCIC				
13	Untreated							0.0	0.0	0.0	0.0
	LSD (P=.05)							0.00	1.57	0.00	0.00
	Standard Deviation							0.00	1.10	0.00	0.00
	CV							0.0	1.27	0.0	0.0
	Replicate F							0.000	3.600	0.000	0.000
	Replicate Prob(F)							1.0000	0.0226	1.0000	1.0000
	Treatment F							0.000	2355.933	0.000	0.000
	Treatment Prob(F)							1.0000	0.0001	1.0000	1.0000

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Trt No	Treatment Name	Form Amt	Fm Ds	Rate	Rate Unit	Appl Code	Method/ Timing	AMARE 06/28/2004 E CO PT 45 A TRAN	NIOTA 07/13/2004 P ST PT 60 A TRAN	NIOTA 07/13/2004 P SR PT 60 A TRAN	AMARE 07/13/2004 E CO PT 60 A TRAN
	Parameter Code										
	Date										
	Flag										
	Pest Stage										
	Eval										
	Size/Eval Type										
	AN										
	Day/Before After										
	Activity										
	Trt-Eval Interval										
	PRM Data Type										
	# Subsamples, Dec.										
1	Spartan	4	F	0.1875	lb ai/A	A	PPBCPR	82.5	0.0	0.0	55.0
2	Spartan	4	F	0.1875	lb ai/A	B	PPBCIC	85.0	0.0	0.0	58.3
3	Spartan	4	F	0.1875	lb ai/A	A	PPBCPR	90.0	0.0	0.0	78.3
3	Sustain			1	qt pr/A	A	PPBCPR				
4	Spartan	4	F	0.1875	lb ai/A	B	PPBCIC	90.0	0.0	0.0	77.5
4	Sustain			1	qt pr/A	B	PPBCIC				
5	Spartan	4	F	0.25	lb ai/A	A	PPBCPR	90.0	0.0	0.0	65.0
6	Spartan	4	F	0.25	lb ai/A	B	PPBCIC	92.5	0.0	0.0	77.5
7	Spartan	4	F	0.25	lb ai/A	A	PPBCPR	95.0	0.0	0.0	77.5
7	Sustain			1	qt pr/A	A	PPBCPR				
8	Spartan	4	F	0.25	lb ai/A	B	PPBCIC	98.3	0.0	0.0	75.0
8	Sustain			1	qt pr/A	B	PPBCIC				
9	Spartan	4	F	0.3125	lb ai/A	A	PPBCPR	95.0	0.0	0.0	80.0
10	Spartan	4	F	0.3125	lb ai/A	B	PPBCIC	100.0	0.0	0.0	82.5
11	Spartan	4	F	0.3125	lb ai/A	A	PPBCPR	100.0	0.0	0.0	82.5
11	Sustain			1	qt pr/A	A	PPBCPR				
12	Spartan	4	F	0.3125	lb ai/A	B	PPBCIC	100.0	0.0	0.0	85.0
12	Sustain			1	qt pr/A	B	PPBCIC				
13	Untreated							0.0	0.0	0.0	0.0
	LSD (P=.05)							2.77	0.00	0.00	6.86
	Standard Deviation							1.80	0.00	0.00	4.57
	CV							2.09	0.0	0.0	6.65
	Replicate F							0.302	0.000	0.000	1.175
	Replicate Prob(F)							0.8233	1.0000	1.0000	0.3501
	Treatment F							867.521	0.000	0.000	98.310
	Treatment Prob(F)							0.0001	1.0000	1.0000	0.0001

ATTACHMENT 26

SUSTAIN® with Herbicides on Flue Cured Tobacco.

FMC Ag Products Group 2004 Field Trials.

FMC Ag Products Group

Spartan/Sustain - Tobacco

roject Code: Master Prt #: Discipline: H
Trial Number: 2004WDM02 Location: Nathalie, VA
Cooperator: Carr By: W.D. Martin

Trial Status: INCOMPLETE **Trial Reliability:** H

Objective(s): WEED CONTROL AND CROP PHYTO

Conclusion(s):

TRIAL MANAGEMENT

Trial Number: 2004WDM02 **Trial Type, Category:** HERBICIDE , FIELD
City: NATHALIE **County:** HALIFAX **State:** VA **Country:** USA

Principal Investigator: MARTIN. W.D.
Affiliation: PRIVATE
HALIFAX, VA

Study Director: MARTIN, W.D.
Affiliation: PRIVATE
HALIFAX, VA

STUDY DESCRIPTION

Study Design: RCB **Reps:** 4 **Plot Type:** SMALL PLOT
lot Width/Area, Unit: 30.6, FT **Length, Unit:** 30 , FT
Treated Width/Area, Unit: 7.67, FT **Length, Unit:** 30 , FT
Tillage Type: CONVENTIONAL **Carryover From Trial No.:**

Field Preparation: FIELD WAS DISCED AND BEDDED PRIOR TO APPLICATIONS AND TRANSPLANTING

Fertilizer Applied: 1000 LB/A OF 6-12-18 PRIOR TO PLANTING

Study Description Comments: ALL FIELD PREPARATION AND CULTURAL PRACTICES NORMAL TO TOBACCO PRODUCTION WERE FOLLOWED THROUGH OUT THE SEASON

CROP DESCRIPTION

Multicrop Trial (Y/N): N

Crop 1:TOBACCO, FLUE CURED **Variety:** NC 72 **Planting Date:** 05/14/2004

Planting Method: TRANSPLANT **Depth, Unit:** 4 , IN **Row Spacing, Unit:** 46 , IN

Perennial Age, Unit: , **YEAR**

Field History:

Year Crop	Pesticide	,Type	Year Crop	Pesticide	,Type
	,		,		
	,		,		
	,		,		
	,		,		
	,		,		
	,		,		

08/16/2004 (2004WDM02)

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APPLICATION INFORMATION

A

Method/Timing: PPBCPR
Application Date: 04/30/2004
Time of Day: 0130
Air Temp., Unit: 76 , F
% Relative Humidity: 40
Wind Velocity, Unit: 2 , MPH
Wind Direction: SE
Dew Presence (Y/N): N
Soil Temp., Unit: 62 , F
Soil Moisture: Optimum
% Cloud Cover: 20

B

Method/Timing: PPBCIC
Application Date: 04/30/2004
Time of Day: 0130
Air Temp., Unit: 76 , F
% Relative Humidity: 40
Wind Velocity, Unit: 2 , MPH
Wind Direction: SE
Dew Presence (Y/N): N
Soil Temp., Unit: 62 , F
Soil Moisture: Optimum
% Cloud Cover: 20

APPLICATION EQUIPMENT INFORMATION

A

Appl. Equipment: SPRAYER
Spray Pressure, Unit: 35 , PSI
Nozzle Type: FLAT FAN
Nozzle Size: 8003
Nozzle Spacing, Unit: 18 , IN
Nozzles/Row: 2.5
Band Width, Unit: , IN
Swath Width, Unit: 7 , FT
Boom Height, Unit: 24 , IN
Ground Speed, Unit: 3 , MPH
Incorporation Equip.:
Hours to Incorp.:
Incorp. Depth, Unit: 2 , IN
Carrier: WATER
Spray Volume, Unit: 20 , GPA

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B

Appl. Equipment: SPAYER
Spray Pressure, Unit: 35 , PSI
Nozzle Type: FLAT FAN
Nozzle Size: 8003
Nozzle Spacing, Unit: 18 , IN
Nozzles/Row: 2.5
Band Width, Unit: , IN
Swath Width, Unit: 7 , FT
Boom Height, Unit: 24 , IN
Ground Speed, Unit: 3 , MPH
Incorporation Equip.: PERFECTOR
Hours to Incorp.: 1
Incorp. Depth, Unit: 2 , IN
Carrier: WATER
Spray Volume, Unit: 20 , GPA

Application Comments:

Treatment Comments:

FMC Ag Products Group

Summary Comments:
TOBACCO WAS CULTIVATED ON 6/23/2004 AND LAYBY WAS PERFORMED ON 06/30/2004. BOTH OF THESE OPERATIONS DISRUPTED WEED CONTROL AND SUBSEQUENT GROWTH. PLOT DATA AFTER THESE TWO EVENTS WERE EVALUATED BASED ON SKIP ROWS BESIDE PLOTS. DATA ENTRY WITH A (.) INDICATES NO AREA FOR EVALUATION AS COMPARED TO PLOTS AVAILABLE. GRASS AND WILD RADISH POPULATIONS INCREASED AFTER THESE CULTIVATION EVENTS AND DATA WILL BE REPORTED AT THE 100 DAT AND AFTER HARVEST INTERVALS.

FMC Ag Products Group

SOIL INFORMATION

Soil Analyzed by: A&L LABS

Soil pH : 5.5 **CEC (meq/100g):** 3.1
Buffer pH: 6.9

Phosphorus (P): 139 **Rate Unit:** PPM
Potassium (K) : 82 PPM **% Saturation** **% Composition**
Calcium (Ca) : 311 PPM **% K :** 6.7 **% Sand:** 64
Magnesium (Mg): 67 PPM **% Ca:** 49.5 **% Silt:** 26
% Mg: 17.8 **% Clay:** 10
Organic Matter (%): 1.5 **% H :** 26
ENR (lb/a) :
Texture: SANDY LOAM

RAINFALL INFORMATION

Measurement Type: ACTUAL **Location:** NATHALIE, VA

Seasonal Moisture: MOIST **Rainfall Units:** IN **Distance to Station:** 1 **Unit:** MI

Date	Amt	Remark/Activity
05/01/2004	0.45	
05/02/2004	0.48	
5/03/2004	0.38	
5/16/2004	0.67	
05/19/2004	0.12	
05/25/2004	0.64	
06/04/2004	1.86	
06/22/2004	0.47	
06/23/2004	0.22	
06/25/2004	0.14	
06/26/2004	0.11	
06/27/2004	2.76	
06/28/2004	0.85	
06/30/2004	0.24	
07/04/2004	1.65	
07/10/2004	0.26	
07/11/2004	1.32	
07/14/2004	0.15	
07/18/2004	0.18	
07/21/2004	0.20	
07/25/2004	0.62	
07/26/2004	1.94	
07/30/2004	0.52	

ATTACHMENT 27

Miller 6064

Rice Herbicide Retention Study.

Sills Ag Consulting Group
Summer 2001.

To: Miller Chemical & Fertilizer Co.
Michael D. Fiery

From: Dave Sills, Sills Ag Consulting, Inc., Researcher

Reported Study: Miller 6064 Rice Herbicide Retention

Date of Study: Summer, 2001

Goal of Study

- To evaluate soil retention performance of Miller 6064 when tank mixed with California registered pre-flood rice herbicides.

Applications, Rates, and Conditions of Study

This study was designed to provide data establishing the chronology (days to flooding after treatment) using Miller 6064 to stabilize pre-flood herbicides and thereby improving both efficacy and crop safety.

A 1.5 acre rice station was constructed specifically for this study. This rice station was located at Sopwith Farms located in south Sutter County, 25 miles north of Sacramento. Three basins were constructed within the station in order to provide the timeline of initial flood. That timeline was to flood basin 1 @ 24 hrs after treatment, basin 2 @ 72 hrs after treatment, and basin 3 @ 120 hrs after treatment.
Within basin no. 3, one pass of drill seeded rice was planted pretreatment and preflood. The balance of the rice station was water seeded with pre-soaked seed, as is the common

practice in commercial rice production. The following information will be divided into 2 sections; water-seeded applications and drill-seeded applications.

Water Seeded Applications

All three basins of water seeded rice had the following pre plant, pre-initial flood applications:

1. Ordram 8E @ 5 pints per acre.
2. Abolish 8E @ 4 pints per acre.
3. Ordram 8E @ 5 pints and Abolish 8E @ 3 pints per acre tank mixed.
4. Ordram 8E @ 5 pints plus Miller 6064 @ 1 pint per acre.
5. Abolish 8E @ 4 pints plus Miller 6064 @ 1 pint per acre.
6. Ordram 8E @ 5 pints and Abolish 8E @ 3 pints per acre plus Miller 6064 @ 1 pint per acre tank mixed.

Drill Seeded Applications (third basin)

1. Prowl @ 2.4 pints per acre.
2. Prowl @ 5 pints per acre.
3. Prowl @ 2.4 pints plus Miller 6064 @ 1 pint per acre.
4. Prowl @ 5 pints plus Miller 6064 @ 1 pint per acre.
5. Prowl @ 2.4 pints and Ordram 8E @ 5 pints per acre.
6. Prowl @ 2.4 pints, Ordram 8E @ 5 pints and Miller 6064 @ 1 pint per acre.

Results and Conclusions (Water Seeded Basins)

Miller 6064 does exhibit value at retention of soil applied herbicides. In this study, efficacy of volatile and movement oriented materials was certainly improved and/or enhanced. In regard to volatility, specifically Ordram 8E, performance begins to fall off beyond the 3-day period. The same is true regarding Abolish 8E. The consistently superior tank mix is Ordram 8E @ 5 pints, Abolish8E @ 3 pints with Miller 6064 @ 1 pint as a tank mix. Under this mix, weed control was excellent and persistent, even when flushed several times. The only weed to escape this combination was red stem (purple ammannia). This weed is easily controlled with post emergent broadleaf herbicides.

In the plots where no Miller 6064 was included, weed control was inconsistent.

Results and Conclusions (Drill Seeded Basin)

The central focus of this basin was to evaluate efficacy and safety of Prowl and Ordram8E when applied post plant, but pre flush, directly to the soil surface. Drill seeding depth was 1" to 1.5" from soil surface and herbicide layer.

The Prowl @ 2.4 pints and Miller 6064 @ 1 pint per acre did not cause phytotoxicity. Rice emergence was normal and growth unrestricted. Prowl @ 5 pints with Miller 6064 @ 1 pint per acre did, however, cause significant stand reduction. (Too much total A.I. to hold to the surface?)

Early observations of the Prowl and Miller 6064 combinations indicated similar efficacy to that of the Prowl @ 2.4 pints, Miller 6064 @ 1 pint and Ordram 8E @ 5 pints. Observations at 3 weeks, however, demonstrated that the addition of the 5-pint rate of Ordram 8E to the 6064/Prowl mix gave much longer and sustained performance that made the addition of the Ordram worth the extra expense. The addition of Abolish caused a stand reduction.

Recommendations and Conclusions

This is a successful project. The addition of Miller 6064 to soil applied rice herbicides, under water seeded conditions, is an excellent way to insure stability and performance of a grower's investment and a relatively inexpensive insurance program.

Drill seeded usage of Miller 6064 to keep Prowl tight to the soil surface needs more research and repetition before I would recommend marketing. This potential is not just improvement of efficacy, but centrally, the elimination of phytotoxicity. This safety consistency must be proven and reliable in order to avoid liability issues.

For the year 2002, a supplemental label for Nu-film P should be pursued for soil surface applied rice herbicides in water seeded rice if Miller 6064 is not registered. From a marketing perspective, the program could be promoted this winter at PCA and grower meetings. There are several attractive advantages to the grower that can be exposed:

1. Reduced water hold due to pre flood advantage.
2. Elimination of aerial application cost(s).
3. Grower control over applications.
4. Tank mix flexibility.
5. Possible elimination of steel ground rig applications.

The point still must be emphasized that applications, even with the Miller 6064, should be made as close as is functional, to the initial head of water. Miller 6064 will reduce volatility and leaching of herbicides but not eliminate them entirely.

ATTACHMENT 28

Nu-Film 17, Pinolene B and SUSTAIN®.

Evaluate the Efficacy of Different Surfactants with Pristine for Powdery Mildew on Squash.

Glades Crop Care, Inc. Spring 2004.

GLADES CROP CARE, INC.
Agricultural Consultants

OFFICE (561) 746-3740
FAX (561) 746-3775
www.gladescropcare.com

949 TURNER QUAY
JUPITER, FLORIDA 33458

"Established 1972"

Evaluate the Efficacy of Different Surfactants with Pristine for Powdery Mildew on Squash

Sponsor: Miller Chemical

Spring, 2004

Glades Crop Care Internal No. 04-40

July, 2004

Objective

To determine the efficacy of different surfactants with Pristine for powdery mildew on squash.

General Trial Information

- **Location:** Kitching Creek Research Facility, Hobe Sound, Florida
- **Soil Type:** EauGallie Fine Sand
- **Crop/Variety:** Squash "Medallion"
- **Planting Date:** April 28, 2004

Trial Design	complete randomized block
Plot Size:	25 row feet
Replications:	4
Output Per Treated Acre:	26.4 to 36.2 GPA
Production System:	raised bed with white on black plastic mulch and drip irrigation

General Treatment Information

Treatments	Rates	Application dates
1. Untreated check	-	-
2. Pristine	12.5 oz/A	May 25, June 2, 8 & 15, 2004.
3. Pristine + Nu-Film 17	12.5 oz/A 8 fl oz/A	
4. Pristine + Pinolene B	12.5 oz/A 1 pt/A	
5. Pristine + Sustain	12.5 oz/A 8 fl oz/A	

Evaluations

Disease Evaluations: Weekly observations for disease incidence and severity were made. Twenty-five leaves per plot were examined. The following rating scale was used to evaluate severity. The evaluation is based on leaf area showing disease symptoms.

0 - 1 = 1-5%
1 = 6-15%
2 = 16-30%
3 = 31-50%
4 = 51-75%
5 = 76-100%

All data collected was entered into FieldPro for statistical analysis.

Results and Discussion

All treatments showed better powdery mildew control then the untreated. Until June 21 evaluation, treatments 2, 4 and 5 provided the best control. Pristine combined with Pinolene B provided the best control.

Powdery Mildew Incidence per 25 Leaves Means					
Trt	**May 25**	**Jun 1**	**Jun 7**	**Jun 14**	**Jun 21**
1	0.0	3.3	21.3	24.5	24.8
2	0.0	0.0	3.8	5.3	18.3
3	0.0	1.8	12.0	12.5	16.3
4	0.0	0.0	2.0	2.5	11.3
5	0.0	1.0	3.8	3.5	13.5

Powdery Mildew Severity per 25 Leaves Means					
Trt	**May 25**	**Jun 1**	**Jun 7**	**Jun 14**	**Jun 21**
1	0.00	0.13	1.33	2.55	4.79
2	0.00	0.00	0.15	0.21	1.51
3	0.00	0.07	0.59	0.71	1.31
4	0.00	0.00	0.08	0.10	0.70
5	0.00	0.04	0.15	0.14	1.07

No phytotoxicity was observed in any of the treatments.

Maintenance Applications

Applications for control of whiteflies and worms included Actara, Avaunt, M-Pede, and Assail.

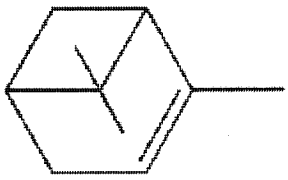
ATTACHMENT 29

α-Pinene and its Insecticidal Properties

« Previous Compound Next Compound »

Compound - alpha-pinene

Discuss this Compound



2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene

Formula:	C10H16
CAS#:	80-56-8
MW:	136.23

[MS spectra]

Species utilize 2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene in its chemical communication system

For help just move the cursor over the abbreviations in green or the red text below

Coleoptera, Chrysomelidae		
Leptinotarsa decemlineata	K	Colorado potato beetle
Coleoptera, Cleridae		
Thanasimus dubius	A	
Thanasimus formicarius	A	European red-bellied clerid
Thanasimus undatulus	A	
Thanasimus undatulus	K	
Coleoptera, Curculionidae		
Anthonomus grandis	A	Cotton boll weevil
Hylobius abietis	A	Large pine weevil
Smicronyx fulvus	A	Sunflower seed weevil
Coleoptera, Nitidulidae		
Epuraea pygmaea	A	
Coleoptera, Scolytidae		
Conophthorus coniperda	K	White pine cone beetle
Dendroctonus frontalis	A	Southern pine beetle
Dendroctonus pseudotsugae	K	Douglas fir beetle
Dendroctonus simplex	A	Eastern larch beetle
Dendroctonus valens	A	Red turpentine beetle
Dryocoetes autographus	K	
Gnathotrichus retusus	A	

Gnathotrichus retusus	K	
Gnathotrichus sulcatus	K	
Hylastes longicollis	A	
Hylastes macer	A	Root-feeding bark beetle
Hylastes nigrinus	A	
Hylastes nigrinus	K	
Hylastes ruber	K	
Hylurgops porosus	A	
Hylurgops subcostulatus	A	
Ips caelatus	A	
Ips latidens	A	
Ips perturbatus	A	Northern spruce engraver
Ips pini	A	Pine engravers
Ips typographus	P	Spruce bark beetle
Phloeotribus scarabaeoides	A	Olive bark beetle
Pseudohylesinus grandis	K	Silver fir beetle
Pseudohylesinus nebulosus	K	
Scolytus ventralis	K	Fir engraver
Tomicus piniperda	K	Pine shoot beetle
Trypodendron lineatum	K	Striped ambrosia beetle
Trypodendron lineatum	P	Striped ambrosia beetle
Coleoptera, Tenebrionidae		
Artystona sp.	Al	
Coleoptera, Trogositidae		
Temnochila chlorodia	A	
Diptera, Dolichopodidae		
Medetera signaticornis	A	
Homoptera, Aphididae		
Megoura viciae	P	Vetch aphid
Isoptera, Rhinotermitidae		
Reticulitermes flavipes	Al	Eastern subterranean termite
Reticulitermes santonensis	Al	
Isoptera, Termitidae		
Nasutitermes costalis	P	
Nasutitermes ephratae	P	
Nasutitermes nigriceps	P	
Nasutitermes princeps	P	
Nasutitermes rippertii	P	
Velocitermes velox	Al	
Velocitermes velox	P	
Coleoptera, Cerambycidae, Lamiinae		
Monochamus alternatus	K	Japanese pine sawyer
Diptera, Tephritidae, Dacinae		
Bactrocera oleae	A	Olive fruit fly
Bactrocera oleae	P	Olive fruit fly
Lepidoptera, Noctuidae, Amphipyrinae		
Spodoptera frugiperda	K	Fall armyworm
Lepidoptera, Papilionidae, Papilioninae		

Papilio demodocus	P	Citrus swallowtail butterfly
Lepidoptera, Pieridae, Pierinae		
Pieris melete	P	White butterfly
Pieris napi japonica	P	
Hymenoptera, Formicidae, Myrmicinae, Myrmicariini		
Myrmicaria natalensis	Al	
Thysanoptera, Phlaeothripidae, Phlaeothripinae, Haplothripini		
Dolichothrips sp.	P	

Free plugin Chime is required to view the molecule in 3D

Page created on 4-November-2004

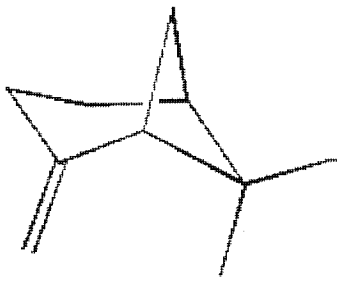
ATTACHMENT 30

β-Pinene and its Insecticidal Properties

« Previous Compound Next Compound »

Compound - beta-pinene

Discuss this Compound



6,6-Dimethyl-2-methylenebicyclo [3.1.1]heptane

Formula:	C10H16
CAS#:	127-91-3
MW:	136.24

[MS spectra]

Species utilize 6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane in its chemical communication system

For help just move the cursor over the abbreviations in green or the red text below

Coleoptera, Cleridae		
Thanasimus formicarius	A	European red-bellied clerid
Thanasimus undatulus	K	
Coleoptera, Curculionidae		
Smicronyx fulvus	A	Sunflower seed weevil
Coleoptera, Nitidulidae		
Epuraea pygmaea	A	
Coleoptera, Scolytidae		
Dendroctonus pseudotsugae	K	Douglas fir beetle
Dendroctonus valens	A	Red turpentine beetle
Dryocoetes autographus	K	
Gnathotrichus retusus	A	
Gnathotrichus retusus	K	
Gnathotrichus sulcatus	K	
Hylastes longicollis	A	
Hylastes macer	A	Root-feeding bark beetle
Hylastes nigrinus	A	
Hylastes nigrinus	K	
Hylastes ruber	K	
Hylurgops palliatus	K	

Hylurgops porosus	A	
Hylurgops subcostulatus	A	
Ips latidens	A	
Ips pini	A	Pine engravers
Ips typographus	P	Spruce bark beetle
Phloeotribus scarabaeoides	A	Olive bark beetle
Pseudohylesinus grandis	K	Silver fir beetle
Pseudohylesinus nebulosus	K	
Scolytus ventralis	K	Fir engraver
Tomicus piniperda	K	Pine shoot beetle
Trypodendron lineatum	K	Striped ambrosia beetle
Coleoptera, Trogositidae		
Temnochila chlorodia	A	
Diptera, Dolichopodidae		
Medetera signaticornis	A	
Heteroptera, Scutelleridae		
Hotea gambiae	Al	
Homoptera, Aphididae		
Megoura viciae	P	Vetch aphid
Isoptera, Rhinotermitidae		
Reticulitermes flavipes	Al	Eastern subterranean termite
Reticulitermes santonensis	Al	
Isoptera, Termitidae		
Nasutitermes costalis	P	
Nasutitermes rippertii	P	
Velocitermes velox	Al	
Coleoptera, Cerambycidae, Lamiinae		
Monochamus alternatus	K	Japanese pine sawyer
Lepidoptera, Pieridae, Pierinae		
Pieris melete	P	White butterfly
Pieris napi japonica	P	
Hymenoptera, Formicidae, Myrmicinae, Myrmicariini		
Myrmicaria natalensis	Al	
Thysanoptera, Phlaeothripidae, Phlaeothripinae, Haplothripini		
Dolichothrips sp.	P	

Free plugin Chime is required to view the molecule in 3D

ATTACHMENT 31

α-Pinene Polymer

PAN Pesticide Database

Identification, Toxicity, Use, Water Pollution Potential, Ecological Toxicity and Regulatory Information

PAN Pesticides Database - Chemicals

Alpha-pinene polymer - Identification, toxicity, use, water pollution potential, ecological toxicity and regulatory information

Note: See Working with the Information on this Page section below for important notes about this data.

Chemical ID	Identifying information, including synonyms, ID numbers, use type, chemical classification, a link to a list of all products containing this chemical and a list of the top crops this pesticide is used on in California.
Poisoning Symptoms	Signs and symptoms of poisoning, first aid, and links to treatment information for this chemical.
Toxicity	Toxicity to humans, including carcinogenicity, reproductive and developmental toxicity, neurotoxicity, and acute toxicity.
Regulatory	Links to world-wide registration status as well as regulatory information for the U.S. and California.
Water	Water quality standards and physical properties affecting water contamination potential.
Ecotoxicity	Toxicity to aquatic organisms.
Related Chems	List of chemicals in the same family, including breakdown products, salts, esters, isomers, and other derivatives.

Chemical Identification and Use for Alpha-pinene polymer

Top

Basic Identification Information About This Chemical

Chemical Name:	Alpha-pinene polymer
CAS Number:	31393-98-3
U.S. EPA PC Code:	
CA DPR Chem Code:	3996
Use Type:	Insecticide
Chem Class:	Polymer , Polymer

View Related Chemicals

Additional Resources About This Chemical Class and Use Type

See the Global Pesticide Resources page for many additional links.

Other Names for this Chemical

About Chemical Synonyms

03996 (CA DPR Chem Code) , 31393-98-3 (CAS Number) , 31393983 (CAS Number) , 3996 (CA DPR Chem Code) , Alpha-

pinene polymer , Alphapinenepolymer

Signs and Symptoms of Alpha-pinene polymer Poisoning

Top

NOTE! There may be other diseases and chemicals that have similar symptoms.

If you have a poisoning emergency in the United States call 1-800-222-1222. If the victim has collapsed or is unconscious, call 911.

Sorry! No symptoms for this chemical or chemical group are available. See related chemicals for possible additional information.

Toxicity Information for Alpha-pinene polymer

Top

Note: Information for many chemicals is incomplete and may not be fully representative of effects on humans. Why?

Summary Toxicity Information

PAN Bad Actor Chemical [1]	Acute Toxicity [2]	Carcinogen	Cholinesterase Inhibitor	Ground Water Contaminant	Developmental or Reproductive Toxin	Endocrine Disruptor
Not Listed	?	?	No	?	?	?

Indicates high toxicity in the given toxicological category.

Indicates no available weight-of-the-evidence summary assessment. For additional information on toxicity from scientific journals or registration documents, see the "Additional Resources for Toxicity " section of the chemical detail page.

1. **PAN Bad Actors** are chemicals that are one or more of the following: highly acutely toxic, cholinesterase inhibitor, known/probable carcinogen, known groundwater pollutant or known reproductive or developmental toxicant. NOTE! Because there are no authoritative lists of Endocrine Disrupting (ED) chemicals, EDs are not yet considered PAN Bad Actor chemicals.

2. The acute toxicity reported on this page is of the pure chemical ingredient only and may not reflect the acute toxicity of individual pesticide products. To view acute toxicity of individual products, click on 'View Products' link in the 'Chemical Identification' section above.

Additional Resources about the Toxicity of this Chemical

Additional Toxicity Info for this Chemical

See the Global Pesticide Resources page for many additional links.

Detailed Toxicity Information	This Chemical	Parent Chemical
Acute Toxicity [2]	Alpha-pinene polymer	Pinene
WHO Acute Hazard	Not Listed	Not Listed
TRI Acute Hazard	Not Listed	Not Listed
Material Safety Data Sheets	Not Available	Not Available
Acute rating from U.S. EPA product label	No Consensus Value	No Consensus Value
U.S. NTP Acute Toxicity Studies	No NTP Studies	Slightly Toxic
View Studies		
Cholinesterase Inhibitor	No	No

2. The acute toxicity reported on this page is of the pure chemical ingredient only and may not reflect the acute toxicity of individual pesticide products. To view acute toxicity of individual products, click on 'View Products' link in the 'Chemical Identification' section above.

Cancer Information

IARC Carcinogens	Not Listed	Not Listed
U.S. NTP Carcinogens	Not Listed	Not Listed
California Prop 65 Known Carcinogens	Not Listed	Not Listed
U.S. EPA Carcinogens	Not Listed	Not Listed
TRI Carcinogen	Not Listed	Not Listed

Endocrine Disruption

Illinois EPA list	Not Listed	Not Listed
Keith list	Not Listed	Not Listed
Colborn list	Not Listed	Not Listed
Benbrook list	Not Listed	Not Listed

Reproductive and Developmental Toxicity

CA Prop 65 Developmental Toxin	Not Listed	Not Listed
U.S. TRI Developmental Toxin	Not Listed	Not Listed
CA Prop 65 Female Reproductive Toxin	Not Listed	Not Listed
CA Prop 65 Male Reproductive Toxin	Not Listed	Not Listed
U.S. TRI Reproductive Toxin	Not Listed	Not Listed

Chemicals of Special Concern

PAN Bad Actors	Not Listed	Not Listed
PAN Dirty Dozen list	Not Listed	Not Listed

Water Pollution Potential and Criteria for Alpha-pinene polymer

Top

Water Pollution Potential	This Chemical	Parent Chemical

	Alpha-pinene polymer	Pinene
PAN Ground Water Contaminant Rating	Insufficient Data	Insufficient Data

Sorry, no water quality standards or criteria have been established for this chemical by the U.S. or Canadian governments; however, there may be criteria established for related chemicals.

Regulatory Information for Alpha-pinene polymer

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International Regulatory Status	This Chemical	Parent Chemical
Worldwide Registration	Worldwide Registration for: Alpha-pinene polymer	Worldwide registration for: Pinene
Click on link at right to view registration information for different countries -->	Number of countries where this chemical is: Banned, Restricted or Cancelled: 0 Not legal for import: 0	Number of countries where this c Banned, Restricted or Cancell Not legal for import: 0

UNEP Persistent Organic Pollutant (POP)	Not Listed	Not Listed
UNEP Prior Informed Consent Chemical (PIC)	Not Listed	Not Listed
WHO Obsolete Pesticide	Not Listed	Not Listed

U.S. and California Regulatory Status

U.S. EPA Registered	No	No
U.S. EPA Hazardous Air Pollutant	Not Listed	Not Listed
U.S. EPA Minimum Risk Pesticide (25b list)	No	No
CA Registered	No	No
CA Groundwater Contaminant	Not Listed	Not Listed
CA Toxic Air Contaminant	Not Listed	Not Listed

Maximum Tolerance and Residue Levels

Codex Alimentarius (UN FAO Maximum Residue Limits)	Go to web site
U.S. Maximum Tolerance Levels	Go to web site
European Union Maximum Residue Levels	Go to web site

Ecotoxicity for Alpha-pinene polymer

Top

Note! Information for many chemicals is incomplete and may not be fully representative of effects on the environment. Why? Click on underlined terms for definitions and additional information.

Aquatic Ecotoxicity

All Toxic Effects for Organism Group	
Organism Group	Effects Noted
Sorry, no ecotoxicity data available for this chemical. Try related chemicals.	

Summary of Acute Toxicity for Organism Group
Sorry, no acute ecotoxicity data available for this chemical. Try related chemicals.

Terrestrial Ecotoxicity

We have recently secured funding from the U.S. EPA to incorporate terrestrial ecotoxicity data. Watch this space!

Related Chemicals for Alpha-pinene polymer

Top

CAS Number	Relation	Reason	Chemical Name	Chem Detail	Registration	Symptoms	California Use	Chem Use Type	U.S EPA Reg
80-56-8, 127-91-3, 1330-16-1	Parent	P	Pinene	View	View	View	View	Insecticide	No
31393-98-3	Related	16	Alpha-pinene polymer	View	View	View	View	Insecticide	No
127-91-3	Related	6	beta-Pinene	View	View	View	View	Insecticide	No
68240-09-5	Related	6, 16	Beta-pinene polymer	View	View	View	View	Insecticide	No
25719-60-2	Related	6, 16	Beta-pinene polymer -toluene and xylene alkylated with dicyclopentadiene	View	View	View	View		No
	Related	6, 16	Beta-pinene-maleamide copolymer	View	View	View	View		No
53404-49-2	Related	2	Ethylene glycol ether of pinene	View	View	View	View	Insecticide	No
	Related	16	Polymerized pinene	View	View	View	View	Insecticide	No

Working with the Information on this Page

Click on underlined terms for definitions or go to the Pesticide Tutorial overview page.

Any underlined term with a book icon has additional information.

* Data marked with an asterisk indicates that this chemical is not explicitly listed on the corresponding list. Instead, it belongs to a group of chemicals that IS designated on the list. For example, if an agency assigns a classification of reproductive toxicant to "mercury compounds", that classification is applied to all mercury compounds in the PAN Pesticide database, which are then marked with an asterisk.

To print this page, choose **Print**. To export this data, choose **Save As** 'HTML Source' and open it in Excel or equivalent program.

Citation: S. Orme and S. Kegley.*PAN Pesticide Database*. Pesticide Action Network, North America (San Francisco, CA. 2004).
http:www.pesticideinfo.org.

Related Chemicals for Alpha-pinene polymer

Top

CAS Number	Relation	Reason	Chemical Name	Chem Detail	Registration	Symptoms	California Use	Chem Use Type	U.S. EPA Reg	PAN Bad Actor
80-56-8, 127-91-3, 1330-16-1	Parent	P	Pinene	View	View	View	View	Insecticide	No	Not Listed
31393-98-3	Related	16	Alpha-pinene polymer	View	View	View	View	Insecticide	No	Not Listed
127-91-3	Related	6	beta-Pinene	View	View	View	View	Insecticide	No	Not Listed
68240-09-5	Related	6, 16	Beta-pinene polymer	View	View	View	View	Insecticide	No	Not Listed
25719-60-2	Related	6, 16	Beta-pinene polymer -toluene and xylene alkylated with dicyclopentadiene	View	View	View	View		No	Not Listed
	Related	6, 16	Beta-pinene-maleamide copolymer	View	View	View	View		No	Not Listed
53404-49-2	Related	2	Ethylene glycol ether of pinene	View	View	View	View	Insecticide	No	Not Listed
	Related	16	Polymerized pinene	View	View	View	View	Insecticide	No	Not Listed

Working with the Information on this Page

Click on underlined terms for definitions or go to the Pesticide Tutorial overview page.

Any underlined term with a book icon has additional information.

* Data marked with an asterisk indicates that this chemical is not explicitly listed on the corresponding list. Instead, it belongs to a group of

chemicals that IS designated on the list. For example, if an agency assigns a classification of reproductive toxicant to "mercury compounds", that classification is applied to all mercury compounds in the PAN Pesticide database, which are then marked with an asterisk.

To print this page, choose **Print**. To export this data, choose **Save As** 'HTML Source' and open it in Excel or equivalent program.

Related Chemicals for Alpha-pinene polymer

Top

CAS Number	Relation	Reason	Chemical Name	Chem Detail	Registration	Symptoms	California Use	Chem Use Type	U.S EPA Reg
80-56-8, 127-91-3, 1330-16-1	Parent	P	Pinene	View	View	View	View	Insecticide	No
31393-98-3	Related	16	Alpha-pinene polymer	View	View	View	View	Insecticide	No
127-91-3	Related	6	beta-Pinene	View	View	View	View	Insecticide	No
68240-09-5	Related	6, 16	Beta-pinene polymer	View	View	View	View	Insecticide	No
25719-60-2	Related	6, 16	Beta-pinene polymer -toluene and xylene alkylated with dicyclopentadiene	View	View	View	View		No
	Related	6, 16	Beta-pinene-maleamide copolymer	View	View	View	View		No
53404-49-2	Related	2	Ethylene glycol ether of pinene	View	View	View	View	Insecticide	No
	Related	16	Polymerized pinene	View	View	View	View	Insecticide	No

Working with the Information on this Page

Click on underlined terms for definitions or go to the Pesticide Tutorial overview page.

Any underlined term with a book icon has additional information.

* Data marked with an asterisk indicates that this chemical is not explicitly listed on the corresponding list. Instead, it belongs to a group of chemicals that IS designated on the list. For example, if an agency assigns a classification of reproductive toxicant to "mercury compounds", that classification is applied to all mercury compounds in the PAN Pesticide database, which are then marked with an asterisk.

To print this page, choose **Print**. To export this data, choose **Save As** 'HTML Source' and open it in Excel or equivalent program.

ATTACHMENT 32

β-Pinene Polymer

PAN Pesticide Database

Identification, Toxicity, Use, Water Pollution Potential, Ecological Toxicity and Regulatory Information

PAN Pesticides Database - Chemicals

Beta-pinene polymer - Identification, toxicity, use, water pollution potential, ecological toxicity and regulatory information

Note: See Working with the Information on this Page section below for important notes about this data.

Chemical ID	Identifying information, including synonyms, ID numbers, use type, chemical classification, a link to a list of all products containing this chemical and a list of the top crops this pesticide is used on in California.
Poisoning Symptoms	Signs and symptoms of poisoning, first aid, and links to treatment information for this chemical.
Toxicity	Toxicity to humans, including carcinogenicity, reproductive and developmental toxicity, neurotoxicity, and acute toxicity.
Regulatory	Links to world-wide registration status as well as regulatory information for the U.S. and California.
Water	Water quality standards and physical properties affecting water contamination potential.
Ecotoxicity	Toxicity to aquatic organisms.
Related Chems	List of chemicals in the same family, including breakdown products, salts, esters, isomers, and other derivatives.

Chemical Identification and Use for Beta-pinene polymer

Top

Basic Identification Information About This Chemical

Chemical Name:	Beta-pinene polymer
CAS Number:	68240-09-5
U.S. EPA PC Code:	
CA DPR Chem Code:	3998
Use Type:	Insecticide
Chem Class:	Polymer , Polymer

View Related Chemicals

Additional Resources About This Chemical Class and Use Type

See the Global Pesticide Resources page for many additional links.

Historical Use of this Chemical

Top five crops and sites for this pesticide in California

Wine Grapes | Tomatoes for Processing | Almonds | Table and Raisin Grapes | Strawberries
View All Crops and Sites

Other Names for this Chemical
About Chemical Synonyms

03998 (CA DPR Chem Code) , 3998 (CA DPR Chem Code) , 68240-09-5 (CAS Number) , 68240095 (CAS Number) , Beta-pinene polymer , Betapinene Polymer , Betapinenepolymer

Signs and Symptoms of Beta-pinene polymer Poisoning

Top

NOTE! There may be other diseases and chemicals that have similar symptoms.

If you have a poisoning emergency in the United States call 1-800-222-1222. If the victim has collapsed or is unconscious, call 911.

Sorry! No symptoms for this chemical or chemical group are available. See related chemicals for possible additional information.

Toxicity Information for Beta-pinene polymer

Top

Note: Information for many chemicals is incomplete and may not be fully representative of effects on humans. Why?

Summary Toxicity Information

PAN Bad Actor Chemical [1]	Acute Toxicity [2]	Carcinogen	Cholinesterase Inhibitor	Ground Water Contaminant	Developmental or Reproductive Toxin	Endocrine Disruptor
Not Listed	?	?	No	?	?	?

Indicates high toxicity in the given toxicological category.

? Indicates no available weight-of-the-evidence summary assessment. For additional information on toxicity from scientific journals or registration documents, see the "Additional Resources for Toxicity " section of the chemical detail page.

1. **PAN Bad Actors** are chemicals that are one or more of the following: highly acutely toxic, cholinesterase inhibitor, known/probable carcinogen, known groundwater pollutant or known reproductive or developmental toxicant. NOTE! Because there are no authoritative lists of Endocrine Disrupting (ED) chemicals, EDs are not yet considered PAN Bad Actor chemicals.

2. The acute toxicity reported on this page is of the pure chemical ingredient only and may not reflect the acute toxicity of individual pesticide products. To view acute toxicity of individual products, click on 'View Products' link in the 'Chemical Identification' section above.

Additional Resources about the Toxicity of this Chemical

Additional Toxicity Info for this Chemical

See the Global Pesticide Resources page for many additional links.

Detailed Toxicity Information	This Chemical	Parent Chemical
Acute Toxicity [2]	Beta-pinene polymer	Pinene
WHO Acute Hazard	Not Listed	Not Listed
TRI Acute Hazard	Not Listed	Not Listed
Material Safety Data Sheets	Not Available	Not Available
Acute rating from U.S. EPA product label	No Consensus Value	No Consensus Value
U.S. NTP Acute Toxicity Studies	No NTP Studies	Slightly Toxic
View Studies		
Cholinesterase Inhibitor	No	No

2. The acute toxicity reported on this page is of the pure chemical ingredient only and may not reflect the acute toxicity of individual pesticide products. To view acute toxicity of individual products, click on 'View Products' link in the 'Chemical Identification' section above.

Cancer Information

IARC Carcinogens	Not Listed	Not Listed
U.S. NTP Carcinogens	Not Listed	Not Listed
California Prop 65 Known Carcinogens	Not Listed	Not Listed
U.S. EPA Carcinogens	Not Listed	Not Listed
TRI Carcinogen	Not Listed	Not Listed

Endocrine Disruption

Illinois EPA list	Not Listed	Not Listed
Keith list	Not Listed	Not Listed
Colborn list	Not Listed	Not Listed
Benbrook list	Not Listed	Not Listed

Reproductive and Developmental Toxicity

CA Prop 65 Developmental Toxin	Not Listed	Not Listed
U.S. TRI Developmental Toxin	Not Listed	Not Listed
CA Prop 65 Female Reproductive Toxin	Not Listed	Not Listed
CA Prop 65 Male Reproductive Toxin	Not Listed	Not Listed
U.S. TRI Reproductive Toxin	Not Listed	Not Listed

Chemicals of Special Concern

PAN Bad Actors	Not Listed	Not Listed
PAN Dirty Dozen list	Not Listed	Not Listed

Water Pollution Potential and Criteria for Beta-pinene polymer

Top

Water Pollution Potential	This Chemical	Parent Chemical
	Beta-pinene polymer	Pinene
PAN Ground Water Contaminant Rating	Insufficient Data	Insufficient Data

Sorry, no water quality standards or criteria have been established for this chemical by the U.S. or Canadian governments; however, there may be criteria established for related chemicals.

Regulatory Information for Beta-pinene polymer

Top

International Regulatory Status	This Chemical	Parent Chemical
Worldwide Registration Click on link at right to view registration information for different countries -->	Worldwide Registration for: Beta-pinene polymer Number of countries where this chemical is: Banned, Restricted or Cancelled: 0 Not legal for import: 0	Worldwide registration for: Pinene Number of countries where this c Banned, Restricted or Cancell Not legal for import: 0
UNEP Persistent Organic Pollutant (POP)	Not Listed	Not Listed
UNEP Prior Informed Consent Chemical (PIC)	Not Listed	Not Listed
WHO Obsolete Pesticide	Not Listed	Not Listed

U.S. and California Regulatory Status

U.S. EPA Registered	No	No
U.S. EPA Hazardous Air Pollutant	Not Listed	Not Listed
U.S. EPA Minimum Risk Pesticide (25b list)	No	No
CA Registered	Yes	No
CA Groundwater Contaminant	Not Listed	Not Listed
CA Toxic Air Contaminant	Not Listed	Not Listed

Maximum Tolerance and Residue Levels

Codex Alimentarius (UN FAO Maximum Residue Limits)	Go to web site
U.S. Maximum Tolerance Levels	Go to web site
European Union Maximum Residue Levels	Go to web site

Ecotoxicity for Beta-pinene polymer

Top

Note! Information for many chemicals is incomplete and may not be fully representative of effects on the environment. Why? Click on underlined terms for definitions and additional information.

Aquatic Ecotoxicity

All Toxic Effects for Organism Group	
Organism Group	Effects Noted
Sorry, no ecotoxicity data available for this chemical. Try related chemicals.	

Summary of Acute Toxicity for Organism Group
Sorry, no acute ecotoxicity data available for this chemical. Try related chemicals.

Terrestrial Ecotoxicity

We have recently secured funding from the U.S. EPA to incorporate terrestrial ecotoxicity data. Watch this space!

Related Chemicals for Beta-pinene polymer

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CAS Number	Relation	Reason	Chemical Name	Chem Detail	Registration	Symptoms	California Use	Chem Use Type	U.S EPA Reg
80-56-8, 127-91-3, 1330-16-1	Parent	P	Pinene	View	View	View	View	Insecticide	No
31393-98-3	Related	16	Alpha-pinene polymer	View	View	View	View	Insecticide	No
127-91-3	Related	6	beta-Pinene	View	View	View	View	Insecticide	No
68240-09-5	Related	6, 16	Beta-pinene polymer	View	View	View	View	Insecticide	No
25719-60-2	Related	6, 16	Beta-pinene polymer -toluene and xylene alkylated with dicyclopentadiene	View	View	View	View		No
	Related	6, 16	Beta-pinene-maleamide copolymer	View	View	View	View		No
53404-	Related	2	Ethylene glycol	View	View	View	View	Insecticide	No

49-2			ether of pinene						
	Related	16	Polymerized pinene	View	View	View	View	Insecticide	No

Working with the Information on this Page

Click on underlined terms for definitions or go to the Pesticide Tutorial overview page.

Any underlined term with a book icon has additional information.

* Data marked with an asterisk indicates that this chemical is not explicitly listed on the corresponding list. Instead, it belongs to a group of chemicals that IS designated on the list. For example, if an agency assigns a classification of reproductive toxicant to "mercury compounds", that classification is applied to all mercury compounds in the PAN Pesticide database, which are then marked with an asterisk.

To print this page, choose **Print**. To export this data, choose **Save As** 'HTML Source' and open it in Excel or equivalent program.

Citation: S. Orme and S. Kegley. *PAN Pesticide Database*. Pesticide Action Network, North America (San Francisco, CA. 2004).
http.www.pesticideinfo.org.

PAN Pesticides Database - California Pesticide Use

Beta-pinene polymer - Pesticide use statistics for 2002

Note: See Working with the Information on this Page section below for important notes about this data.

Chemical ID	Identifying information for this chemical, including synonyms, ID numbers, use type, and chemical classification.
Summary Toxicity	Summary toxicity to humans, including carcinogenicity, reproductive and developmental toxicity, neurotoxicity, and acute toxicity.
Regional Use	Use of this pesticide by county for all counties in California, with information on gross pounds used, application rate, acres planted, and number of applications.
Top Crops/Sites	Top crops and sites use of this chemical in 2002, with information on gross pounds used, application rate, acres planted, and number of applications.

Basic Chemical Information for Beta-pinene polymer

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For detailed chemical information see the chemical detail page.

Basic Identification Information About This Chemical

Chemical Name	Beta-pinene polymer
CAS Number	68240-09-5
U.S. EPA PC Code	
CA DPR Chem Code	3998
Use Type	Insecticide
Chem Class	Polymer, Polymer

Synonyms

Chemical versus Common Names

03998 (CA DPR Chem Code) , 3998 (CA DPR Chem Code) , 68240-09-5 (CAS Number) , 68240095 (CAS Number) , Beta-pinene polymer , Betapinene Polymer , Betapinenepolymer

Summary Toxicity Information for Beta-pinene polymer

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For detailed chemical information see the chemical detail page.

Note: Information for many chemicals is incomplete and may not be fully representative of effects on humans. Why?

Summary Toxicity Information

PAN Bad Actor Chemical [1]	Acute Toxicity [2]	Carcinogen	Cholinesterase Inhibitor	Ground Water Contaminant	Developmental or Reproductive Toxin	Endocrine Disruptor
Not Listed			No			

? ? ? ? ?

Indicates high toxicity in the given toxicological category.

Indicates no available weight-of-the-evidence summary assessment. For additional information on toxicity from scientific journals or registration documents, see the "Additional Resources for Toxicity " section of the chemical detail page.

1. **PAN Bad Actors** are chemicals that are one or more of the following: highly acutely toxic, cholinesterase inhibitor, known/probable carcinogen, known groundwater pollutant or known reproductive or developmental toxicant. NOTE! Because there are no authoritative lists of Endocrine Disrupting (ED) chemicals, EDs are not yet considered PAN Bad Actor chemicals.

2. The acute toxicity reported on this page is of the pure chemical ingredient only and may not reflect the acute toxicity of individual pesticide products. To view acute toxicity of individual products, click on 'View Products' link in the 'Chemical Identification' section above.

Top 50 Crops and Sites for for Beta-pinene polymer use in California in 2002

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Crop or Site (Commodity Code)	Gross Pounds	Application Rate pounds per acre treated	Acres Planted where all or part has been sprayed	Acres Treated	Application Count
All Sites (00)	7,645	0.17	36,031	46,282	1,916
Wine Grapes (29143)	1,898	0.08	10,138	22,837	1,108
Tomatoes for Processing (29136)	1,324	0.17	7,696	7,777	61
Almonds (3001)	735.3	0.33	2,153	2,250	35
Table and Raisin Grapes (29141)	675.2	0.34	2,748	1,993	61
Strawberries (1016)	522.0	0.29	788.5	1,804	37
Outdoor Container Nursery (154)	471.1	0.95	504.0	493.4	46
Head Lettuce (13045)	264.8	0.16	1,477	1,634	72
Apricots (5001)	227.3	0.29	599.5	796.5	26
Corn for Forage (22005)	190.9	0.26	838.0	741.0	21
Mustard (29123)	150.0	0.29	705.0	516.7	55
Cherries (5002)	127.3	0.21	208.0	597.0	20
Oranges (2006)	111.7	0.44	363.0	252.0	4
Walnuts (3009)	90.9	0.28	347.0	323.0	11
Celery (29113)	89.9	0.26	1,374	348.6	27
Leaf Lettuce (13031)	88.8	0.16	651.2	544.4	63
Collards (13009)	79.3	0.32	665.0	251.3	21
Kale (13011)	71.8	0.18	665.0	396.2	67
Onions (14011)	59.8	0.17	349.0	349.0	3
Cotton (29121)	57.6	0.17	336.0	336.0	3
Turnips (29137)	45.9	0.29	455.0	159.8	35

Bell Peppers (11003)	45.8	0.10	306.0	450.5	11
Alfalfa for Forage (23001)	44.7	0.29	152.0	151.7	3
Potatoes (14013)	44.6	0.34	260.0	131.0	3
Cabbage (13007)	31.7	0.15	250.0	206.0	25
Asparagus (16002)	27.0	0.08	317.7	317.7	11
Greenhouse Plants (153)	26.3	0.64	100.0	41.0	5
Cauliflower (13008)	26.0	0.17	120.8	149.2	23
Outdoor Flower Nursery (152)	25.5	1.69	15.1	15.1	3
Apples (4001)	21.1	0.41	27.0	51.0	6
Pears (4003)	18.7	0.20	188.0	94.0	5
Tomatoes (11005)	18.6	0.17	38.0	109.0	6
Broccoli (13005)	7.51	0.17	34.0	44.0	6
Nectarines (5003)	6.68	0.26	13.0	26.0	2
Chinese Cabbage (13010)	6.36	0.16	190.5	40.8	8
Radishes (14014)	3.51	0.18	225.0	20.0	4
Right of Way (40)	3.36	-	-	-	5
Bok Choy (13502)	2.41	0.16	139.2	15.2	9
Swiss Chard (13025)	1.92	0.19	370.0	10.0	2
Leeks (14010)	1.53	0.17	225.0	9.00	2
Landscape (30)	0.13	-	-	-	1

Regional Use for Beta-pinene polymer on All Sites in 2002

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Region (County Code)	Gross Pounds	Application Rate pounds per acre treated	Acres Planted where all or part has been sprayed	Acres Treated	Application Count
California (00)	7,645	0.17	36,031	46,282	1,916
Fresno (10)	1,287	0.17	7,932	7,706	90
Ventura (56)	984.1	0.27	5,815	3,682	272
Monterey (27)	860.6	0.07	4,197	12,982	778
Stanislaus (50)	756.3	0.26	2,534	2,870	67
Tulare (54)	588.0	0.71	904.0	828.0	21
Orange (30)	497.3	0.93	600.0	534.0	50
Sacramento (34)	493.2	0.17	2,716	2,911	63
Kern (15)	394.0	0.27	1,610	1,441	29
Kings (16)	359.1	0.17	2,243	2,097	9
San Benito (35)	299.8	0.06	1,088	4,870	233
Santa Barbara (42)	273.0	0.16	2,047	1,716	122
San Joaquin (39)	226.0	0.21	1,608	1,090	23
San Luis Obispo (40)	189.9	0.11	932.0	1,687	97
Merced (24)	166.2	0.21	636.0	782.0	25
Yolo (57)	109.5	0.30	386.0	368.0	11

Solano (48)	51.8	0.23	238.0	223.7	8
Amador (03)	48.5	0.17	355.9	283.0	6
Imperial (13)	40.5	0.23	163.0	174.0	8
Santa Cruz (44)	19.8	0.55	22.0	36.0	3
San Bernardino (36)	0.04	0.09	4.00	0.46	1

Working with the Information on this Page

* Complete 2001 data for Kern county was never submitted by the Kern County Agricultural Commissioners office. This missing data includes approximately 32,000 records totaling roughly 10 million pounds. An omission of this scale will significantly impact statewide trends.

NOTE! See methodology and documentation for California Pesticide Use Reporting for important qualifications on these numbers. Click on the commodities name links for special caveats on the data for each commodity. For more detailed use information, including data for other years, search our California Pesticide Use database. Original source for all pesticide use data is the California Pesticide Use Report (PUR) dataset, collected and managed by the California Department of Pesticide Regulation. Significant processing of the original dataset is required to generate the summary data presented here; see documentation for a full discussion of data handling.

NOTE! Comments on the accuracy of Acres Planted reflect PAN's analysis of acreage data from the California Agricultural Statistics Service (CASS) and Department of Pesticide Regulation Pesticide Use Reporting data. The method used to assess acreage accuracy is described in the documentation. Accuracy was evaluated for aggregate, statewide California acreage, but not for smaller regions (e.g., counties). See CASS acreage estimates for additional information.

Click on underlined terms for definitions or go to the Pesticide Tutorial overview page.

Any underlined term with a book icon has additional information.

To print this page, choose **Print**. To export this data, choose **Save As** 'HTML Source' and open it in Excel or equivalent program.

Definitions

- Acres Planted is the planted acreage of the crop in the selected region where the selected chemical was applied. This figure may not be the total acreage of the selected crop. To obtain the total planted acreage for a crop or county, see the crop and county pages available from the Pesticide Use Search Page. See documentation for important distinctions between acres treated and acres planted.
- Acres Treated is the acreage of the crop actually treated with the pesticide. Gross Pounds applied divided by Acres Treated is the application rate of the pesticide. The difference between Acres Planted and Acres Treated is best explained through an example:If a farmer has a 100-acre field and sprays 50 acres, then the Acres Planted will be 100 and the Acres Treated will be 50. If a farmer sprays 50 of their 100 acres three times, then the Acres Planted will remain at 100 acres, but now the value of Acres Treated will be 150. This figure may not be the total acreage of the selected crop. See documentation for further distinctions between acres treated and acres planted.
- Application Count indicates the number of times this chemical was applied.
- Application Rate indicates the total pounds applied to acreage divided by the total treated acres. Only pounds applied to acreage are used to calculate the application rate. This excludes pesticides used to fumigate stored commodities or other non-cropland applications. See documentation for important distinctions between acres treated and acres planted.

- Chemical Class indicates the chemical classification of this chemical.
- Chemical Code is the California Department of Pesticide Regulation code number assigned as an identifier for each chemical.
- Chemical Uses indicates the major uses for this chemical, with the most common use listed first.
- County Code is a code number that various California State agencies use to identify each California county. There are 58 total counties.
- Field Count is the number of fields where this chemical was applied.
- Gross Pounds indicates the total pounds used, including both pounds used on acreage and pounds used in other applications such as commodity fumigation.
- PAN Bad Actor indicates whether this chemical belongs to the group of most toxic pesticides or not. See link for a full definition of this term.

Citation: S. Orme and S. Kegley,*PAN Pesticide Database*, Pesticide Action Network, North America (San Francisco, CA. 2004).

http:www.pesticideinfo.org.

ATTACHMENT 33

EPA Scientific Assessment for α- and β-Pinene Chemicals

Kathryn Boyle
Registration Division, EPA
April 11, 2003

opp-2005-0110-0002

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

APR 20 2005

April 11, 2005

MEMORANDUM

FROM: Kathryn Boyle, Inerts Team
Minor Use, Inerts, and Emergency Response Branch
Registration Division

THROUGH: Pauline Wagner, Inerts Coordinator
Registration Division

TO: Dan Rosenblatt, Chief
Minor Use, Inerts, and Emergency Response Branch
Registration Division

SUBJECT: Science Assessment for alpha- and beta-Pinene Chemicals

The attached science assessment discusses the toxicity of pure alpha- and beta-pinene, and alpha- and/or beta-pinene polymers. This document serves the dual purpose of supporting the tolerance reassessment process for the existing two tolerance exemptions for alpha-pinene (40 CFR 180.920 and 180.930) and the one tolerance exemption for beta-pinene polymers (40 CFR 180.910), and Pesticide Petition 6E4782 to amend the existing exemption for beta-pinene polymers to include alpha- and/or beta-pinene polymers.

The Agency is performing a qualitative assessment. Given the low acute toxicity by the oral, dermal and inhalation routes, the low subchronic toxicity, the lack of reproductive or developmental effects at high dose levels, and the extensive naturally-occurring (primarily inhalation and oral) exposures, a quantitative approach is not needed. Based on this qualitative assessment, EPA finds that exempting alpha- and/or beta-pinene polymers from the requirement of a tolerance will be safe, and that the three existing tolerance exemptions can be reassessed.

Science Assessment for
Alpha- and Beta-Pinene Compounds

I. Executive Summary

This assessment evaluates pure alpha- and beta-pinene, and alpha- and/or beta-pinene polymers. Currently two tolerance exemptions are established for alpha-pinene (40 CFR 180.920 and 180.930), and one tolerance exemption for beta-pinene polymers (40 CFR 180.910). These exemptions must be reassessed under the Food Quality Protection Act. The Agency has been petitioned (6E4782) to amend the existing exemption for beta-pinene polymers to include alpha- and/or beta-pinene polymers.

The data considered in this assessment included information submitted by the petitioner, and information located by OPP on the internet, primarily information prepared by the National Toxicology Program (NTP) and the robust summaries for bicyclic terpene hydrocarbons submitted in 2002 to EPA by the Terpene Consortium of the Flavor and Fragrance High Production Volume Consortia (FFHPVC). The Agency has not conducted a review of the original study, unless specifically stated.

Alpha- and beta-pinene are the major components of turpentine. The two chemicals are closely related, having the same empirical formula of $C_{10}H_{16}$ and the same basic ring structure. The predominant uses of the pure forms of alpha- and beta-pinene are as fragrances.

Alpha- and beta-pinene are of low acute toxicity. Both are irritants to the skin, eye and mucous membranes. The subchronic toxicity of alpha- and beta-pinene compounds appears to be low. In a subchronic oral toxicity study there were no effects at approximately 800 mg/kg/day.

Genotoxicity study summaries indicated no evidence of mutagenicity. No chronic/carcinogenicity studies were identified; however, alpha- and beta-pinene are not structurally related to any known carcinogens.

In three developmental toxicity studies no maternal or developmental effects were noted in mice, hamsters, or rats at the highest dose levels, 560, 600, or 260 mg/kg/day, respectively. Alpha- and beta-pinene are not structurally related to any known developmental/reproductive toxicants.

Most of the turpentine produced in the United States is made up primarily of alpha-pinene (75 to 85%). Turpentine is known to act as a central nervous system (CNS) depressant. Given the relationship of turpentine to alpha-pinene, and the relationship of alpha- to beta-pinene, there could be solvent toxicity concerns for pinene chemicals in the workplace.

The polymers composed of alpha and beta-pinene monomers are of too low a molecular weight to be exempted from the requirement of a tolerance using the criteria specified for

defining a low-risk polymer in 40 CFR 723.250. However, any polymerization process would increase the molecular weight beyond that of the pinene monomers, and therefore decrease absorption. These alpha- and/or beta-pinene polymers should therefore be even less toxic than pure alpha- and beta-pinene.

Exposure to alpha- and beta-pinene can occur from use as a fragrance in consumer products and as a flavoring in foods. However, alpha- and beta-pinene occur naturally in the atmosphere as a result of release from forests, and are present in a variety of foods commonly consumed in the human diet. Alpha- and beta- pinene could be present in sources of drinking water, but are not persistent and would be expected to readily volatilize to the atmosphere. These naturally-occurring exposures are more extensive than such anthropogenic exposures.

The Agency is performing a qualitative assessment. Given the low acute toxicity by the oral, dermal and inhalation routes, the low subchronic toxicity, the lack of reproductive or developmental effects at high dose levels, and the extensive naturally-occurring (primarily inhalation and oral) exposures, a quantitative approach is not needed. Based on this qualitative assessment, EPA finds that exempting alpha- and/or beta-pinene polymers from the requirement of a tolerance will be safe, and that the three existing tolerance exemptions can be reassessed.

Alpha-and beta-pinene are acutely toxic to aquatic organisms, and although documented environmental concentrations are <100 ppb, they may present an acute concern for marine/estuarine invertebrates. Bioconcentration of alpha- and beta-pinene in aquatic organisms may occur. This information was a significant factor in the decision to classify the pinene chemicals as List 4B.

II. Introduction

This review serves two purposes. First, it is conducted to reassess the existing tolerance exemptions as presented in Table 1.

Table 1: Tolerance Exemptions Being Reassessed in this Document

Tolerances Exemption Expression	40 CFR ◊	Uses
beta-Pinene polymers	180.910	Surfactants, related adjuvants of surfactants
alpha-Pinene	180.920 and 180.930	Stabilizer Limitation: Not more than 2% of formulation by weight

◊Residues listed in 40 CFR 180.910[formerly 180.1001(c)] are exempted from the requirement of a tolerance when used in accordance with good agricultural practice as inert (or occasionally active) ingredients in pesticide formulations applied to growing crops or to raw agricultural commodities after harvest.

Residues listed in 40 CFR 180.920 [formerly 180.1001(d)] are exempted from the requirement of a tolerance when used in accordance with good agricultural practice as inert (or occasionally active) ingredients in pesticide formulations applied to growing crops only.

Residues listed in 40 CFR 180.930 [formerly 180.1001(e)] are exempted from the requirement of a tolerance when used in accordance with good agricultural practice as inert (or occasionally active) ingredients in pesticide formulations applied to animals.

Second, this review supports the petition to amend the existing exemption from tolerance for beta-pinene polymers to include alpha- and/or beta-pinene polymers (Petition 6E4782). The chemicals considered include:

Table 2: Chemicals Considered

Common Chemical Name	CAS Nomenclature	CAS Reg. No.	List Classification
alpha-pinene	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl- (9CI) 2-Pinene (8CI)	80-56-8	4B
L-alpha-pinene	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-, (1S,5S) (9CI) 2-Pinene, (1S,5S)-(-)- (8CI)	7785-26-4	---
beta-pinene	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene- (9CI) 2(10)-Pinene (8CI)	127-91-3	---
(S)-beta-pinene	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S,5S)- (9CI) 2(10)-Pinene, (1S,5S)-(-)- (8CI)	18172-67-3	3
Oil of turpentine, alpha-pinene fraction	Terpenes and terpenoids, turpentine oil, alpha-pinene fraction	65996-96-5*	---
Oil of turpentine, beta-pinene fraction	Terpenes and terpenoids, turpentine oil, beta-pinene fraction	65996-97-6**	---

Table 2: Chemicals Considered			
Common Chemical Name	CAS Nomenclature	CAS Reg. No.	List Classification
alpha-pinene polymer	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-, homopolymer	25766-18-1	---
beta-pinene polymer	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, homopolymer (9CI)	25719-60-2	4B
copolymer of alpha- and beta-pinene	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-, polymer with 6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane (9CI)	31393-98-3	---
Polymerized alpha- pinene fraction from turpentine	Terpenes and Terpenoids, turpentine oil, alpha-pinene fraction, polymd.	70750-57-1	---

* Defined by CAS as "The hydrocarbon fraction distilled from oil of turpentine. Contains greater than 80% [alpha]-pinene, the remainder being other terpene hydrocarbons."
** Defined by CAS as "The hydrocarbon fraction distilled from oil of turpentine or produced by the isomerization of [alpha]-pinene. Contains greater than 70% [beta]-pinene"

The Lower Risk Pesticide Chemical Focus Group served as the review body for this assessment of alpha- and beta- pinene compounds.

III. Use Pattern of Alpha and Beta-Pinene

The predominant (non-pesticidal) uses of the pure forms of alpha- and beta-pinene are as fragrances. They are approved by the Food and Drug Administration as food additives (synthetic flavoring substances and adjuvants) for direct addition to food for human consumption (21 CFR 172.515: Synthetic flavoring substances and adjuvants). Alpha-pinene is used as a solvent for protective coatings, polishes, and waxes and in the synthesis of other chemicals such as camphene, camphor, synthetic pine oil and terpine esters. Beta-pinene is used in polyterpene resins and as a feedstock for preparation of menthol, and other flavors and fragrances.

The predominant pesticidal use of the pinene chemicals is as an adjuvant. Adjuvants are used to enhance the performance of a spray application of a pesticide product. Adjuvants are added in the field to the tank mix containing the active pesticidal ingredient. Under 21 CFR 182.99 and 582.99, chemicals used as adjuvants on agricultural food crops must have an

exemption from the requirement of a tolerance under 40 CFR part 180 as established by the Environmental Protection Agency.

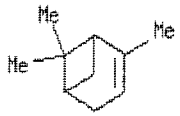
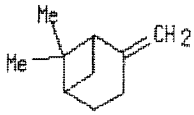
IV. Physical/Chemical Properties of alpha and beta-Pinene

Alpha- and beta-pinene are the major components of turpentine which is manufactured from the resinous sap of pine trees. "Turpentine is a mixture of constituents. The type and amount of specific constituents is dependent on the type of pine tree, the geographical location of the trees, and the season of tree harvest. Turpentine produced in the United States is made up primarily of alpha-pinene (75 to 85%) with varying amounts of beta-pinene (up to 3%)..." New Zealand turpentine is 30 to 50% alpha-pinene and 40 to 60% beta-pinene. (NTPb)

Alpha- and beta-pinene are separated from the turpentine by fractional distillation. Further fractionation in needed to separate the pinenes. They are colorless, transparent, liquids with a turpentine odor. Chemically, they are bicyclic, unsaturated, monoterpene hydrocarbons. The two chemicals are closely related, having the same empirical formula of $C_{10}H_{16}$ and the same basic ring structure, differing only in the placement of a carbon-carbon double bond. Alpha-pinene can be chemically converted to beta-pinene.

Various physical/chemical property data for alpha- and beta-pinene and their structures are presented in Table 3.

Table 3: Chemical/Physical Properties of Alpha- and Beta-Pinene

Property	Alpha-Pinene	Beta-Pinene
Structure		
Molecular Weight	136.24	136.24
Melting point	- 55 °C	----
Boiling point	156.2 °C	----
Vapor Pressure	10 mm Hg at 37.3 °C 4.75 mm Hg at 25 °C	2.93 mm Hg at 25 °C
Log Kow	4.83	4.35 (E)
Koc	1204 (E)	1204 (E)
Water solubility	1.891 mg/L at 25 °C	4.886 mg/L at 25 °C (E)
Henry's Law Constant	2.94×10^{-1} atm-m^3/mole	9.2×10^{-2} atm-m^3/mole (E)
Hydrolysis/Photodegradation	Not expected to occur	Not expected to occur
Biodegradation	Ultimate: weeks to months (E) Primary: days to weeks (E) Complete removal in 250 hours in 3 different soil slurries from Georgia	Ultimate: weeks to months (E) Primary: days to weeks (E)

E= Estimated value as reported in the 2002 EFED Tolerance Review

V. Toxicity Reviews and Evaluation by the Office of Pesticide Programs (OPP)

In August 2001, the Health Effects Division of OPP reviewed the toxicity data submitted in support of Pesticide Petition 6E4782. The following information on dermal irritation, sensitization and subchronic toxicity was extracted from that review and evaluation.

Beta-pinene polymer (CAS Reg. No. 25719-60-2): The amount of absorption is dependent on the molecular weight of the polymer; however, it is considered that beta-pinene polymer is not well-absorbed via any route. No concerns were noted for developmental/reproductive effects, carcinogenicity, or mutagenicity: overall low concern.

VII. Toxicity Reviews and Evaluation by the Terpene Consortium of the Flavor and Fragrance High Production Volume Consortia (FFHPVC)

Alpha- and beta-pinene are sponsored by The Flavor and Fragrance High Production Volume Consortia/The Terpene Consortium under the High Production Volume (HPV) Challenge Program. HPV chemicals are those that are manufactured or imported into the United States in volumes greater than one million pounds per year. Twenty-one companies are current members of the Terpene Consortium. Additional information on the HPV Challenge Program can be found at http://www.epa.gov/chemrtk/volchall.htm.

Alpha-pinene is also sponsored by the Flavor and Fragrance High Production Volume Consortia/The Terpene Consortium in the Voluntary Children's Chemical Evaluation Program. (See http://www.epa.gov/chemrtk/vccep/terpenecons.pdf for the sponsorship letter.) The Agency's website does not indicate the submission of any additional information under VCCEP.

The HPV Test Plan for the Bicyclic Terpene Hydrocarbons was submitted to the Agency in February 2002. (AR201-13610A). HPV submissions are prepared by the submitters. As stated on the Agency's website (see http://www.epa.gov/chemrtk/viewsrch.htm), the submissions are not edited by the Agency. The Agency is unaware of the type of review process used by the submitter in the preparation of the robust summaries.

Summaries of the applicable information on alpha-pinene, beta pinene, l-alpha-pinene, and the pinene fractions of turpentine oil are presented in Tables 4, 5 and 6. Only data rated as reliability code 1 (reliable without restrictions), or reliability code 2 (reliable with restrictions) are included in the tables below. Reliability code 2 is generally assigned if the study was not conducted according to today's standards, but the methodology was comparable, and the results of the study were published in a peer-reviewed journal.

Table 4: Acute Toxicity Studies (FFHPVC-Prepared Robust Summaries for Bicyclic Terpene Hydrocarbons)

Test Substance	Doses	Type of Study (species)	Results	Reliability Code
alpha-Pinene	0, 2020, 3200, 5000, 7800 mg/kg bw	Oral (Rat)	LD_{50} = 3700 mg/kg bw (95% C.L. 2300-5100 mg/kg bw)	1

Table 4: Acute Toxicity Studies
(FFHPVC-Prepared Robust Summaries for Bicyclic Terpene Hydrocarbons)

Test Substance	Doses	Type of Study (species)	Results	Reliability Code
	5000 mg/kg bw	Dermal (Rabbit)	LD_{50} > 5000 mg/kg bw	1
beta- Pinene	5000 mg/kg bw	Oral (Rat)	LD_{50} > 5000 mg/kg bw	1
	5000 mg/kg bw	Dermal (Rabbit)	LD_{50} > 5000 mg/kg bw	1

Table 5: Summary of Toxicological Data for alpha- and beta-Pinene (FFHPVC-Prepared Robust Summaries for Bicyclic Terpene Hydrocarbons)

Test Substance	Doses	Type of Study (species)	Results	Reliability Code
Developmental and Reproductive Toxicity				
Mixture of terpene hydrocarbons (20-25% alpha-pinene and 15 to 18% beta-pinene)	0 (control), 6, 26, 120, 560 mg/kg bw/day	Developmental (Mouse)	"There was no evidence of maternal toxicity or developmental toxicity at dose levels up to and including 560 mg/kg bw/day of test material." Maternal NOAEL is equal to or greater than 560 mg/kg bw/day Maternal LOAEL was not determined, but would be greater than 560 mg/kg bw/day Developmental NOAEL is equal to or greater than 560 mg/kg bw/day Developmental LOAEL was not determined, but would be greater than 560 mg/kg bw/day	2
Mixture of terpene hydrocarbons (20-25% alpha-pinene and 15 to 18% beta-pinene)	0 (control), 6, 28, 130, 600 mg/kg bw/day	Developmental (Hamster)	"The administration of up to and including 600 mg/kg bw/day of test article FDA 71-28 to pregnant golden hamsters on days 6 through 10 of gestation had no effects on nidation, maternal survival or fetal survival. The number and types of abnormalities seen in tissues of the dam or pups of the test groups did not differ for the number and type occurring spontaneously in the positive or negative controls." Maternal NOAEL is equal to or greater than 600 mg/kg bw/day Maternal LOAEL was not determined, but would be greater than 600 mg/kg bw/day Developmental NOAEL is equal to or greater than 600 mg/kg bw/day Developmental LOAEL was not determined, but would be greater than 600 mg/kg bw/day	2

Test Substance	Doses	Type of Study (species)	Results	Reliability Code
Mixture of terpene hydrocarbons (20-25% alpha-pinene and 15 to 18% beta-pinene)	0 (control), 3, 12, 56, 260 mg/kg bw/day	Developmental (Rat)	"The administration of up to and including 260 mg/kg bw/day of test article FDA 71-28 to pregnant Wistar rats on days 6 through 15 of gestation had no effects on nidation, maternal survival or fetal survival. The number and types of abnormalities seen in tissues of the dam or pups of the test groups did not differ for the number and type occurring spontaneously in the positive or negative controls." Maternal NOAEL is equal to or greater than 260 mg/kg bw/day Maternal LOAEL was not determined, but would be greater than 260 mg/kg bw/day	2

It is noted that the reference given for these three developmental toxicity studies indicate that these studies were performed for the Food and Drug Administration.

Table 6: Mutagenicity Studies (FFHPVC-Prepared Robust Summaries for Bicyclic Terpene Hydrocarbons)

Test Substance	Concentration	Species/Strain	Type of Study	Results	Reliability Code
alpha-Pinene	0.5- 300 µL/plate	*Salmonella typhimurium* TA100 and TA98	Ames/ microsome assay Reverse Mutation	No evidence of mutagenicity	2
	4.08, 40.8, 408, and 4080 µg/plate	*Salmonella typhimurium* TA100 and TA98	Ames/ microsome assay Reverse Mutation	No evidence of mutagenicity	2
	25000 µg/plate	*Salmonella typhimurium* TA1535, TA1537, TA1538, TA100 and TA98	Ames/ microsome assay Reverse Mutation	No evidence of mutagenicity	1
	0, 0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, and 25 µL/plate	*Salmonella typhimurium* TA1535, TA1537, TA1538, TA100 and TA98	Ames/ microsome assay Reverse Mutation	No evidence of mutagenicity	1
	0.001, 0.003, 0.01, 0.03, 0.1, 10 µL/mL	Rat hepatocytes	Unscheduled DNA Synthesis Assay	No evidence of genotoxicity	1
beta-Pinene	0, 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0 µL/plate	*Salmonella typhimurium* TA1535, TA1537, TA1538, TA100 and TA98	Ames/ microsome assay Reverse Mutation	No evidence of mutagenicity	1
	Up to 5000 µg/plate	*Salmonella typhimurium* TA1535, TA1537, TA1538, TA100 and TA98	Ames/ microsome assay Reverse Mutation	No evidence of mutagenicity	1
	4.08, 40.8, 408, and 4080 µg/plate	*Salmonella typhimurium* TA100 and TA98	Ames/ microsome assay Reverse Mutation	No evidence of mutagenicity	2
	0, 3.3, 10, 33.3, 100, 333, 1000 µM	Chinese hamster ovary (CHO) cells	Sister Chromatid Exchange	Test substance did not induce sister chromatid exchange in CHO cells	1

VIII. Toxicity Summaries by the National Toxicology Program (NTP)

Health and Safety Summary for alpha-Pinene

The Health and Safety summary for alpha-pinene prepared by NTP indicates that this chemical is a "moderate irritant to skin, eyes and mucous membrane and via oral, inhalation and dermal routes."

Of significant interest is the information that NTP is currently conducting or planning to conduct toxicity studies using alpha-pinene. (NTPc). The 14-day inhalation toxicity study in rats is listed as **on test.** A 13-week inhalation toxicity study in rats and a 2-year inhalation carcinogenicity study in rats are listed as **assigned.** However, given that the background document on which this testing is based is the Turpentine Review (discussed below), the Agency believes that the test substance is actually turpentine.

Turpentine Review of Toxicological Literature

Although this report researched turpentine toxicological literature, given the chemical relationship of turpentine to alpha- and beta-pinene, toxicity data generated using alpha- and beta-pinene were used for assessing the toxicity of turpentine. The results of the author's data search which are not duplicative to previously reported information are discussed below.

Solvent Toxicity:

Turpentine (which is mostly alpha-pinene) is known to act as a central nervous system (CNS) depressant. Typical symptoms of CNS can include headache; fatigue; dizziness; an effect similar to that of ethanol intoxication; difficulty in breathing; irritation of the skin, eyes, nose, and mucous membranes; and can progress to convulsions and death. OSHA's permissible exposure limit (PEL), as an 8-hour time-weighted average, for turpentine is 100 ppm or 560 mg/m^3 as measured in breathing-zone air samples.

Inhalation Acute Toxicity:

Table 7: Acute Inhalation Toxicity Studies (Taken from Table 18 - Toxicological Summary for Turpentine)

Species Tested	Chemical Tested	Test Results
Mouse, rat, mouse, guinea pig	α-pinene from wood turpentine	LC_{100} = 4666 ppm (26,000 mg/m^3) (5 h) (all species)

Mouse, rat, mouse, guinea pig	α-pinene from sulfate turpentine	LC_{100} = 5025 ppm (28,000 mg/m^3) (5 h) (all species)
Mouse, rat, guinea pig	β-pinene from sulfate turpentine	LC_{100} = 3517 ppm (19,596 mg/m^3) (5 h) (all species)
Mouse	(+)- β-pinene	RD_{50} = 1279 ppm (7126 mg/m^3) (30 min)
Mouse	(-)- β-pinene	RD_{50} = 4663 ppm (25,981 mg/m^3) (30 min)
Rat	α-pinene	LCLo = 625 mg/kg (4.59 mmol/kg)
Guinea pig	α-pinene	LCLo = 0.572 mg/m^3) (103 ppb)

LC_{100} is the concentration (dose) that is lethal to 100% of the test animals;
LCLo is the lowest concentration (dose) that resulted in the death of test animals;
RD_{50} is the concentration that causes 50% decrease in respiratory frequency

Short-Term Inhalation: Wistar rats were treated with a commercial turpentine mixture that was 95% alpha-pinene. The dose level was 300 ppm (1670 mg/m^3), 6 hours/day, 5 days/week for up to 8 weeks. "No behavioral abnormalities were observed in treated rats. Exposures resulted in the accumulation of alpha-pinene in fat. One and two week exposures resulted in a reduction of RNA content in the brain, similar to the effects of other solvents."

Chronic/carcinogenic: No alpha-pinene chronic or carcinogenicity studies were identified.

IX. Hazard Characterization

The toxicity of alpha- and beta-pinene is defined by studies from open-literature conducted with alpha-pinene, beta-pinene and various alpha- and beta pinene mixtures and/or polymers. There is also a structure-activity-relationship (SAR) assessment. The database is sufficient for the purposes of this document. Alpha- and beta-pinene are of low acute toxicity via the oral, dermal and inhalation routes. Both alpha-and beta-pinene are irritants to the skin, eye and mucous membranes. Alpha- and beta-pinene are well-absorbed by all routes of exposure.

The subchronic toxicity of alpha- and beta-pinene compounds appears to be low. A subchronic oral toxicity study reviewed by HED during evaluation of Petition 6E4782 indicated minor changes in liver and thryoid weights at the two higher dose levels, which were not considered treatment related. There were no effects at approximately 800 mg/kg/day.

Genotoxicity study summaries indicated no evidence of mutagenicity in several *Salmonella typhimurium* reverse mutation assays, one unscheduled DNA assay, and one sister

Dermal Irritation

"Undiluted [alpha] pinene applied to the backs of hairless mice and swine was not irritating. However, once applied to intact or abraded rabbit skin for 24 hr under occlusion it was a **moderate irritant**. When tested in 10% petroleum it produced no irritation after a 48 hr close patch test on two different panels of human subjects. Beta pinene applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was a **moderate irritant**. When tested in 12% petroleum it produced no irritation after a 48 hr close patch test on human subjects."

Dermal Sensitization

"In a dermal human sensitization study, [alpha] and [beta]-pinene produced no dermal sensitization when tested at concentration of 10% and 12% in petroleum, respectively."

Subchronic toxicity

In a 3-month oral toxicity study, rats were fed an alpha pinene resin or pinene polymer made predominantly from alpha-pinene. (The ratio of alpha- and beta-pinene was 10:1.) The dose levels were 0, 1, 3 or 5% in the diet. Effects seen at 5% (3967 mg/kg/day) included an increase in relative liver weight in both sexes, and absolute liver weight in females only. Increased relative thyroid weights in males were noted at the 3 and 5% dose levels. In the absence of histopathological alterations, these changes were not considered treatment related. No effects were noted at 1%, which corresponds to roughly 800 mg/kg/day.

VI. Structure-Activity-Relationship (SAR) Assessments Performed by OPPT

In the early 1990s, the Office of Pollution Prevention and Toxic Substances (OPPT) performed as part of an OPP inert ingredient list reclassification project SARs for several hundred inert ingredients including alpha-pinene and beta-pinene polymers. Toxicity for these two chemicals was assessed by a process called structure-activity relationship. In this process, the chemical's structural similarity to other chemicals (for which data are available) is used to determine toxicity. For human health, this process can be used to assess absorption and metabolism, mutagenicity, carcinogenicity, developmental and reproductive effects, neurotoxicity, systemic effects, immunotoxicity, and sensitization and irritation. This is a qualitative assessment using terms such as good, not likely, poor, moderate, or high. The conclusions of the team performing the SAR are as follows.

alpha-Pinene (CAS Reg. No. 80-56-8): Alpha-pinene is well-absorbed via the skin, lungs, and gastro-intestinal tract. No concerns were noted for developmental/reproductive effects, carcinogenicity, or mutagenicity.

chromatid exchange assay. No chronic/carcinogenicity studies were identified; however, alpha- and beta-pinene are not structurally related to any known carcinogens.

A mixture of alpha- and beta-pinene (and other terpene hydrocarbons) were tested in three developmental toxicity studies. Summaries of the results of these studies report that no maternal or developmental effects were noted in mice, hamsters, or rats at the highest dose levels, 560, 600, or 260 mg/kg/day, respectively. Alpha- and beta-pinene are not structurally related to any known developmental/reproductive toxicants.

The available information does not indicate that any of these chemicals are of higher toxicity. For alpha- and beta-pinene, the irritation effects are of concern. Additionally, given the fact that the turpentine produced in the United States is made up primarily of alpha-pinene (75 to 85%), and that turpentine is known to act as a CNS depressant, by extrapolation, there could be solvent neurotoxicity concerns for pinene chemicals from dermal and inhalation exposures. Exposures generally need to be "high" and/or "prolonged" for these solvent toxicity effects to occur. Also, for acute exposures such effects, generally, are reversible. Concerns are for occupational exposures since the potential for day in/day out exposure can occur in the workplace.

The polymers composed of alpha and beta-pinene monomers are of a low molecular weight, and thus cannot be exempted from the requirement of a tolerance using the criteria specified for defining a low-risk polymer in 40 CFR 723.250. An MSDS for a pinene polymer (CAS Reg. No. 31393-98-3) describes the chemical as a viscous liquid. Processes that could increase the molecular weight beyond that of alpha- or beta-pinene include formation of a dimer (two "pinenes" in a single molecule), formation of a trimer (three "pinenes" in a single molecule), or polymerization. Greater molecular weight means decreased absorption. Alpha- and/or beta-pinene dimers, trimers, or polymers should therefore be even less toxic than pure alpha- and beta-pinene.

X. Exposure Assessment

Exposure to alpha- and beta-pinene can occur from use as a fragrance in consumer products and as a flavoring in foods. However, as discussed below, the naturally-occurring exposures to alpha- and beta-pinene are more extensive. For this section, generally only the exposures of alpha-pinene are discussed. According to Toxnet's beta-pinene summary (TOXNETb), beta-pinene naturally occurs with the alpha-pinene, but at lower concentrations.

Atmospheric

Total US emission of alpha-pinene from deciduous and coniferous forests amounted to 6.6 mega tons annually. An estimated emission rate of alpha-pinene from natural sources to the atmosphere is 1.84×10^{-10} g/sq cm/sec. (TOXNETa)

In a compilation of published and non-published data on the atmospheric concentration of volatile organic compounds determined between 1970 to 1987, the daily mean concentration of alpha-pinene in suburban and urban areas is 0.147 ppb and 0.120 ppb, respectively. the daily mean concentration of alpha-pinene in remote and rural areas is 0.035 ppb, 0.030 ppb, respectively. (USEPA as cited in TOXNETa)

Dietary

According to Toxnet's alpha-pinene summary, alphà-pinene is a component of trees, fruits, grasses, bushes, fungi, herbs, and flowers. Alpha-pinene has been detected in filberts, chicken, mangos, fresh grapefruit juice (0.054 ppm), guava, carrots, pistachio, safflower, sorghum, tomato, walnut, ginger, celery, unpasteurized orange juice (0.10-1.09 ppm), shrimp, and crab. Alpha-pinene is also a constituent of over 400 essential oils.

Environmental Fate Characterization/ Drinking Water Consideration

Alpha-pinene is not expected to persist in the environment. Alpha-pinene has been detected in the river water and sea water. Concentrations were reported in the low parts per billion (<100 ppb). Alpha-pinene will readily volatilize from soil and flowing water to the atmosphere within hours, and within days from lakes. Once in the atmosphere, alpha-pinene degrades with an estimated half-life of 4 hours. Reaction with ozone occurs with a half-life of approximately 40 minutes. Night-time reactions with nitrate radicals occur with a half-life of 6 minutes. Biological degradation is expected to occur rapidly in aerobic soils, and can proceed to complete removal. Degradation in seawater samples occurred with a half-life of approximately 6-8 hours.

Beta-pinene is also not expected to persist in the environment, and will readily volatilize from soil and flowing water to the atmosphere within hours and within days from lakes. Once in the atmosphere, beta-pinene will degrade with photochemically-produced hydroxyl radicals with an estimated half-life of 4.9 hours. Reaction with ozone occurs with a half-life of approximately 22 hours. Biological degradation is expected to occur rapidly in aerobic soils.

Neither alpha- nor beta-pinene are persistent in the environment. Given the ready volatilization and rapid degradation of alpha- and beta-pinene, it is unlikely to be present in any significant amounts in sources of drinking water.

XI. Aggregate Exposures

In examining aggregate exposure, section 408 of the FFDCA directs EPA to consider available information concerning exposures from the pesticide residue in food and all other non-occupational exposures, including drinking water from ground water or surface water and exposure through pesticide use in gardens, lawns, or buildings (residential and other indoor uses).

Given their volatility, pinenes are present in the atmosphere. They are present in the foods that are consumed on a daily basis. They could be present in sources of drinking water, but are not persistent and would be expected to readily volatilize to the atmosphere. The uses regulated by EPA are much smaller than the naturally-occurring exposures.

XII. Cumulative Exposure

Section 408(b)(2)(D)(v) of the FFDCA requires that, when considering whether to establish, modify, or revoke a tolerance, the Agency consider "available information" concerning the cumulative effects of a particular pesticide's residues and "other substances that have a common mechanism of toxicity."

EPA has not made a common mechanism of toxicity finding as to alpha- or beta-pinene and any other substances. They do not appear to produce a toxic metabolite produced by other substances. For the purposes of this tolerance action, therefore, EPA has not assumed that alpha- and beta-pinene have a common mechanism of toxicity with other substances. For information regarding EPA's efforts to determine which chemicals have a common mechanism of toxicity and to evaluate the cumulative effects of such chemicals, see the policy statements released by EPA's Office of Pesticide Programs concerning common mechanism determinations and procedures for cumulating effects from substances found to have a common mechanism on EPA's website at http://www.epa.gov/pesticides/cumulative/.

XIII. Safety Factor for Infants and Children

FFDCA section 408 provides that EPA shall apply an additional tenfold margin of safety for infants and children in the case of threshold effects to account for prenatal and postnatal toxicity and the completeness of the data unless EPA concludes that a different margin of safety will be safe for infants and children. Three developmental toxicity studies (rat, mouse and hamster) conducted using a mixture of alpha- and beta-pinenes at high dose levels did not identify either maternal or developmental NOAELs. There are no indications of increased susceptibility. These pinene chemicals are not structurally related to any known developmental/reproductive toxicants. Therefore, EPA has not used a safety factor analysis to assess the risk. For the same reasons a tenfold safety factor is unnecessary.

XIV. Determination of Safety for US Population, and Infants and Children

The database considered for this action included mostly toxicity data derived using alpha- and beta-pinene. Alpha- and beta-pinene exhibit low acute toxicity by the oral, dermal and inhalation routes, and low subchronic toxicity. Polymers composed of alpha and beta-pinene monomers, even those of low molecular weight, should be even less toxic than alpha- and beta-pinene considering that their absorption is decreased. Based on the available information on toxicity and exposure, EPA concludes that there is a reasonable certainty of no harm from aggregate exposure to residues of alpha-pinene, beta-pinene, and alpha- and/or beta-pinene polymers. EPA finds that amending the existing exemption from the requirement of a tolerance

for beta-pinene polymers to include alpha- and/or beta-pinene polymers will be safe for the general population including infants and children.

XV. Conclusions Pertinent to the Food Quality Protection Act

In Consideration Pesticide Petition 6E4782:

EPA finds that exempting alpha- and/or beta-pinene polymers from the requirement of a tolerance will be safe. The existing exemption for beta-pinene polymers in 40 CFR 180.910 can be amended to include alpha- and/or beta pinene polymers. The following CAS Reg. Nos. have thus far been identified: 25719-60-2, 25766-18-1, 31393-98-3, and 70750-57-1.

In Consideration of Tolerance Reassessment:

The existing exemptions for alpha-pinene and beta-pinene polymers can be reassessed. There are several forms of alpha-pinene (CAS Reg. Nos. 80-56-8, 7785-26-4, and 65996-96-5), all of which are included in the reassessment. The limitation of 2% by weight in the formulation is to remain; however, the exemption should be corrected to indicate that the use of alpha-pinene is as a solvent or fragrance component, not as a stabilizer.

Toxicologically speaking, there is little difference between alpha- and beta-pinene. Given the available information a tolerance exemption could be established for beta-pinene (CAS Reg. Nos. 127-91-3, 18172-67-3, and 65996-97-6). The limitation and use pattern is the same as that for alpha-pinene.

XVI. Ecotoxicity Assessment Developed by OPP/EFED

Alpha-pinene is very highly toxic to aquatic organisms on an acute basis, and may present an acute concern for marine/estuarine invertebrates based on documented environmental concentrations near the levels of predicted toxicity. Acute toxicity estimates are 0.72 mg/L for freshwater fish, 0.51 mg/L for marine/estuarine fish, 0.93 mg/L for *Daphnia magna*, 0.042 mg/L for mysid shrimp, and 0.66 mg/L for green algae. Chronic toxicity estimates for freshwater fish is 0.138 mg/L. Terrestrial animal toxicity based on available rat data would indicate that alpha-pinene is practically non-toxic on an acute basis.

Beta-pinene is very highly toxic to aquatic organisms on an acute basis, and may present an acute concern for marine/estuarine invertebrates based on probable environmental concentrations at or near the levels of predicted toxicity. Acute toxicity estimates are 0.62 mg/L for freshwater fish, 0.45 mg/L for marine/estuarine fish, 0.79 mg/L for *Daphnia magna*, 0.034 mg/L for mysid shrimp, and 0.56 mg/L for green algae. Chronic toxicity estimates for freshwater fish is 0.117 mg/L. Terrestrial animal toxicity based on available rat data would indicate that β-pinene is practically non-toxic on an acute basis.

Bioconcentration of alpha-pinene in aquatic organisms may occur based on an estimated BCF of 2800. For beta-pinene, bioconcentration in aquatic organisms may occur based on an estimated BCF of 444.

XVII. List Reclassification:

Given the ecotoxicity assessment which indicated concerns for aquatic organisms and bioconcentration, and the specification in the tolerance exemption for a limitation of 2% by weight in the formulation, all CAS Reg. No.s discussed in this document are confirmed or reclassified as List 4B.

XVIII. References

EPA/OPP; Environmental Fate and Effects Division (EFED) Tolerance Review of Compounds of Group 13, Lignins, Cellulose and Pinenes as Inert Ingredients in Terrestrial and/or Aquatic Agricultural and Non-Agricultural Uses; April 10, 2002; Memorandum from Sid Abel (EFED) to Kathryn Boyle (RD).

EPA/OPP; Health Effects Division (HED): An Evaluation of Hercules Inc. Petition for the Expansion of the Existing Exemption from Tolerance for β-Pinene Polymers to Include Polymers Made with α-Pinenes and Mixtures of α and β Pinenes (Petition #: 6E04782); August 16, 2001; Memorandum from Waheeda Mani Tehseen (HED) to Robert Forest (RD).

FFHPVC (Flavor and Fragrance High Production Volume Consortia); The Terpene Consortium; Robust Summaries for Bicyclic Terpene Hydrocarbons; AR201-13610B; accessed February 19, 2002; http://www.epa.gov/chemrtk/bictrphy/c13610rs.pdf

NTPa (National Toxicology Program); Health and Safety Information for alpha-Pinene (CAS No. 80-56-8);(accessed March 14, 2002). Note: Information has since been removed to http://ntp-db.niehs.nih.gov/htdocs/H&S_archive.zip

NTPb; Turpentine, Toxicological Literature Review; accessed February 28, 2005; http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/turpentine.pdf

NTPc; Testing Status: Alpha Pinene, accessed February 28, 2005; http://ntp.niehs.nih.gov/INDEX.CFM?OBJECTID=07105185-B741-02BD-FE4334DA286D3041

TOXNETa. Hazardous Substance Data Bank (HSDB). On-line Scientific Search Engine, National Library of Medicine, National Institute of Health; (http://www.toxnet.nlm.nih.gov.) Search terms: 80-56-8

TOXNETb. Hazardous Substance Data Bank (HSDB). On-line Scientific Search Engine, National Library of Medicine, National Institute of Health; (http://www.toxnet.nlm.nih.gov.) Search terms: 127-91-3

Acknowledgement: The Agency was assisted in the preparation of this document by Versar, Inc. Under GSA Contract Number EP-05-W-000253.

ATTACHMENT 34

Acute Oral Toxicity Study in Rats on Miller 6064

STILLMEADOW
INCORPORATED

VOLUME __ OF __ OF SUBMISSION

Miller 6064

FINAL REPORT

ACUTE ORAL TOXICITY STUDY IN RATS

OPPTS NO. 870.1100

AUTHOR:

Janice O. Kuhn, Ph.D., D.A.B.T.

STUDY INITIATION DATE: 26 December 2000
STUDY COMPLETION DATE: 26 March 2001

CONDUCTED BY:
STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, TX 77478

LABORATORY STUDY NUMBER:

6206-00

VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 19

SUBMITTED TO:
Miller Chemical and Fertilization Corp.
P.O. Box 333
Hanover, PA 17331

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA § 10 (d) (1) (A), (B) or (C).

Company: Miller Chemical and Fertilization Corp.

Company Agent: _____ Date: _____

_____ _____

Title Signature

These data are the property of Miller Chemical and Fertilization Corp., and as such, are considered to be confidential for all purposes other than compliance with FIFRA § 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other statute or in any other country.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was designed and performed in STILLMEADOW, Inc.'s AAALAC accredited laboratory in compliance with:

- United States Environmental Protection Agency FIFRA; GLP Standards 40 CFR 160 with exception of Sec. 160.31 (d), and 160.105 (b)(e): stability information was not provided
- United States Environmental Protection Agency TSCA 40 CFR 792 with exception of Sec. 792.31 (d), and 792.105 (b)(e): stability information was not provided
- Organization for Economic Cooperation & Dev. Principles of GLP, Annex 2, C(97)186 with exception of Sec. 6.2 (4): stability information was not provided
- Japan Ministry of Agriculture, Forestry & Fisheries, Notification No. 59 Nohsan 3850, Director-General of Agricultural Prod. Bureau, 10 Aug 84 with exception of Art. 5 (2)(9), and 21 (3): stability information was not provided

Janice O. Kuhn, Ph.D., D.A.B.T.
Study Director, STILLMEADOW, Inc.

26 Mar 01
Date

Signature of Agent of Sponsor

Date

Agent Name
Sponsor: Miller Chemical and Fertilization Corp.

Signature of Agent of Submitter

Date

Agent Name
Submitter: Mandava Associates

TABLE OF CONTENTS

QUALITY ASSURANCE STATEMENT

Study Number: 6206-00
Test Substance: Miller 6064
Study Title: Acute Oral Toxicity Study in Rats

The study report and data have been audited in accordance with STILLMEADOW, Inc. Standard Operating Procedures (SOPs). The findings from the inspection and audit were reported to the Study Director and management as follows:

Phase of Study Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
Dosing	10 Jan 01	11 Jan 01	11 Jan 01
Report/Data Audit	20 Feb 01	20 Feb 01	20 Feb 01

Darrell Zeno

Darrell Zeno, M.S.
Quality Assurance Unit, STILLMEADOW, Inc.

26 Mar 01

Date

SUMMARY

The test substance, Miller 6064, was evaluated for its acute oral toxicity potential in albino rats when administered as a single gavage dose at a level of 5050 mg/kg to males and females. No mortality occurred during the study. Clinical signs included diarrhea, nasal discharge, polyuria and salivation, which were no longer evident by Day 6. There was no effect on body weight gain. The gross necropsy conducted at termination of the study revealed no observable abnormalities, except a herniated liver in one animal. The acute oral LD_{50}, as indicated by the data, was determined to be greater than 5050 mg/kg.

INTRODUCTION

The objective of this study was to assess the acute oral toxicity potential of the test substance when administered by gavage to rats in accordance with US EPA OPPTS 870.1100, which is intended to meet testing requirements of FIFRA 7 USC 136, et seq, and TSCA 15 USC 2601. This study was conducted for Miller Chemical and Fertilization Corp., according to the approved protocol and STILLMEADOW, Inc. SOPs. There were no deviations from the protocol which affected the quality or outcome of the study. All procedures used in this study are in compliance with Animal Welfare Act Regulations. The protocol, raw data and this report are archived at STILLMEADOW, Inc. The animals were treated as follows:

Dose		Male Treatment		Female Treatment		In-life Termination Date	
mg/kg	mL/kg	Date	Time	Date	Time	Males	Females
5050	5.50	10 Jan 01	0920	10 Jan 01	0925	24 Jan 01	24 Jan 01

TEST SUBSTANCE

Identification:	Miller 6064
Date & Quantity Received:	19 Dec 00; 2 x 1 gal
Physical Description:	Amber liquid
Storage:	Room temperature
Density:	0.9179 g/mL
Purity & Composition:	Refer to Certificate of Analysis (Appendix A)
Stability:	Not provided by sponsor

Records pertaining to stability, characterization and verification of test substance identity are the responsibility of the sponsor. A copy of the Certificate of Analysis is included in the report.

TEST SYSTEM

Experimental Animals

Species & Strain:	Albino rat; Sprague-Dawley
Justification of Species:	The rat is a representative rodent species preferred by various regulatory agencies for use in an acute oral study.
Source:	Texas Animal Specialties, Humble, TX
Date Received:	4 Jan 01
Quarantine Period:	5 days
Quantity & Sex:	5 males and 5 females (nulliparous and non-pregnant) were selected for testing
Group Identification:	Cage cards
Animal Identification:	Ear punch
Fasted Wt on Dosing Day:	Males: 236-244 g; Females: 159-181 g
Date of Birth:	14 Nov 00

Animal Husbandry

Cage Type:	Suspended, wire bottom, stainless steel
Housing:	1 per cage
Environmental Controls Set to Maintain:	· Temperature Range 22°C±3° · Humidity Range 30-70% · 12-hour light/dark cycle · 10-12 air changes/hour
Food:	PMI Feeds Inc.™ Formulab #5008; available *ad libitum* except for approximately 16 hours before dosing
Water:	Municipal water supply analyzed by TNRCC Water Utilities Division; available *ad libitum* from automatic water system.

Animal husbandry and housing at STILLMEADOW, Inc. comply with Animal Welfare Act Regulations. No contaminants were expected to have been present in the feed or water which would have interfered with or affected the results of the study.

PROCEDURES

Test Substance Administration

The test substance was administered as received and was not diluted. An individual dose was calculated for each animal based on its fasted body weight and administered by gavage at a volume of 5.50 mL/kg. Each dose was administered using an appropriately sized syringe and stainless steel ball-tipped intubation needle. The animals were returned to their cages immediately after dosing.

In-life Observations

Observations for mortality and clinical/behavioral signs of toxicity were made at least three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14.

Postmortem Observations

On Day 14 after dosing, each animal was euthanized by an overdose of CO_2. All study animals were subjected to gross necropsy and all abnormalities were recorded.

RESULTS AND DISCUSSION

Mortality/Estimated Lethality Values

There was no mortality during the study. The estimated acute oral LD_{50}, as indicated by the data, was determined to be greater than 5050 mg/kg.

Clinical Signs

Clinical signs are presented in Table 2. Clinical signs included diarrhea in both sexes, and nasal discharge, polyuria and salivation in females. Animals were asymptomatic by Day 6.

Body Weights

Individual body weights are presented in Table 1. Body weight gain was unaffected by the administration of the test substance.

Necropsy Findings

Individual necropsy findings are presented in Table 1. The gross necropsy conducted at termination of the study revealed no observable abnormalities, except for a herniated liver in one male.

CONCLUSION

The test substance, Miller 6064, was evaluated for its acute oral toxicity potential when administered to albino rats. The acute oral LD_{50}, as indicated by the data, is greater than 5050 mg/kg in males and females.

Study Director: Janice O. Kuhn, Ph.D., D.A.B.T. — Date: 26 Mar 01
Senior Toxicologist, STILLMEADOW, Inc.

STUDY PERSONNEL

Technical Staff: Carol Morris, B.A.; Hector Fuentes; Paul Siemens, B.A.; Michelle Gantt, B.S.

Data Services: Connie Pavatte, Report Preparation

TABLE 1
ACUTE ORAL TOXICITY STUDY IN RATS
Body Weights, Time of Death, and Gross Necropsy
Test Substance: Miller 6064
Dose Level: 5050 mg/kg (5.50 mL/kg)

Animal Number	Body Weights (g)			Time of Death*	Gross Necropsy Findings
	Day 0	Day 7	Final		
111-M	237	284	341	Day 14	NOA
112-M	236	274	309	Day 14	NOA
113-M	244	295	307	Day 14	NOA
114-M	240	300	342	Day 14	NOA
115-M	238	283	322	Day 14	Liver herniated through diaphragm.
116-F	169	201	226	Day 14	NOA
117-F	166	193	215	Day 14	NOA
118-F	172	197	229	Day 14	NOA
119-F	181	223	249	Day 14	NOA
120-F	159	186	215	Day 14	NOA

* - Day of dosing considered Day 0; Day 14 is terminal sacrifice.
M - Male; F - Female; NOA - No Observable Abnormalities

TABLE 2
ACUTE ORAL TOXICITY STUDY IN RATS
Pharmacologic and/or Toxicologic Signs
Test Substance: Miller 6064
Dose Level: 5050 mg/kg (5.50 mL/kg)
Sex: Males and Females

	Time After Treatment																
	HOURS			DAYS													
Reaction and Severity	1	2	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Males																	
Diarrhea (s-m)	1	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Females																	
Polyuria (v-m)	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0
Salivation (m)	2	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Clear nasal discharge (m)	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diarrhea (v-m)	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0

v - very slight; s - slight; m - moderate; e - extreme
Note: Digits indicate number of animals exhibiting reaction.

APPENDIX A

MILLER® CHEMICAL & FERTILIZER CORPORATION

P.O. BOX 333, 120 RADIO ROAD
HANOVER, PENNSYLVANIA 17331 U.S.A.
TELEPHONE: 717-632-8921
FAX NO.: 717-632-4581

1/18/01

CERTIFICATE OF ANALYSIS

Product Name: MILLER 6064
Quantity Produced: 7.4 kilograms
Batch Number: 150386
Date of Formulation: 12/13/00

Appearance: Yellow to Amber Liquid
Odor: Terpenic
Moisture: Negligible (non-aqueous)
Specific Gravity: 0.92 - 0.94 @ 20c
Viscosity @ 20c: 100 to 500 cps
pH(1% solution): 6.0 to 8.0
Solubility in Water: Insoluble (Emulsifies)
Flash Point: over 200 f
Purity: 100%

Signature:

Name: Andrew G. Smith
Title: Compliance Officer

APPENDIX B

STILLMEADOW
INCORPORATED

PROTOCOL FOR STUDY 6206-00

Study Title: ACUTE ORAL TOXICITY STUDY IN RATS (OPPTS 870.1100)

Test Substance: MILLER 6064

Test Facility: STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, Texas 77478

Approved: _____ 26 Dec 00
Janice O. Kuhn, Ph.D., D.A.B.T. Date
Study Director
STILLMEADOW, Inc.

Approved: _____ 6 Dec 00
Mark S. Holbert Date
Vice President
STILLMEADOW, Inc.

Reviewed: _____ 6 Dec 2000
Vicki S. Crutchfield, RQAP Date
Director, Quality Assurance Unit
STILLMEADOW, Inc.

Sponsor: Miller Chemical and Fertilization Corp.
P.O. Box 333
Hanover, PA 17331

Sponsor Representative: Mandava Associates
1730 M Street, N.W., Suite 906
Washington, DC 20036

Approved: _____ December 26, 2000
N. Bhushan Mandava Date
Agent to Miller Chemical and Fertilization Corp.

APPENDIX B (cont.)

PROTOCOL FOR STUDY 6206-00

A. GENERAL

1. Study Title: ACUTE ORAL TOXICITY STUDY IN RATS

2. Purpose: To assess the acute oral toxicity potential of the test substance when administered by the oral route (gavage) to rats.

3. Regulatory Compliance: This study will be conducted according to OPPTS 870.1100, which is intended to meet testing requirements of both FIFRA (7 U.S.C. 136, *et seq.*) and TSCA (15 U.S.C. 2601).

This study will be conducted in compliance with Good Laboratory Practice Standards:
1. EPA FIFRA: 40 CFR 160
2. EPA TSCA: 40 CFR 792
3. OECD: C(97)186
4. Japanese MAFF

All procedures in this protocol are in compliance with Animal Welfare Act Regulations. All methods can be found in STILLMEADOW, Inc. Standard Operating Procedures (SOPs).

4. Quality Assurance: The Protocol is reviewed by the Quality Assurance Unit (QAU). The study information will be entered into the Master Schedule. The study will be inspected at least once during its progress. Further inspections may be scheduled as needed to ensure the integrity of the study. Any deviations from SOPs, the Protocol, or Good Laboratory Practice Standards will be immediately reported to the Study Director and Management. The report will be audited and a statement prepared and signed which shall specify the dates inspections were made and findings reported to Management and to the Study Director.

5. Test Substance: MILLER 6064. Test substance identification should include the name, batch number, and purity. Information regarding safety, stability, storage conditions, and disposal should also be provided by the Sponsor. The Sponsor assumes responsibility for purity and stability determinations. Analysis and archiving of reserve samples will be the responsibility of the Sponsor.

6. Proposed Schedule: Testing will begin within approximately three weeks of receipt of the test substance and authorization to conduct the study.

Proposed Start Date: 20 Dec 00
Proposed End Date: 03 Jan 01

The study will be extended if several dose levels are required.

APPENDIX B (cont.)

A. GENERAL (cont.)

7.	Study Director:	Janice O. Kuhn, Ph.D., D.A.B.T.
8.	Experimental Summary:	The test substance will be administered to rats orally by gavage. The animals will be observed three times on the day of dosing for mortality and signs of pharmacologic and/or toxicologic effects and once daily thereafter for at least 14 days. If a sufficient number of dose levels are tested, an LD_{50} with slope function and 95% confidence limits will be calculated, and/or a Toxicity Category will be assigned.
9.	Protocol Amendments:	Any alteration in the Protocol will be justified, approved by the Study Director, and recorded in writing.
10.	Sponsor Audits:	The Sponsor may send an authorized representative to inspect the test system and/or data on the STILLMEADOW, Inc. premises during normal working hours.

B. EXPERIMENTAL DESIGN

1. Animals

a. Species:	Albino rat
b. Strain/Source:	Sprague-Dawley (Texas Animal Specialties, Humble Texas or other suitable source)
c. Justification of Species:	The rat is conventionally used to provide an index of toxicity on which human hazard can be judged, and is the species preferred by the regulatory agencies.
d. Quantity and Sex:	Five males and five females (nulliparous and non-pregnant) for the initial dose level and 5/sex for any additional dose levels, if required (see B.3.g.).
e. Age/Weight:	Young adult (8 - 12 weeks) Males: approximately 225 - 330 grams Females: approximately 175 - 250 grams. Weight variation should not exceed ±20% of the mean for each sex.
f. Identification:	Ear punch
g. Acclimation and Health Status:	Animals will be acclimated for at least five days prior to testing. Normal weight gain, appearance, and behavior will be factors used to select healthy animals for testing. Only naive animals will be selected.
h. Randomization:	Unless a control group is required (see B.3.f.) no formal randomization procedure will be required. If a control group is necessary, a weight-stratified randomization procedure will be employed.

2. Animal Husbandry

a. Cages:	Stainless steel, suspended, wire bottom
b. Number per Cage:	Animals will be housed individually during the study.
c. Food:	PMI Feeds, Inc.™ Formulab #5008, available *ad libitum* prior to fasting and after dosing, or equivalent. Analyzed by manufacturer for nutritional content.
d. Water:	Tap water; available *ad libitum* (automatic system). Municipal water supply analyzed by Texas Natural Resource Conservation Commission (TNRCC) Water Utilities Division.
e. Contaminants:	There are no known contaminants in the feed or water available to laboratory animals that would be expected to interfere with this study.
f. Environment:	Target temperature: approximately 22°C ± 3°C. Target relative humidity: approximately 30 - 70%. 12-hour light/dark cycle (regulated automatically). Room ventilation of approximately 10 - 12 air changes per hour.

APPENDIX B (cont.)

B. EXPERIMENTAL DESIGN (cont.)

3. Test Substance Administration

a. Preparation of Animals: Animals will be fasted for at least 16 hours prior to dosing. Food will be made available immediately after dosing.

b. Reason for Route of Administration: Historically, the oral route has been a route of choice for evaluation of the toxicity potential of a test substance and is a potential route of human exposure.

c. Assignment of Animals to Groups: Animals will be randomly selected for dose groups so that individual body weights will not exceed ±20% of the mean weight for each sex.

d. Preparation of Test Substance: The test substance will be administered as received, if possible, or diluted in an appropriate vehicle to the most concentrated workable dilution. If the substance is an aerosol, it will be discharged into a container and administered as a liquid. All animals in a dose group will receive the same concentration of the test substance. Maximum dose volume will not exceed 1 mL/100 g, or for aqueous solutions, 2 mL/100 g. The dosing solutions will be prepared on the day of dosing and the prepared dosing solutions will be stored at room temperature until administration, within three hours after mixing.

e. Dosing: The animals will be dosed by gavage with an appropriately-sized stainless steel ball-tipped dosing needle and syringe. Individual doses will be calculated based upon the animal's body weight on the day of test substance administration.

f. Control Groups: If the test substance is administered as received, a control group is not necessary. If the test substance is administered in a vehicle for which the toxicity is not known, then a vehicle control group (five males and five females) will be required.

g. Dose Level: Unless available toxicologic information indicates otherwise, a single dose level of approximately 5050 mg/kg (or 2020 mg/kg if requested by Sponsor) will be administered to five animals per sex. If no mortality occurs at this level, no further testing is required.

If mortality meets or exceeds 40% in either or both sexes at the level tested, then at least two additional dose levels may be tested for those sexes. There will be at least five animals (five males and/or five females) per dose level. The number and spacing of dose levels will be chosen so that an LD_{50} and/or a Toxicity Category can be determined. If both sexes are tested at a given dose level, then the group will consist of an equal number of males and females. If animals of one sex are markedly more sensitive, testing in animals of the other sex may be dispensed with.

B. EXPERIMENTAL DESIGN (cont.)

4. Observations

a. Clinical Signs: Observations for mortality and signs of pharmacologic and/or toxicologic effects will be made three times on the day of dosing, and once daily thereafter for 14 days. The duration of the study should be determined by the toxic reactions and may be extended beyond 14 days when considered necessary. The nature, onset, severity, and duration of all gross or visible pharmacologic or toxicologic signs will be recorded.

Observations will include but not necessarily limited to evaluation of skin, fur, eyes and mucous membranes, respiratory and circulatory effects, autonomic effects such as salivation, lacrimation, excessive urination, diarrhea, central nervous system effects, including tremors, and convulsions, changes in the level of activity, gait and posture, reactivity to handling or sensory stimuli, altered strength, and stereotypies or bizarre behavior (e.g., self mutilation, walking backwards).

b. Body Weights: Body weights will be recorded on the day prior to dosing (Day -1), the day of dosing (Day 0), and weekly thereafter (Days 7 and 14), or at the time of discovery after death.

c. Sacrifice of Animals: All surviving animals will be euthanized by an overdose of carbon dioxide.

d. Necropsy: A gross necropsy will be conducted on each animal at termination of the study or at the time of discovery after death, and the results recorded. The gross necropsy shall include terminal body weight and gross observations of external surfaces; all orifices; and thoracic abdominal, and pelvic cavities.

5. Evaluation of Results: Unless only a single dose level is tested, an LD_{50} with slope function and 95% confidence limits will be calculated for males, females, and males and females combined (if necessitated by mortality in one or both sexes) by the method of Litchfield and Wilcoxon (Litchfield, J.T., Jr., and Wilcoxon, F.: A Simplified Method of Evaluating Dose-Effect Experiments, J. Pharm. & Exp. Ther., 96, 99-115, 1949) or other appropriate method. If requested by the Sponsor, a Toxicity Category may be determined instead of an LD_{50} or in addition to an LD_{50}.

6. Test Substance Accountability: A comprehensive inventory of test substance received and used will be kept. The test substance container(s) will be weighed when received at this facility, and a record of all test substance use will be maintained. Test substance and test substance dosing solutions will be stored in the original containers, or in the equivalent thereof, or in glass containers with Teflon-lined caps.

APPENDIX B (cont.)

B. EXPERIMENTAL DESIGN (cont.)

7. Disposal of Unused Test Substance:

Unused test substance will be returned or disposed of at the Sponsor's expense after the termination of the study. The Sponsor should specify return or disposal at the time of study authorization. If returned, the recipient of the test substance will be notified in advance of shipping. The sample will be accompanied by a chain of custody letter describing the sample(s) contained in the shipment.

8. Safety Precautions:

General safety precautions required by laboratory SOPs will be followed. The Sponsor will supply basic toxicity data on the test substance to be used. However, since the toxicity of test substances is often not well characterized, this laboratory will be conservative in setting safety procedures. The Sponsor or Sponsor's Representative shall be notified of any exposures requiring a physician's examination or care.

C. DATA MANAGEMENT

1. Records:

The following records will be maintained during the study and transferred to the STILLMEADOW, Inc. archives upon study termination.

a. Protocol and Protocol Amendments (if any).
b. Final report and amendments (if any).
c. Study correspondence.
d. Animal receipt/acclimation data.
e. Test substance receipt, identification as supplied by Sponsor, preparation, administration, and disposition.
f. Test animal information: number, sex, source, strain.
g. Body weight data.
h. Daily observation data for signs of pharmacologic and/or toxicologic effects.
i. Mortality data, gross necropsy findings, and histopathology findings, if requested.
j. Calculations (if any) of the LD_{50} and slope determinations with 95% confidence limits.
k. Other pertinent data.

2. Data Storage:

All raw data will be retained at STILLMEADOW, Inc without charge to the Sponsor for a period of five years.

APPENDIX B (cont.)

C. DATA MANAGEMENT (cont.)

3. Data Reporting:

The final report will include all data as described in the Good Laboratory Practice Standards, including:

a. Statement from the Quality Assurance Unit.
b. Signature of the Study Director.
c. A GLP Compliance Statement signed by the Study Director.
d. Names of scientific personnel involved in the study.
e. Dates of study initiation and termination.
f. Identification, description, preparation, and storage of the test substance.
g. All pertinent animal data, animal husbandry, dosing information, and observation methods.
h. Description of the test procedures.
i. If calculated, the LD_{50} and slope function data with 95% confidence limits for males, females, and males and females combined (if necessitated by mortality in one or both sexes), and/or the Toxicity Category.
j. Individual body weights.
k. Observations on the nature, onset, severity, and duration of all gross or visible pharmacologic and/or toxicologic signs. Nonroutine findings will be addressed in a discussion section in which the relationship to treatment and historical data will be evaluated.
l. Individual mortality data, gross necropsy findings, and histopathology findings, if requested.
m. A reference to this Protocol.

4. Report Submission:

A report will be submitted after termination of the in-life portion of the study (subject to completion of histopathology, if requested).

ATTACHMENT 35

Acute Dermal Toxicity Study in Rabbits on Miller 6064

STILLMEADOW
INCORPORATED

VOLUME _ OF _ OF SUBMISSION

Miller 6064

FINAL REPORT

ACUTE DERMAL TOXICITY STUDY IN RABBITS

OPPTS NO. 870.1200

AUTHOR:

Janice O. Kuhn, Ph.D., D.A.B.T.

STUDY INITIATION DATE: 26 December 2000
STUDY COMPLETION DATE: 26 March 2001

CONDUCTED BY:
STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, TX 77478

LABORATORY STUDY NUMBER:

6207-00

VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 22

SUBMITTED TO:
Miller Chemical and Fertilization Corp.
P.O. Box 333
Hanover, PA 17331

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA § 10 (d) (1) (A), (B) or (C).

Company: Miller Chemical and Fertilization Corp.

Company Agent: _____ Date: _____

_____ _____

Title Signature

These data are the property of Miller Chemical and Fertilization Corp., and as such, are considered to be confidential for all purposes other than compliance with FIFRA § 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other statute or in any other country.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was designed and performed in STILLMEADOW, Inc.'s AAALAC accredited laboratory in compliance with:

- United States Environmental Protection Agency FIFRA: GLP Standards, 40 CFR 160 with exception of Sec. 160.31 (d), and 160.105 (b)(e): stability information was not provided
- United States Environmental Protection Agency TSCA 40 CFR 792 with exception of Sec. 792.31 (d), and 792.105 (b)(e): stability information was not provided
- Organization for Economic Cooperation & Dev. Principles of GLP, Annex 2, C(97)186 with exception of Sec. 6.2 (4): stability information was not provided
- Japan Ministry of Agriculture, Forestry & Fisheries, Notification No. 59 Nohsan 3850, Director-General of Agricultural Prod. Bureau, 10 Aug 84 with exception of Art. 5 (2)(9), and 21 (3): stability information was not provided

Janice O. Kuhn — 26 Mar 01

Janice O. Kuhn, Ph.D., D.A.B.T.
Study Director, STILLMEADOW, Inc.

Date

Signature of Agent of Sponsor

Date

Agent Name
Sponsor: Miller Chemical and Fertilization Corp.

Signature of Agent of Submitter

Date

Agent Name
Submitter: Mandava Associates

TABLE OF CONTENTS

QUALITY ASSURANCE STATEMENT

Study Number: 6207-00
Test Substance: Miller 6064
Study Title: Acute Dermal Toxicity Study in Rabbits

The study report and data have been audited in accordance with STILLMEADOW, Inc. Standard Operating Procedures (SOPs). The findings from the inspection and audit were reported to the Study Director and management as follows:

Phase of Study Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
Dosing	4 Jan 01	9 Jan 01	9 Jan 01
Report/Data Audit	14 Feb 01	14 Feb 01	14 Feb 01

Darrell Zeno

Darrell Zeno, M.S.
Quality Assurance Unit, STILLMEADOW, Inc.

26 Mar 01
Date

SUMMARY

The test substance, Miller 6064, was evaluated for its dermal toxicity potential and relative skin irritancy when a single undiluted dose of 5050 mg/kg was applied to the intact skin of albino rabbits. No mortality occurred during the study. Clinical signs included decreased defecation, soft feces and not eating, which were no longer observed on Day 4 of the study. Signs of dermal irritation included erythema, edema, atonia, desquamation and shallow fissuring. There was no effect on body weight gain, with the exception of three animals that lost or failed to gain weight during the first week. The gross necropsy conducted at termination of the study revealed no observable abnormalities, except for discolored lungs in six animals. The estimated LD_{50}, as indicated by the data, was determined to be greater than 5050 mg/kg.

INTRODUCTION

The objective of this study was to assess the systemic toxicity potential and relative skin irritancy of the test substance when administered to rabbits in accordance with US EPA OPPTS 870.1200, which is intended to meet testing requirements of FIFRA 7 USC 136, et seq, and TSCA 15 USC 2601. This study was conducted for Miller Chemical and Fertilization Corp., according to the approved protocol and STILLMEADOW, Inc. SOPs. There were no deviations from the protocol that affected the quality or outcome of the study. All procedures used in this study are in compliance with Animal Welfare Act Regulations. The protocol, raw data and this report are archived at STILLMEADOW, Inc. The animals were treated as follows:

Dose		Male Treatment		Female Treatment		In-life Termination Date	
mg/kg	mL/kg	Date	Time	Date	Time	Males	Females
5050	5.50	4 Jan 01	0940	4 Jan 01	0946	18 Jan 01	18 Jan 01

TEST SUBSTANCE

Identification:	Miller 6064
Date & Quantity Received:	19 Dec 00; 2 x 1 gal
Physical Description:	Amber liquid
Storage:	Room temperature
Measured Density:	0.9179 g/mL
Purity & Composition:	Refer to Certificate of Analysis (Appendix A)
Stability:	Not provided by sponsor

Records pertaining to stability, characterization and verification of test substance identity are the responsibility of the sponsor. A copy of the Certificate of Analysis is included in the report.

TEST SYSTEM

Experimental Animals

Species & Strain:	Albino rabbit; New Zealand White
Justification of Species:	The rabbit is a representative species preferred by various regulatory agencies for use in acute dermal testing.
Source:	Ray Nichols Rabbitry; Lumberton, TX
Date Received:	28 Dec 00
Quarantine Period:	5 days
Quantity & Sex:	5 males and 5 females (nulliparous and non-pregnant) were selected for testing
Group Identification:	Cage cards
Animal Identification:	Ear tag
Weight on Dosing Day:	Males: 2.075-2.950 kg; Females: 2.275-2.475 kg
Date of Birth:	8 Oct 00

Animal Husbandry

Cage Type:	Suspended, wire bottom, stainless steel
Housing:	1 per cage
Environmental Controls Set to Maintain:	· Temperature Range 20°C± 3° · Humidity Range 30-70% · 12-hour light/dark cycle · 10-12 air changes/hour
Food:	PMI Feeds, Inc.™ Lab Rabbit Diet #5321, in measured amounts
Water:	Municipal water supply analyzed by TNRCC Water Utilities Division; tap water, available *ad libitum* (automatic system)

Animal husbandry and housing at STILLMEADOW, Inc. comply with Animal Welfare Act Regulations. No contaminants were expected to have been present in the feed or water which would have interfered with or affected the results of the study.

PROCEDURES

Test Substance Administration

Healthy albino rabbits were released from quarantine. Each animal was prepared on the day prior to treatment by clipping the dorsal surface of the trunk free of hair to expose not less than 10% of the total body surface area. Care was taken to avoid abrading the skin. Only those animals with exposure areas free of pre-existing skin irritation or defects were used for this study. All animals were treated with 5050 mg/kg (5.50 mL/kg) of undiluted test substance. An individual dose was calculated for each animal based on its Day 0 body weight just before exposure. The test substance was applied evenly to each exposure area in a thin, uniform layer. The area of application was covered with an appropriately sized surgical gauze patch (4 ply, 8 x 4 in) and secured with non-irritating adhesive tape. The trunk of each animal was then wrapped with orthopedic stockinette which was secured in place with non-irritating adhesive tape to prevent possible ingestion of the test substance.

PROCEDURES (cont.)

Removal of Test Substance

After 24 hours, the wrappings were removed. The test sites were gently washed with room temperature tap water and a clean cloth to remove as much residual test substance as possible.

In-life Observations

Observations for mortality and clinical/behavioral signs of toxicity were made at least three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14.

Dermal Irritation Observations

Observations for evidence of dermal irritation were made at approximately 60 minutes after removal of wrappings, and on Days 4, 7, 11 and 14.

Postmortem Observations

On Day 14 after dosing, animals were euthanized by an intracardiac injection of Fatal Plus® (Vortech Pharmaceuticals, Dearborn, MI 48126). All study animals were subjected to gross necropsy and all abnormalities were recorded. After necropsy, the animal carcasses were discarded.

RESULTS AND DISCUSSION

Mortality/Estimated LD_{50} Values

There was no mortality during the study. The estimated acute dermal LD_{50}, as indicated by the data, was determined to be greater than 5050 mg/kg body weight.

Clinical Signs

Clinical signs are presented in Table 2. Prominent in-life observations included decreased defecation, soft feces and not eating. Animals were asymptomatic by Day 4.

Dermal Irritation

Signs of dermal irritation are presented in Table 3. Irritation included very slight to well-defined erythema, very slight edema, atonia, desquamation and shallow fissuring.

Body Weights

Individual body weights are presented in Table 1. Body weight gain was unaffected by the administration of the test substance, with the exception of two males that failed to gain weight and one male that lost weight during the first week of the study.

RESULTS AND DISCUSSION (cont.)

Necropsy Findings

Individual necropsy findings are presented in Table 1. The gross necropsy conducted at termination of the study revealed no observable abnormalities, except for discolored lungs in four males and two females.

CONCLUSION

The test substance, Miller 6064, was evaluated for its acute dermal toxicity potential and relative skin irritancy when administered to albino rabbits. The acute dermal LD_{50}, as indicated by the data, is greater than 5050 mg/kg in males and females.

Study Director: Janice O. Kuhn 26 Mar 01

Janice O. Kuhn, Ph.D., D.A.B.T.
Senior Toxicologist, STILLMEADOW, Inc.

Date

STUDY PERSONNEL

Technical Staff: Carol Morris, B.A.
Hector Fuentes
Paul Siemens, B.A.
Michelle Gantt, B.S.

Data Services: Connie Pavatte, Report Preparation

TABLE 1
ACUTE DERMAL TOXICITY STUDY IN RABBITS
Body Weights, Time of Death, and Gross Necropsy
Test Substance: Miller 6064
Dose Level: 5050 mg/kg (5.50 mL/kg)

Animal Number	Body Weights (kg)			Time of Death*	Gross Necropsy Findings
	Day 0	Day 7	Final		
2308-M	2.350	2.325	2.450	Day 14	NOA
2310-M	2.600	2.725	2.825	Day 14	Lungs pale.
2316-M	2.950	2.950	3.200	Day 14	Lungs pale.
2318-M	2.075	2.075	2.275	Day 14	Lungs mottled.
2322-M	2.800	3.000	3.175	Day 14	Lungs pale.
2317-F	2.400	2.450	2.675	Day 14	NOA
2321-F	2.400	2.625	2.775	Day 14	NOA
2323-F	2.475	2.750	2.850	Day 14	Lungs mottled.
2325-F	2.325	2.500	2.800	Day 14	Lungs pale.
2327-F	2.275	2.425	2.600	Day 14	NOA

* - Day of dosing considered Day 0; Day 14 is terminal sacrifice.
M - Male; F - Female; NOA - No Observable Abnormalities

TABLE 2
ACUTE DERMAL TOXICITY STUDY IN RABBITS
Pharmacologic and/or Toxicologic Signs
Test Substance: Miller 6064
Dose Level: 5050 mg/kg (5.50 mL/kg)
Sex: Males and Females

	Time After Treatment															
	HOURS			DAYS												
Reaction and Severity	1.0	2.0	4.0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Males																	
Soft feces	0	3	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0
Decreased defecation	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Not eating	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Females																	
Soft feces	0	1	1	4	4	0	0	0	0	0	0	0	0	0	0	0	0
Not eating	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0

Note: Digits indicate number of animals exhibiting reaction.

TABLE 3
ACUTE DERMAL TOXICITY STUDY IN RABBITS
Signs of Dermal Irritation
Test Substance: Miller 6064
Dose Level: 5050 mg/kg (5.50 mL/kg)

Animal Number	Day 1			Day 4			Day 7			Day 11			Day 14		
	Er	Ed	Other	Er	Ed	Other	Er	Ed	Other	Er	Ed	Other	Er	Ed	Other
2308-M	1	0	-	1	0	D	0	0	-	0	0	-	0	0	-
2310-M	1	1	-	1	0	D,W	0	0	-	0	0	-	0	0	-
2316-M	1	0	-	1	0	D,W	0	0	-	0	0	-	0	0	-
2318-M	1	1	-	1	0	D,W	0	0	-	0	0	-	0	0	-
2322-M	2	0	-	1	0	D,W	0	0	-	0	0	-	0	0	-
2317-F	2	0	-	1	0	D,W	0	0	-	0	0	-	0	0	-
2321-F	2	0	-	1	0	A,D,W	0	0	-	0	0	-	0	0	-
2323-F	2	1	-	1	0	A,D,W	0	0	-	0	0	-	0	0	-
2325-F	1	1	-	1	0	A,D,W	0	0	-	0	0	-	0	0	-
2327-F	2	1	-	1	0	A,D,W	0	0	-	0	0	-	0	0	-

Er = Erythema; 0 = None, 1 = Very slight, 2 = Well-defined, 3 =Moderate, 4 = Severe
Ed = Edema; 0 = None, 1 = Very slight, 2 = Slight, 3 = Moderate, 4 = Severe
A = Atonia; D = Desquamation; W = Shallow lateral fissuring
Note: A dash (-) is used if no other irritation is observed.

APPENDIX A

MILLER® CHEMICAL & FERTILIZER CORPORATION

P O. BOX 333, 120 RADIO ROAD
HANOVER, PENNSYLVANIA 17331 U.S.A.
TELEPHONE: 717-632-8921
FAX NO.: 717-632-4581

1/18/01

CERTIFICATE OF ANALYSIS

Product Name: MILLER 6064
Quantity Produced: 7.4 kilograms
Batch Number: 150386
Date of Formulation: 12/13/00

Appearance: Yellow to Amber Liquid
Odor: Terpenic
Moisture: Negligible (non-aqueous)
Specific Gravity: 0.92 - 0.94 @ 20c
Viscosity @ 20c: 100 to 500 cps
pH(1% solution): 6.0 to 8.0
Solubility in Water: Insoluble (Emulsifies)
Flash Point: over 200 f
Purity: 100%

Signature:

Name: Andrew G. Smith
Title: Compliance Officer

APPENDIX B

STILLMEADOW
INCORPORATED

PROTOCOL FOR STUDY 6207-00

Study Title: ACUTE DERMAL TOXICITY STUDY IN RABBITS
(OPPTS 870.1200)

Test Substance: MILLER 6064

Test Facility: STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, Texas 77478

Approved: _____ 26 Dec 00
Janice O. Kuhn, Ph.D., D.A.B.T. Date
Study Director
STILLMEADOW, Inc.

Approved: _____ 6 Dec 00
Mark S. Holbert Date
Vice President
STILLMEADOW, Inc.

Reviewed: _____ 6 Dec. 2000
Vicki S. Crutchfield, RQAP Date
Director, Quality Assurance Unit
STILLMEADOW, Inc.

Sponsor: Miller Chemical and Fertilization Corp.
P.O. Box 333
Hanover, PA 17331

Sponsor Representative: Mandava Associates
1730 M Street, N.W., Suite 906
Washington, DC 20036

Approved: _____ December 26, 2000
N. Bhushan Mandava Date
Agent to Miller Chemical and Fertilization Corp.

PROTOCOL FOR STUDY 6207-00

A. GENERAL

1. Study Title: ACUTE DERMAL TOXICITY STUDY IN RABBITS

2. Purpose: To assess the systemic toxicity and relative skin irritancy of a test substance when applied to the skin of rabbits.

3. Regulatory Compliance: This study will be conducted according to OPPTS 870.1200, which is intended to meet testing requirements of both FIFRA (7 U.S.C., 136 *et seq.*) and TSCA (15 U.S.C. 2601).

This study will be conducted in compliance with Good Laboratory Practice Standards:

1. EPA FIFRA: 40 CFR 160
2. EPA TSCA 40 CFR 792
3. OECD: C(97)186
4. Japanese MAFF

All procedures in this protocol are in compliance with Animal Welfare Act Regulations. All methods can be found in STILLMEADOW, Inc. Standard Operating Procedures (SOPs).

4. Quality Assurance: The Protocol is reviewed by the Quality Assurance Unit (QAU). The study information will be entered into the Master Schedule. The study will be inspected at least once during its progress. Further inspections may be scheduled as needed to ensure the integrity of the study. Any deviations from SOPs, the Protocol, or Good Laboratory Practice Standards will be immediately reported to the Study Director and Management. The report will be audited and a statement prepared and signed which shall specify the dates inspections were made and findings reported to Management and to the Study Director.

5. Test Substance: MILLER 6064. Test substance identification will include the name, batch number, and purity. Information regarding safety, stability, storage conditions, and disposal will also be provided by the Sponsor. The Sponsor assumes responsibility for purity and stability determinations. Analysis and archiving of reserve samples will be the responsibility of the Sponsor.

6. Proposed Schedule: Testing will begin within approximately three weeks of receipt of the test substance and authorization to conduct the study.

Proposed Start Date: 21 Dec 00
Proposed End Date: 04 Jan 01

The study will be extended if several dose levels are required.

A. GENERAL (cont.)

7. Study Director:	Janice O. Kuhn, Ph.D., D.A.B.T.
8. Experimental Summary:	The test substance will be applied to the intact skin of albino rabbits and maintained in contact with the skin for 24 hours. The animals will be observed three times on the day of dosing and once daily thereafter for mortality and for signs of pharmacologic and/or toxicologic effects, for at least 14 days. If a sufficient number of dose levels are tested, an LD_{50} with slope function and 95% confidence limits will be calculated and/or a Toxicity Category will be assigned.
9. Protocol Amendments:	Any alteration in the Protocol will be justified, approved by the Study Director, and recorded in writing.
10. Sponsor Audits:	The Sponsor may send an authorized representative to inspect the test system and/or data on the STILLMEADOW, Inc. premises during normal working hours.

APPENDIX B (cont.)

B. EXPERIMENTAL DESIGN

1. Animals

a. Species:	Albino rabbit
b. Strain/Source:	New Zealand White (Ray Nichols Rabbitry, Lumberton, Texas or other suitable source)
c. Justification of Species:	The rabbit is conventionally used in dermal toxicity tests to provide information on which human hazard can be judged, and is one of the species preferred by the regulatory agencies.
d. Quantity and Sex:	At least five males and five females (nulliparous and non-pregnant) for the initial dose level; additional animals may be required (refer to B.3.g.).
e. Age/Weight:	Young adult (12 weeks - 6 months); approximately 2 - 4 kg. Weight variation should not exceed ±20% of the mean for each sex.
f. Identification:	Ear tag
g. Acclimation and Health Status:	Animals will be acclimated for at least five days prior to testing. Normal weight gain, appearance, and behavior will be factors used to select healthy animals for testing. Only naive animals will be selected.
h. Randomization:	Unless a control group is required (see B.3.f.) no formal randomization procedure will be required. If a control group is necessary, a weight-stratified randomization procedure will be employed.

2. Animal Husbandry

a. Cages:	Stainless steel, suspended, wire bottom
b. Number per Cage:	Animals will be individually housed.
c. Food:	A measured amount of PMI Feeds, Inc.™ Laboratory Rabbit Diet #5321. The feed is routinely analyzed by the manufacturer for nutritional components and environmental contaminants.
d. Water:	Tap water; available *ad libitum* (automatic system). Municipal water supply analyzed by Texas Natural Resource Conservation Commission (TNRCC) Water Utilities Division.
e. Contaminants:	There are no known contaminants in the feed or water available to laboratory animals that would be expected to interfere with this study.
f. Environment:	Target temperature: approximately 20°C ± 3°C. Target relative humidity: approximately 30 - 70%. 12-hour light/dark cycle (regulated automatically), and room ventilation of approximately 10 - 12 air changes per hour.

B. EXPERIMENTAL DESIGN (cont.)

3. Test Substance Administration

a. Preparation of Animals: Animals will be prepared by clipping the back of the trunk of each animal free of hair to expose not less than 10% of the total body surface area. Care will be taken to avoid abrading the skin. Clipping of the animals will be done at least 12 hours before dosing. Animals with exposure areas free from pre-existing skin irritation or defects will be selected for testing. The animals will be clipped as needed throughout the study.

b. Reason for Route of Administration: Dermal contact is a potential route of human exposure.

c. Assignment of Animals to Groups: Animals will be randomly selected for dose groups so that individual body weights will not exceed ±20% of the mean weight for each sex.

d. Preparation of Test Substance: The test substance will be administered as received. If the substance is an aerosol, it will be discharged into a container and administered as a liquid.

e. Application of Test Substance: All animals will receive a single administration of the test substance on Day 0 based on the body weight on the day of treatment. The test substance will be applied evenly to the exposure area to make as thin and uniform a layer as possible under a 4-ply surgical gauze square. If the test substance is a solid, it will be pulverized, if necessary, and moistened with a sufficient quantity of deionized water or saline to make a thick paste before application. If an aqueous vehicle is not appropriate, corn oil should be considered first. Other acceptable vehicles include gum arabic, ethanol plus water, glycerol, propylene glycol, carboxymethyl cellulose, PEG, vegetable oil, and mineral oil. Justification for use of a vehicle other than water or saline must be supplied in the report. If the test substance is a liquid, it will be applied as received. The area of application will be covered with an appropriately sized 4 ply gauze patch (4 in x 8 in. or larger if necessary) and secured with non-irritating adhesive tape. The trunk of each animal will be wrapped with stockinette and secured in place with non-irritating adhesive tape.

f. Removal of Test Substance: The wrappings will be removed after a 24-hour exposure period. Depending on the presence of test substance residue, the skin may be wiped with a dry cloth or washed with room temperature tap water and dried with a dry cloth to remove as much residual test substance as possible. Control animals, if any, will be treated in a similar manner.

B. EXPERIMENTAL DESIGN (cont.)

3. Test Substance Administration (cont.)

g. Dose Level: Unless available toxicologic information indicates otherwise, a test will be conducted with five animals per sex at a dose level of approximately 5050 mg/kg unless 2020 mg/kg is requested by the Sponsor. If no mortality occurs among these animals, no further testing is necessary.

If mortality meets or exceeds 40% in either or both sexes at the level tested, then at least two additional dose levels may be tested for those sexes. There will be at least five animals (five males or five females) per dose level. The number and spacing of dose levels will be chosen so that an LD_{50} and/or a Toxicity Category can be determined. If both sexes are tested at a given dose level, the group will consist of an equal number of males and females. If animals of one sex are markedly more sensitive, testing of animals of the other sex may be dispensed with.

h. Control Groups: If the test substance is administered as received, a control group is not necessary. If the test substance is administered in a vehicle for which the toxicity is not known, a vehicle control group (five males and five females) will be required.

4. Observations

a. Clinical Signs: Observations for mortality and signs of pharmacologic and/or toxicologic effects will be made three times on the day of dosing, and once daily thereafter for 14 days. The duration should be determined by the toxic reactions and may be extended beyond 14 days when considered necessary. The nature, onset, severity, and duration of all gross or visible pharmacologic or toxicologic signs will be recorded.

Observations will include, but not be limited to evaluation of skin, fur, eyes and mucous membranes, respiratory and circulatory effects, autonomic effects such as salivation, lacrimation, excessive urination, diarrhea, central nervous system effects, including tremors and convulsions, changes in the level of activity, gait and posture, reactivity to handling or sensory stimuli, altered strength, and stereotypies or bizarre behavior (e.g., self mutilation, walking backwards).

b. Body Weights: Body weights will be recorded on the day of dosing (Day 0), and weekly thereafter (Days 7 and 14), or at the time of discovery after death.

c. Dermal Irritation: Approximately 60 minutes after removal of wrappings, the exposure area of each animal will be examined for evidence of dermal irritation (Appendix A). Additional observations for dermal irritation will be made on Study Days 4, 7, 11, and 14.

B. EXPERIMENTAL DESIGN (cont.)

4. Observations (cont.)

d. Sacrifice of Animals: All surviving animals will be sacrificed with a cardiac injection of Fatal Plus® (Vortech Pharmaceuticals, Dearborn, Michigan 48126).

e. Necropsy: A gross necropsy will be conducted on each animal at termination of the study or at the time of discovery after death, and the results recorded. The gross necropsy shall include terminal body weight, and gross observations of external surfaces; all orifices; and thoracic, abdominal, and pelvic cavities.

5. Evaluation of Results: Unless only a single dose level is tested, an LD_{50} with slope function and 95% confidence limits will be calculated for males, females, and males and females combined (if necessitated by mortality in one or both sexes) by the method of Litchfield and Wilcoxon (Litchfield, J.T., Jr., and Wilcoxon, F.: A Simplified Method of Evaluating Dose-Effect Experiments, J. Pharm. & Exp. Ther., 96, 99-115, 1949) or other appropriate method. If requested by the Sponsor, a Toxicity Category may be determined instead of an LD_{50} or in addition to an LD_{50}.

6. Test Substance Accountability: A comprehensive inventory of test substance received and used will be kept. The test substance container(s) will be weighed when received at this facility, and a record of all test substance use will be maintained. Test substance and test substance dosing solutions will be stored in the original containers, or in the equivalent thereof, or in glass containers with Teflon-lined caps.

7. Disposal of Unused Test Substance: Unused test substance will be returned or disposed of at the Sponsor's expense after the termination of the study. The Sponsor should specify return or disposal at the time of study authorization. If returned, the recipient of the test substance will be notified in advance of shipping. The sample will be accompanied by a chain of custody letter describing the sample(s) contained in the shipment.

8. Safety Precautions: General safety precautions required by laboratory SOPs will be followed. The Sponsor will supply basic toxicity data on the test substance to be used. However, since the toxicity of test substances is often not well characterized, this laboratory will be conservative in setting safety procedures. The Sponsor or Sponsor's Representative shall be notified of any exposures requiring a physician's examination or care.

C. DATA MANAGEMENT

1. Records:

The following records will be maintained during the study and transferred to the STILLMEADOW, Inc. archives upon study termination.

a. Protocol and Protocol Amendments (if any).
b. Final report and amendments (if any).
c. Study correspondence.
d. Animal receipt/acclimation data.
e. Test substance receipt, identification as supplied by Sponsor, preparation, administration and disposition.
f. Test animal information: species, strain, sex, source and number.
g. Body weight data.
h. Daily observation data for signs of pharmacologic and/or toxicologic effects.
i. Mortality data, gross necropsy findings, and histopathology findings, if requested.
j. Calculations (if any) of the LD_{50} and slope determinations with 95% confidence limits.
k. Observations for dermal irritation.
l. Other pertinent data.

2. Data Storage:

All raw data will be retained at STILLMEADOW, Inc.

3. Data Reporting:

The final report will include all data as described in the Good Laboratory Practice Standards, including:

a. Statement from the Quality Assurance Unit.
b. Signature of the Study Director.
c. A GLP Compliance Statement signed by the Study Director.
d. Names of scientific personnel involved in the study.
e. Dates of study initiation and termination.
f. Identification, description, and storage of the test substance.
g. All pertinent animal data, animal husbandry, dosing information, and observation methods.
h. Description of the test procedures.
i. If calculated, the LD_{50} and slope function data with 95% confidence limits for males, females, and males and females combined (if necessitated by mortality in one or both sexes), and/or the Toxicity Category.
j. Individual body weights.
k. Observations on the nature, onset, severity, and duration of all gross or visible pharmacologic and/or toxicologic signs. Nonroutine findings will be addressed in a discussion section in which the relationship to treatment and historical data will be evaluated.
l. Individual mortality data, gross necropsy findings, and histopathology findings, if applicable.
m. Dermal irritation observations.
n. A reference to this Protocol.

4. Report Submission:

A report will be submitted after termination of the in-life portion of the study (subject to completion of histopathology, if requested).

APPENDIX B (cont.)

Appendix A
ACUTE DERMAL TOXICITY STUDY IN RABBITS
Evaluation of Skin Reactions

Primary Dermal Irritation Scoring Scale
(Draize Technique)

Erythema and Eschar Formation	Score
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Maximum Possible	4

Edema Formation	Score
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4
Maximum Possible	4

Other observations may be made when needed, for example: Staining of the test site skin, necrosis, blanching, desquamation, sloughing, eschar, coriaceousness (leathery texture), atonia, etc.

ATTACHMENT 36

Acute Inhalation Toxicity Study in Rats on Miller 6064

STILLMEADOW
INCORPORATED

VOLUME _ OF _ OF SUBMISSION

Miller 6064

FINAL REPORT

ACUTE INHALATION TOXICITY STUDY IN RATS

OPPTS NO. 870.1300

AUTHOR:

Lori Carter, B.A.

STUDY INITIATION DATE: 26 December 2000
STUDY COMPLETION DATE: 22 March 2001

CONDUCTED BY:
STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, TX 77478

LABORATORY STUDY NUMBER:

6208-00

VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 29

SUBMITTED TO:
Miller Chemical and Fertilization Corp.
P.O. Box 333
Hanover, PA 17331

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA § 10 (d) (1) (A), (B) or (C).

Company: Miller Chemical and Fertilization Corp.

Company Agent: _____ Date: _____

_____ _____

Title Signature

These data are the property of Miller Chemical and Fertilization Corp., and as such, are considered to be confidential for all purposes other than compliance with FIFRA § 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other statute or in any other country.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was designed and performed in STILLMEADOW, Inc.'s AAALAC accredited laboratory in compliance with:

- United States Environmental Protection Agency FIFRA; GLP Standards 40 CFR 160 with exception of Sec. 160.31 (d), and 160.105 (b)(e): stability information was not provided
- United States Environmental Protection Agency TSCA 40 CFR 792 with exception of Sec. 792.31 (d), and 792.105 (b)(e): stability information was not provided
- Organization for Economic Cooperation & Dev. Principles of GLP, Annex 2, C(97)186 with exception of Sec. 6.2 (4): stability information was not provided
- Japan Ministry of Agriculture, Forestry & Fisheries, Notification No. 59 Nohsan 3850, Director-General of Agricultural Prod. Bureau, 10 Aug 84 with exception of Art. 5 (2)(9), and 21 (3): stability information was not provided

Lori Carter — 22 Mar 01

Lori Carter, B.A.
Study Director, STILLMEADOW, Inc. — Date

Signature of Agent of Sponsor — Date

Agent Name
Sponsor: Miller Chemical and Fertilization Corp.

Signature of Agent of Submitter — Date

Agent Name
Submitter: Mandava Associates

TABLE OF CONTENTS

QUALITY ASSURANCE STATEMENT

Study Number: 6208-00
Test Substance: Miller 6064
Study Title: Acute Inhalation Toxicity Study in Rats

The study has been inspected and the report and data have been audited in accordance with STILLMEADOW, Inc. Standard Operating Procedures (SOPs). The findings from the inspection and audit were reported to the Study Director and Management as follows:

Phase of Study Inspected	Date Inspected	Reported to Study Director	Reported to Management
Body weights	16 Jan 01	17 Jan 01	17 Jan 01
Report/Data Audit	23 Feb 01	23 Feb 01	23 Feb 01

B. Lynn Murphy, B.S.
Quality Assurance Unit, STILLMEADOW, Inc.

23 March 01
Date

SUMMARY

The test substance, Miller 6064, was evaluated for its acute inhalation toxicity potential in albino rats. Five males and five females were exposed for four hours to an aerosol generated from the undiluted liquid test substance at a level of 5.26 mg/L. There was no mortality during the study. Clinical signs included activity decrease, respiratory gurgle and chirp, which were no longer evident by Day 6. Body weights were unaffected by exposure, except in two animals that lost weight during the first week. The gross necropsy revealed no observable abnormalities. As indicated by the data, the acute inhalation LC_{50} is greater than 5.26 mg/L.

INTRODUCTION

The objective of this study was to determine the acute inhalation toxicity potential of the test substance in accordance with US EPA OPPTS 870.1300, which is intended to meet testing requirements of FIFRA 7 USC 136, et seq, and TSCA 15 USC 2601. This study was conducted for Miller Chemical and Fertilization Corp., according to the approved protocol and STILLMEADOW, Inc. SOPs. There were no deviations from the protocol which affected the quality or outcome of the study. All procedures used in this study are in compliance with Animal Welfare Act Regulations. The protocol, raw data and this report are archived at STILLMEADOW, Inc. The animals were exposed as follows:

Beginning of 4 Hour Exposure					Termination of In-Life Observations	
Dose (mg/L)	Males		Females		Date	
	Date	Time	Date	Time	Males	Females
5.26	9 Jan 01	0745	9 Jan 01	0745	23 Jan 01	23 Jan 01

TEST SUBSTANCE

Identification:	Miller 6064
Date & Quantity Received:	19 Dec 00; 2 x 1 gal
Physical Description:	Amber liquid
Storage:	Room temperature
Purity & Composition:	Refer to Certificate of Analysis (Appendix B)
Stability:	Not provided by sponsor

Records pertaining to stability, characterization, and verification of test substance identity are the responsibility of the sponsor. A copy of the Certificate of Analysis is included in the report.

TEST SYSTEM

Experimental Animals

Species & Strain:	Rat; Sprague-Dawley
Justification of Species:	The rat is a representative rodent species preferred by various regulatory agencies for use in acute inhalation toxicity studies.
Source:	Texas Animal Specialties, Humble, TX
Quarantine Period:	5 days
Date Received:	4 Jan 01
Quantity & Sex:	5 males and 5 females (nulliparous and non-pregnant)
Animal Identification:	Ear punch and cage cards
Weight When Tested:	Males: 268-307 g; Females: 189-194 g
Date of Birth:	14 Nov 00

Animal Husbandry

Cage Type:	Suspended, wire bottom, stainless steel
Housing:	One per cage
Environmental Controls Set to Maintain:	·Temperature Range 22°C±3° ·Humidity Range 30-70% ·12-hour light/dark cycle ·10-12 air changes/hour
Food:	PMI™ Lab Diet Formula #5008, available *ad libitum* except during the exposure period
Water:	Municipal water supply analyzed by TNRCC Water Utilities Division; from automatic water system, available *ad libitum* except during the exposure period

Animal husbandry and housing at STILLMEADOW, Inc. comply with Animal Welfare Act Regulations. No contaminants were expected to have been present in the feed or water which would have interfered with or affected the results of the study.

PROCEDURES

Prestudy Testing

Trial assays were conducted to determine which method(s) of aerosolizing the test substance into the exposure chamber would produce an acceptable concentration and mass median aerodynamic diameter (MMAD).

Exposure Chamber

A 500 L nose-only stainless steel, dynamic flow inhalation chamber was utilized in this experiment (Diagram 1). The body of the chamber has 25 ports in 5 rows. Polycarbonate tubes are inserted into 10 designated individual ports. The test substance is introduced through the opening in the top of the chamber. The bottom section has a corresponding air outlet and a drain valve for cleaning the chamber. The individual polycarbonate tubes are tapered at one end to fit the shape of the animal's head and the back portion is sealed with a rubber cap. The tubes containing the animals fit tightly into the ports, and are sealed with "O" rings.

PROCEDURES (cont.)

Generation of Test Atmosphere

The aerosol was generated by a pressure operated Spraying System Company air atomizer (1/4 JSS) which aspirated the test substance from a container, then sprayed the resulting aerosol directly into the exposure chamber. Air flow into the chamber was maintained through the use of a calibrated orifice plate at a rate of 13.6 air changes per hour. Air flow was recorded at 30 minute intervals during the exposure period, and was sufficient to ensure an oxygen content of at least 19% of the exposure atmosphere. Temperature and humidity were recorded at 30 minute intervals during the exposure period from a Taylor wet bulb/dry bulb hygrometer located in the exposure chamber.

Test Substance Administration

Healthy albino rats were released from quarantine, and five males and five females were selected for testing. The animals were exposed to an aerosol generated from the undiluted liquid test substance for a period of four hours. When 99% concentration (t-99) was attained, the animals which were individually housed in polycarbonate exposure tubes were inserted into a 500 L stainless steel nose-only inhalation chamber for the specified exposure period. At the termination of the exposure period, the animals were returned to their stock laboratory cages.

Determination of Concentration

The concentration of test substance in the exposure atmosphere (taken from the breathing zone of the animals) was determined analytically once per hour and nominally at the end of the exposure. The analytical determination was made using a Beckman DU-65 UV Spectrophotometer (Appendix A). The nominal concentration was determined by dividing the loss in weight of the test substance after the exposure by the total volume of air which passed through the chamber.

Particle Size Distribution

Particle size, taken from the breathing zone of the animals, was determined twice during the exposure, using a cascade impactor, at a rate of 8.5 L/minute for a duration of 30 seconds. The MMAD and particle size distributions are calculated from these data by a computer program utilizing probit analysis.

In-life Observations

Observations for mortality and signs of pharmacologic and/or toxicologic effects were made frequently on the day of exposure and at least once daily thereafter for 14 days (Day 0 is day of exposure). Individual body weights were recorded just prior to the inhalation exposure and on Days 7 and 14.

PROCEDURES (cont.)

Postmortem Observations

On Day 14 after exposure, each animal was euthanized by an intraperitoneal injection of Fatal Plus® (Vortech Pharmaceuticals, Dearborn, MI 48126). All study animals were subjected to gross necropsy, and all abnormalities were recorded.

Statistical Analysis

In order to calculate a mean exposure, the Mean Value Theorem of Calculus was used to properly weight the concentration, since the concentrations could not be measured continuously (see Table 4). This method weights concentrations based on the time span of each concentration. A concentration can be calculated for each minute, which better represents the exposure concentration received by each animal.

RESULTS AND DISCUSSION

Mortality/Estimated LC_{50} Values

There was no mortality during the study. As indicated by the data, the acute inhalation LC_{50} for Miller 6064 is greater than 5.26 mg/L.

Body Weights

Individual body weights are presented in Table 1. Body weight gain was unaffected by the administration of the test substance, except in one male and one female that lost 1-6 g during the first week.

Clinical Signs

Clinical signs are presented in Table 2. Prominent in-life observations included activity decrease, respiratory gurgle and chirp. Animals were asymptomatic by Day 6.

Necropsy Findings

Individual necropsy findings are presented in Table 1. The gross necropsy conducted on each animal at termination of the study revealed no observable abnormalities.

Inhalation Chamber Conditions

Chamber operating parameters are presented in Table 3. Concentration determinations and calculations are presented in Table 4. Particle size distributions are presented in Table 5. Pretest data are presented in Table 6. The exposure concentration of 5.26 mg/L had an average MMAD of 3.6 µm.

CONCLUSION

Miller 6064 was evaluated for its acute inhalation toxicity potential in albino rats. As indicated by the data, the acute inhalation LC_{50} is greater than 5.26 mg/L in males and females.

Lori Carter — 22 Mar 01

Lori Carter, B.A.
Study Director, STILLMEADOW, Inc.

Date

STUDY PERSONNEL

Technical Staff: Liangbao Rao, B.S.
Hector Fuentes
Paul H. Siemens, B.A.

Data Services: Connie Pavatte, Report Preparation

TABLE 1
ACUTE INHALATION TOXICITY STUDY IN RATS
Body Weights, Time of Death, and Gross Necropsy
Test Substance: Miller 6064
Exposure Concentration: 5.26 mg/L

Animal Number	Body Weights (g)			Time of Death*	Gross Necropsy Findings
	Day 0	Day 7	Final		
91-M	276	281	289	Day 14	NOA
92-M	268	262	296	Day 14	NOA
93-M	279	292	319	Day 14	NOA
94-M	274	278	313	Day 14	NOA
95-M	307	318	351	Day 14	NOA
96-F	194	193	220	Day 14	NOA
97-F	193	232	242	Day 14	NOA
98-F	189	199	215	Day 14	NOA
99-F	190	201	251	Day 14	NOA
100-F	192	195	213	Day 14	NOA

* - Day of dosing considered Day 0; Day 14 is terminal sacrifice.
M - Male; F - Female; NOA - No Observable Abnormalities

TABLE 2 (cont.)
ACUTE INHALATION TOXICITY STUDY IN RATS
Pharmacologic and/or Toxicologic Signs
Test Substance: Miller 6064
Exposure Concentration: 5.26 mg/L
Sex: Males and Females

	Time After Exposure Begins																	
	HOURS					DAYS												
Reaction and Severity	0.5	1.0	2.5	4.5	6.0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Males																			
Respiratory gurgle (v-s)	0	0	0	4	5	5	5	5	5	5	0	0	0	0	0	0	0	0	0
Activity decrease (v-s)	0	0	0	0	4	4	5	5	1	0	0	0	0	0	0	0	0	0	0
Females																			
Respiratory gurgle (v)	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
Respiratory chirp (v-s)	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

v - very slight; s - slight; m - moderate; e - extreme
Note: Digits indicate number of animals exhibiting reaction.

TABLE 3
ACUTE INHALATION TOXICITY STUDY IN RATS
Chamber Operating Parameters
Test Substance: Miller 6064

Exposure Concentration: 5.26 mg/L

Time (Hour)	Temp. (°F)	RH (%)	Air Flow (Lpm)
0.0	71	42	113
0.5	71	39	113
1.0	71	39	113
1.5	72	40	113
2.0	72	40	113
2.5	72	40	113
3.0	72	40	113
3.5	72	40	113
4.0	72	40	113
Mean:	72	40	113

t-99 Determination

Initial Chamber Air Flow	113 Lpm
Exposure Chamber Size	500 L
Baffling Chamber	Not used
t-99 Value	21 min

Air Atomizer Setting

Sprayer Air Flow	127 Lpm
Sample Intake	~1.3 mL/min

TABLE 4
ACUTE INHALATION TOXICITY STUDY IN RATS
Analytical Concentration Calculations
Test Substance: Miller 6064

Standard Curve

Standard	Conc. (mg/mL)	Abs. @ 270 nm
A	1.5360	2.2490
B	1.1520	1.7170
C	1.0240	1.5455
D	0.7550	1.1680
E	0.5662	0.9035
F	0.5033	0.8090

Corr (r) = 0.999970 y intercept = 0.114211 slope = 1.391966

Calculation for 5.26 Concentration

Sample No	Abs. @ 270 nm	Conc. (mg/ml)	Multi. Factor*	Chamber Conc. (mg/L)
1	1.5600	1.03867	x 4.99	5.183
2	1.5975	1.06561	x 4.99	5.317
3	1.6010	1.06812	x 4.99	5.330
4	1.5705	1.04621	x 4.99	5.221

* - Multiplication factor (mL/L) calculated from figures in Appendix A using following formula:

$$\frac{\text{Solvent volume}}{(\text{Duration})(\text{Sample rate})} = \text{Multifactor}$$

TABLE 4 (cont.)
ACUTE INHALATION TOXICITY STUDY IN RATS
Analytical Concentration Determination
Test Substance: Miller 6064

	Dose Level: 5.26 mg/L	
Event*	Time Period	Concentration
Start-up	0724	
t-99 (begin exposure)	0745	
Extrapolation calculated	0745-0800	5.183 mg/L
Sample 1 taken	0800-0805	5.183 mg/L
Interpolation calculated	0805-0900	5.250 mg/L
Sample 2 taken	0900-0905	5.317 mg/L
Interpolation calculated	0905-1000	5.324 mg/L
Sample 3 taken	1000-1005	5.330 mg/L
Interpolation calculated	1005-1100	5.275 mg/L
Sample 4 taken	1100-1105	5.221 mg/L
Extrapolation calculated	1105-1145	5.221 mg/L
End exposure	1145	
MEAN EXPOSURE CONC.		5.26 mg/L
Nominal Concentration		29.1 mg/L

* - Sample # taken is the concentration measured during the sampling period.
Extrapolation is the measured concentration of the adjacent event period carried over to the present event period.
Interpolation is the concentration calculated as the average of the measured concentration before and after the present event period.
Mean exposure concentration is the sum of the actual time weighted concentrations divided by the sum of elapsed times and represents the mean value of the exposure concentration.

TABLE 5
ACUTE INHALATION TOXICITY STUDY IN RATS
Particle Size Distribution*
Test Substance: Miller 6064
Concentration: 5.26 mg/L
½ Hour Distribution

Stage	Size Range (µm)	EPD** (µm)	Amount Collected (mg)	% in Size Range	Cumulative % Less Than Size Range
1	>16.7	16.7	0.1	1.09	98.91
2	10.0 - 16.7	10.0	0.9	9.78	89.13
3	4.0 - 10.0	4.0	2.4	26.09	63.04
4	2.4 - 4.0	2.4	2.4	26.09	36.96
5	1.5 - 2.4	1.5	2.6	28.26	8.70
6	0.9 - 1.5	0.9	0.8	8.70	0.00
7	0.5 - 0.9	0.5	0.0	0.00	0.00
8	0.3 - 0.5	0.3	0.0	0.00	0.00
Backup Filter	0.0 - 0.3	0.0	0.0	0.00	0.00

Calculated CHI^2 = 43.0 with 6 Degrees of Freedom.
Values of T and CHI^2 for P=.05 are: T = 2.45 CHI^2 = 12.6

Particle Size (Microns)	% of Particles Collected
≤ 0.3	5
≤ 0.8	16
≤ 3.2	50
≤ 13.5	84
≤ 34.0	95

Mass Median Aerodynamic Diameter = 3.2 µm
Geometric Standard Deviation = 4.2

* - Finney, D.J.: PROBIT ANALYSIS, 3rd ed., Chapters 3 and 4, 1971, Cambridge University Press.
** - Equivalent particle diameter @ 8.5 Lpm

TABLE 5 (cont.)
ACUTE INHALATION TOXICITY STUDY IN RATS
Particle Size Distribution*
Test Substance: Miller 6064
Concentration: 5.26 mg/L
3 ¾ Hour Distribution

Stage	Size Range (µm)	EPD** (µm)	Amount Collected (mg)	% in Size Range	Cumulative % Less Than Size Range
1	>16.7	16.7	0.9	4.69	95.31
2	10.0 - 16.7	10.0	3.0	15.63	79.69
3	4.0 - 10.0	4.0	5.5	28.65	51.04
4	2.4 - 4.0	2.4	4.4	22.92	28.13
5	1.5 - 2.4	1.5	4.1	21.35	6.77
6	0.9 - 1.5	0.9	1.3	6.77	0.00
7	0.5 - 0.9	0.5	0.0	0.00	0.00
8	0.3 - 0.5	0.3	0.0	0.00	0.00
Backup Filter	0.0 - 0.3	0.0	0.0	0.00	0.00

Calculated CHI^2 = 40.2 with 6 Degrees of Freedom.
Values of T and CHI^2 for P=.05 are: T = 2.45 CHI^2 = 12.6

Particle Size (Microns)	% of Particles Collected
≤ 0.3	5
≤ 0.8	16
≤ 4.0	50
≤ 19.0	84
≤ 51.7	95

Mass Median Aerodynamic Diameter = 4.0 µm
Geometric Standard Deviation = 4.7

* - Finney, D.J.: PROBIT ANALYSIS, 3rd ed., Chapters 3 and 4, 1971, Cambridge University Press.
** - Equivalent particle diameter @ 8.5 Lpm

TABLE 6
ACUTE INHALATION TOXICITY STUDY IN RATS
Pretest Data
Test Substance: Miller 6064

Trial assays were conducted to ascertain results, summarized below, under different chamber conditions.

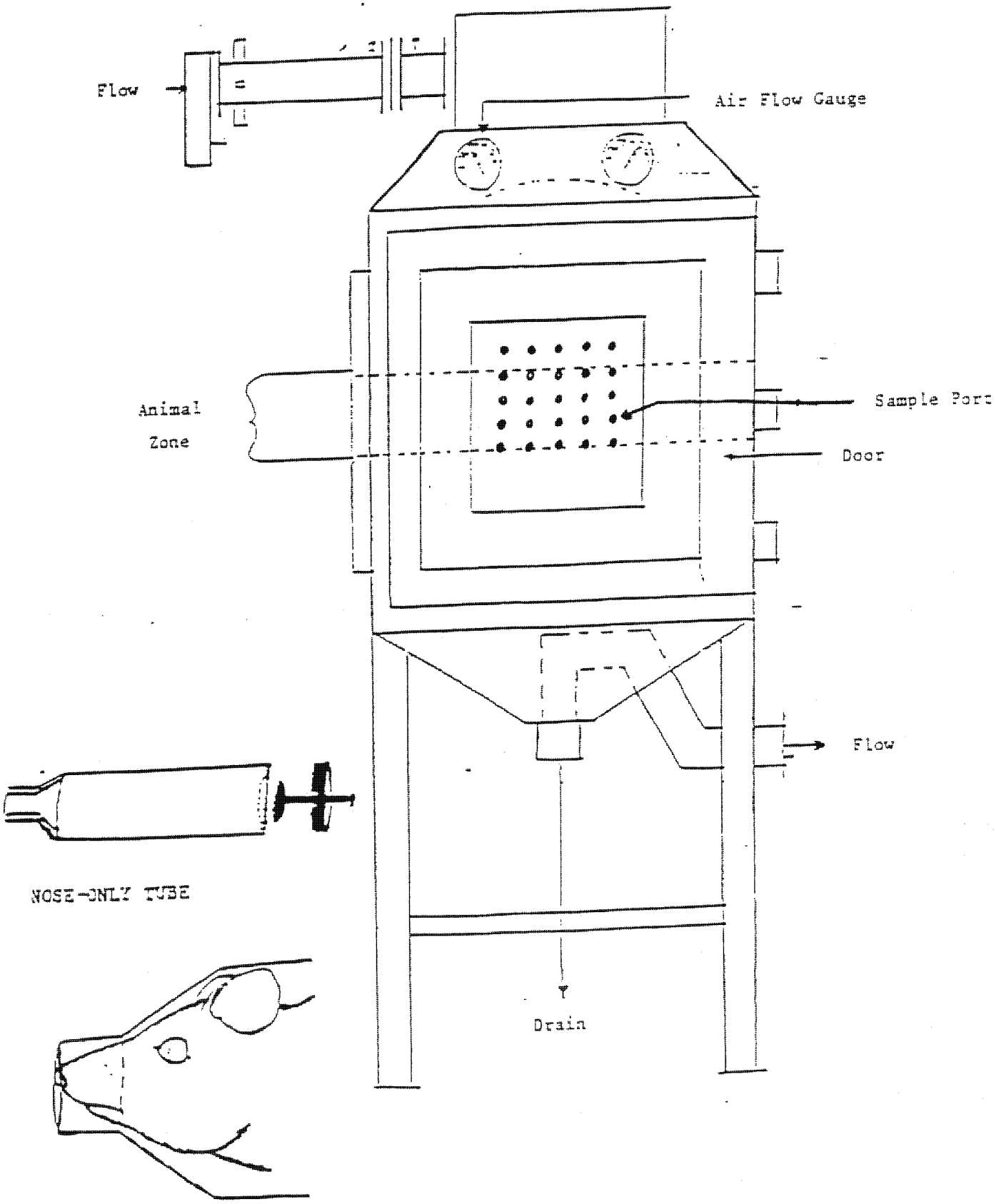
MMAD (um)	Concentration (mg/L)
ND	7.20
4.2	5.02
3.6	3.92

ND - Not determined

Exposure concentration - 5.26 mg/L with an average MMAD of 3.6 µm

Diagram 1

NOSE-ONLY INHALATION CHAMBER



APPENDIX A
ACUTE INHALATION TOXICITY STUDY IN RATS
Operating Parameters for UV Analysis
Test Substance: Miller 6064

Instrument	Beckman DU-65 UV Spectrophotometer
Wavelength	270 nm
Extraction Solvent	1,4 Dioxane
Sampling Method	Double impingement into 25 mL of solvent
Sampling Rate	1.002 Lpm
Sampling Duration	5 minutes
Volume of Chamber Air Sampled	5.01 L

APPENDIX B

MILLER® CHEMICAL & FERTILIZER CORPORATION

P O. BOX 333, 120 RADIO ROAD
HANOVER, PENNSYLVANIA 17331 U.S.A.
TELEPHONE: 717-632-8921
FAX NO.: 717-632-4561

1/18/01

CERTIFICATE OF ANALYSIS

Product Name: MILLER 6064
Quantity Produced: 7.4 kilograms
Batch Number: 150386
Date of Formulation: 12/13/00

Appearance: Yellow to Amber Liquid
Odor: Terpenic
Moisture: Negligble (non-aqueous)
Specific Gravity: 0.92 - 0.94 @ 20c
Viscosity @ 20c: 100 to 500 cps
pH(1% solution): 6.0 to 8.0
Solubility in Water: Insoluble (Emulsifies)
Flash Point: over 200 f
Purity: 100%

Signature:

Name: Andrew G. Smith
Title: Compliance Officer

APPENDIX C

STILLMEADOW
INCORPORATED

PROTOCOL FOR STUDY 6208-00

Study Title: ACUTE INHALATION TOXICITY STUDY IN RATS
(OPPTS 870.1300)

Test Substance: MILLER 6064

Test Facility: STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, Texas 77478

Approved: Lori Carter — 26 Dec 00 (Date)
Lori Carter, B.A.
Study Director
STILLMEADOW, Inc.

Approved: [signature] — 6 Dec 00 (Date)
Mark S. Holbert
Vice President
STILLMEADOW, Inc.

Reviewed: [signature] — 6 Dec 2000 (Date)
Vicki S. Crutchfield, RQAP
Director, Quality Assurance Unit
STILLMEADOW, Inc.

Sponsor: Miller Chemical and Fertilization Corp.
P.O. Box 333
Hanover, PA 17331

Sponsor Representative: Mandava Associates
1730 M Street, N.W., Suite 906
Washington, DC 20036

Approved: N. Bhushan Mandava — December 26, 2000 (Date)
N. Bhushan Mandava
Agent to Miller Chemical and Fertilization Corp.

PROTOCOL FOR STUDY 6208-00

A. GENERAL

1. Study Title: ACUTE INHALATION TOXICITY STUDY IN RATS

2. Purpose: To determine the acute inhalation toxicity potential of the test substance in rats.

3. Regulatory Compliance: This study will be conducted according to OPPTS 870.1300, which is intended to meet testing requirements of both FIFRA (7 U.S.C. 136, *et seq.*) and TSCA (15 U.S.C. 2601).

This study will be conducted in compliance with Good Laboratory Practice Standards:
1. EPA FIFRA: 40 CFR 160
2. EPA TSCA: 40 CFR 792
3. OECD: C(97)186
4. Japanese MAFF

All procedures in this protocol are in compliance with Animal Welfare Act Regulations. All methods can be found in STILLMEADOW, Inc. Standard Operating Procedures (SOPs).

4. Quality Assurance: The Protocol is reviewed by the Quality Assurance Unit (QAU). The study information will be entered into the Master Schedule. The study will be inspected at least once during its progress. Further inspections may be scheduled as needed to ensure the integrity of the study. Any deviations from SOPs, the Protocol, or Good Laboratory Practice Standards will be immediately reported to the Study Director and Management. The report will be audited and a statement prepared and signed which shall specify the dates inspections were made and findings reported to Management and to the Study Director.

5. Test Substance: MILLER 6064. Test substance identification should include the name, batch number and purity. Information regarding safety, stability, storage conditions and disposal should also be provided by the Sponsor. The Sponsor assumes responsibility for purity and stability determinations. Analysis and archiving of reserve samples will be the responsibility of the Sponsor.

6. Proposed Schedule: Definitive study or necessary preliminary analyses will begin within 30 days of receipt of test substance and authorization to conduct study. In the event of a delay, Sponsor will be notified within this 30-day period.

Proposed Start Date: 13 Dec 00
Proposed End Date: 27 Dec 00

The in-life portion of each exposure level generally will be 14 days. The study will be extended if several dose levels are required.

APPENDIX C (cont.)

A. GENERAL (cont.)

7. Study Director:	Lori Carter, B.A.
8. Experimental Summary:	The test substance will be administered for 4 hours in either a 15 L nose-only chamber or a 200 or 500 L stainless steel, dynamic flow test chamber either nose-only or whole body. Nominal, gravimetric, and/or analytical determinations of chamber concentration as well as particle size determinations will be made. The animals will be observed frequently on the day of exposure for mortality and signs of pharmacologic and/or toxicologic effects and once daily thereafter for 14 days. Histopathology will be available upon Sponsor request. If a sufficient number of dose levels are tested, an LC_{50} with slope function and 95% confidence limits will be calculated.
9. Protocol Amendments:	Any alteration in the Protocol will be justified, approved by the Study Director, and recorded in writing.
10. Sponsor Audits:	The Sponsor may send an authorized representative to inspect the test system and/or data on the STILLMEADOW, Inc. premises during normal working hours.

B. EXPERIMENTAL DESIGN

1. Animals

a. Species:	Albino rat
b. Strain/Source:	Sprague-Dawley (Texas Animal Specialties, Humble Texas or other suitable source)
c. Justification of Species:	The rat is conventionally used to provide an index of toxicity on which human hazard can be judged, and is preferred by the regulatory agencies.
d. Quantity and Sex:	Five males and five females (nulliparous and non-pregnant) for the initial dose level and 5/sex for any additional dose levels, if required (see B.3.f.).
e. Age/Weight:	Young adult (8 - 12 weeks of age) Males: approximately 225 - 330 grams Females: approximately 175 - 250 grams. Weight variation should not exceed ±20% of the mean for each sex.
f. Identification:	Ear punch
g. Acclimation and Health Status:	Animals will be acclimated for at least five days prior to testing. Normal weight gain, appearance, and behavior will be factors used to select healthy animals for testing. Only naive animals will be selected.

2. Animal Husbandry

a. Cages:	Stainless steel, suspended, wire bottom
b. Number per Cage:	Animals will be housed individually during exposure and observation periods.
c. Food:	PMI Feeds, Inc.™ Formulab #5008; available *ad libitum* prior to and after exposure. Analyzed by manufacturer.
d. Water:	Tap water (available *ad libitum* prior to and after the exposure period). Automatic system. Municipal water supply analyzed by Texas Natural Resource Conservation Commission (TNRCC) Water Utilities Division.
e. Contaminants:	There are no known contaminants in the feed or water available to laboratory animals that would be expected to interfere with this study.
f. Environment:	Target temperature: approximately 22°C ± 3°C Target relative humidity: approximately 30 - 70% 12-hour light/dark cycle (regulated automatically) Room ventilation of approximately 10 - 12 air changes per hour.

B. EXPERIMENTAL DESIGN (cont.)

3. Test Substance Administration

a. Reason for Route of Administration:	Inhalation is a potential route of human exposure.
b. Test Chamber and Housing:	A test substance atmosphere will be established inside either a 15 L nose-only chamber (animals individually housed in polycarbonate tubes) or a 200 or 500 L New York University design, stainless steel, dynamic flow test chamber either nose-only or whole body (animals individually housed in stainless steel wire mesh cages).
c. Preliminary Analysis:	A variety of techniques will be used in an attempt to attain a concentration of 2 mg/L (EPA) or 5 mg/L (OECD) of the test substance in the chamber while also obtaining a particle size distribution with a mass median aerodynamic diameter of 1 - 4 microns. If the particle size generated is too large and a concentration of 2 mg/L (EPA) or 5 mg/L (OECD) is not attained, the Sponsor will be notified. For gaseous test substances, particle size will not be assayed.
d. Dosing:	The test substance will be administered as either an aerosol or a gas in the test chamber. Filtered air will be used for dilution.
e. Duration of Exposure:	Four hours after equilibration of the chamber conditions.
f. Number of Animals and Selection of Dose Levels:	Ten rats (five males and five females) will be exposed for four hours to the optimum concentration determined by pre-exposure testing and/or Sponsor request. If the LC_{50} is shown to be greater than 2 mg/L (EPA) or 5 mg/L (OECD), no further testing will be required. If mortality meets or exceeds 40% in either or both sexes, then at least two additional concentrations of the test substance will be tested for those sexes. There will be at least five animals (five males and/or five females) per exposure level, and the number and spacing of exposure levels will be chosen so that an LC_{50} can be determined. If both sexes are tested at a given exposure level, the group will contain equal numbers of males and females.
g. Control Groups:	At the request of the Sponsor, a sham control, vehicle control, or negative control group can be run concurrently.

B. EXPERIMENTAL DESIGN (cont.)

3. Test Substance Administration (cont.)

h. Operating Parameters: Operating parameters to be measured include air flow, t-99, temperature, humidity, exposure concentration, and particle size.

Air flow: Monitored through the use of a calibrated orifice plate and will be sufficient to insure adequate oxygen content (at least 19%) of the exposure atmosphere. Air flow will equal at least 12 to 15 air changes per hour.

t-99: Calculated for each exposure and depends on the air flow. Start time of exposure will be adjusted accordingly.

Temperature and humidity: Measurements will be taken at 30 minute intervals during the exposure period. (Targeted conditions are approximately 22°±2°C and 40 - 60% RH).

Analytical concentration determination: Determined at least hourly for each exposure concentration, using the procedures supplied by the Sponsor or developed by STILLMEADOW, Inc.

Gravimetric concentration determination: When applicable, at least once per hour.

Nominal concentration: Determined once for each exposure.

Particle size: Determined at least twice with a cascade impactor. Results reported will include the aerodynamic mass median size and geometric standard deviation (aerosols only).

4. Observations

a. Clinical Signs: Observations for mortality and signs of pharmacologic and/or toxicologic effects will be made frequently on the day of exposure and once daily thereafter for 14 days. The duration should be determined by the toxic reactions and may be extended beyond 14 days when considered necessary. The nature, onset, severity, and duration of all gross or visible pharmacologic or toxicologic signs will be recorded.

Observations will include: skin, fur, eyes and mucous membranes, somatomotor activity, and behavior pattern. Particular attention will be given to tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma.

b. Body Weights: Body weights will be recorded on the day of exposure (Day 0), and on Days 7 and 14, or at the time of discovery after death.

B. EXPERIMENTAL DESIGN (cont.)

4. Observations (cont.)

c. Sacrifice of Animals: All surviving animals will be sacrificed with an IP injection of Fatal Plus® (Vortech Pharmaceuticals, Dearborn, Michigan 48126).

d. Necropsy: A gross necropsy will be conducted on each animal at termination of the study or at the time of discovery after death, and the results recorded. The gross necropsy shall include the following:

1. Terminal body weight.
2. Gross observations of external surfaces; all orifices; and thoracic, abdominal, and pelvic cavities.
3. Upon request of the Sponsor, sections of abnormal tissues may be saved in 10% neutral buffered formalin for possible histopathologic examination. Tissues will be discarded if histopathology is not performed.

5. Evaluation of Results: Unless only a single exposure concentration is tested, an LC_{50} with slope function and 95% confidence limits will be calculated for males, females, and males and females combined (if necessitated by mortality in one or both sexes) by the method of Litchfield, J.T., Jr., and Wilcoxon, F.: A Simplified Method of Evaluating Dose-Effect Experiments, Journal of Pharmacology & Experimental Therapeutics, 96, 99-115, 1949, or other appropriate method.

6. Test Substance Accountability: A comprehensive inventory of test substance received and used will be kept. The test substance container(s) will be weighed when received at this facility, and a record of all test substance use will be maintained. Test substance and test substance dosing solutions will be stored in the original containers, or in the equivalent thereof, or in glass containers with Teflon-lined caps.

7. Disposal of Unused Test Substance: Unused test substance will be returned or disposed of at the Sponsor's expense after the termination of the study. The Sponsor should specify return or disposal at the time of study authorization. If returned, the recipient of the test substance will be notified in advance of shipping. The sample will be accompanied by a chain of custody letter describing the sample(s) contained in the shipment.

8. Safety Precautions: General safety precautions required by laboratory SOPs will be followed. The Sponsor will supply basic toxicity data on the test substance to be used. However, since the toxicity of test substances is often not well characterized, this laboratory will be conservative in setting safety procedures. The Sponsor or Sponsor's Representative shall be notified of any exposures requiring a physician's examination or care.

APPENDIX C (cont.)

C. DATA MANAGEMENT

1. Records:

The following records will be maintained during the study and transferred to the STILLMEADOW, Inc. archives upon study termination.

a. Protocol and Protocol Amendments (if any).
b. Final report and amendments (if any).
c. Study correspondence.
d. Animal receipt/acclimation data.
e. Test substance receipt, identification as supplied by Sponsor, administration, and disposition.
f. Test animal information: number, species, age, sex, source, strain.
g. Body weight data.
h. Daily observation data for signs of pharmacologic and/or toxicologic effects.
i. Mortality data and gross necropsy findings, and histopathology data, if requested.
j. Calculations (if any) of the LC_{50} and slope determinations with 95% confidence limits.
k. Chamber operating parameters.
l. Other pertinent data.

2. Data Storage:

All raw data will be retained at STILLMEADOW, Inc.

3. Data Reporting:

The final report will include all data as described in the Good Laboratory Practice Standards, including:

a. Statement from the Quality Assurance Unit.
b. Signature of the Study Director.
c. A GLP Compliance Statement signed by the Study Director.
d. Names of scientific personnel involved in the study.
e. Dates of study initiation and termination.
f. Identification, description, preparation, and storage of the test substance.
g. All pertinent animal data, animal husbandry, exposure information, operating parameters, and observation methods.
h. Description of the test procedures.
i. If calculated, the LC_{50} and slope function data with 95% confidence limits for males, females, and males and females combined (if necessitated by mortality in one or both sexes).
j. Individual body weights.
k. Observations on the nature, onset, severity, and duration of all gross or visible pharmacologic and/or toxicologic signs. Nonroutine findings will be addressed in a discussion section in which the relationship to treatment and historical data will be evaluated.
l. Individual mortality data, gross necropsy findings, and histopathology findings, if applicable.
m. Chamber operating parameters.
n. A reference to this Protocol.

4. Report Submission:

A report will be submitted after termination of the in-life portion of the study (subject to completion of histopathology, if requested).

ATTACHMENT 37

Acute Dermal Irritation Study in Rabbits on Miller 6064

STILLMEADOW
INCORPORATED

VOLUME __ OF __ OF SUBMISSION

Miller 6064

FINAL REPORT

ACUTE DERMAL IRRITATION STUDY IN RABBITS

OPPTS NO. 870.2500

AUTHOR:

Janice O. Kuhn, Ph.D., D.A.B.T.

STUDY INITIATION DATE: 26 December 2000
STUDY COMPLETION DATE: 26 March 2001

CONDUCTED BY:
STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, TX 77478

LABORATORY STUDY NUMBER:

6210-00

VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 23

SUBMITTED TO:
Miller Chemical & Fertilization Corp.
P.O. Box 333
Hanover, PA 17331

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA § 10 (d) (1) (A), (B) or (C).

Company: Miller Chemical & Fertilization Corp.

Company Agent: _____ Date: _____

_____ _____

Title Signature

These data are the property of Miller Chemical & Fertilization Corp., and as such, are considered to be confidential for all purposes other than compliance with FIFRA § 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other statute or in any other country.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was designed and performed in STILLMEADOW, Inc.'s AAALAC accredited laboratory in compliance with:

- United States Environmental Protection Agency FIFRA: GLP Standards, 40 CFR 160 with exception of Sec. 160.31 (d), and 160.105 (b)(e): stability information was not provided
- United States Environmental Protection Agency TSCA 40 CFR 792 with exception of Sec. 792.31 (d), and 792.105 (b)(e): stability information was not provided
- Organization for Economic Cooperation & Dev. Principles of GLP, Annex 2, C(97)186 with exception of Sec. 6.2 (4): stability information was not provided
- Japan Ministry of Agriculture, Forestry & Fisheries, Notification No. 59 Nohsan 3850, Director-General of Agricultural Prod. Bureau, 10 Aug 84 with exception of Art. 5 (2)(9), and 21 (3): stability information was not provided

Janice O Kuhn — 26 Mar 01

Janice O. Kuhn, Ph.D., D.A.B.T.
Study Director, STILLMEADOW, Inc.

Date

Signature of Agent of Sponsor

Date

Agent Name
Sponsor: Miller Chemical & Fertilization Corp.

Signature of Agent of Submitter

Date

Agent Name
Submitter: Mandava Associates

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QUALITY ASSURANCE STATEMENT

Study Number: 6210-00
Test Substance: Miller 6064
Study Title: Acute Dermal Irritation Study in Rabbits

The study report and data have been audited in accordance with STILLMEADOW, Inc. Standard Operating Procedures (SOPs). The findings from the inspection and audit were reported to the Study Director and management as follows:

Phase of Study Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
Dosing	2 Jan 01	2 Jan 01	2 Jan 01
Report/Data Audit	7 Feb 01	7 Feb 01	7 Feb 01

Darrell Zeno

Darrell Zeno, M.S.
Quality Assurance Unit, STILLMEADOW, Inc.

26 Mar 01
Date

SUMMARY

A primary dermal irritation study was conducted on three albino rabbits using test substance Miller 6064. There was one intact test site per animal. Each test site was treated with 0.5 mL of the undiluted test substance and covered with a semi-permeable dressing. The test substance was maintained in contact with the skin for 4 hours. Observations for dermal irritation and defects were made at 1, 24, 48 and 72 hours after removal of the dressings.

Irritation scores derived from the respective erythema and edema scores through the 72 hour observations for each animal are presented below.

	Erythema Hours after Unwrap				**Edema** Hours after Unwrap				Irritation Scores
	1	24	48	72	1	24	48	72	
2280-M	1	1	0	0	0	0	0	0	0.50
2279-F	0	0	0	0	0	0	0	0	0.00
2281-F	1	1	0	0	0	0	0	0	0.50
Primary Irritation Index (PII)=									0.3

Based on the PII of 0.3, the test substance is rated slightly irritating. Based on the scores at the 72 hour observation only, the test substance is assigned to Toxicity Category IV.

INTRODUCTION

The objective of this study was to assess the relative primary skin irritation level of the test substance on rabbits in accordance with US EPA OPPTS 870.2500, which is intended to meet testing requirements of FIFRA 7 USC 136, et seq, and TSCA 15 USC 2601. This study was conducted for Miller Chemical & Fertilization Corp., according to the approved protocol and STILLMEADOW, Inc. SOPs. There were no deviations from the protocol which affected the quality or outcome of the study. All procedures used in this study are in compliance with Animal Welfare Act Regulations. The protocol, raw data and this report are archived at STILLMEADOW, Inc. The animals were treated with the test substance between 1120 and 1124 on 2 Jan 01. The in-life portion of the study was terminated on 5 Jan 01.

TEST SUBSTANCE

Identification:	Miller 6064
Date & Quantity Received:	19 Dec 00; 2 x 1 gal
Physical Description:	Amber liquid
Storage:	Room temperature
Purity & Composition:	Refer to Certificate of Analysis (Appendix A)
Stability:	Not provided by sponsor

Records pertaining to stability, characterization and verification of test substance identity are the responsibility of the sponsor. A copy of the Certificate of Analysis is included in the report.

TEST SYSTEM

Experimental Animals

Species & Strain:	Albino rabbit; New Zealand White
Justification of Species:	The rabbit is preferred by the various regulatory agencies for use in primary dermal irritation testing.
Source:	Ray Nichols Rabbitry; Lumberton, TX
Date Received:	29 Nov 00
Quarantine Period:	5 days
Quantity & Sex:	1 male and 2 females
Group Identification:	Cage cards
Animal Identification:	Ear tag
Initial Body Weight:	Male: 2.600 kg; Females: 2.775-3.025 kg
Date of Birth:	10 Sep 00

Animal Husbandry

Cage Type:	Suspended, wire bottom, stainless steel
Housing:	1 per cage
Environmental Controls Set to Maintain:	· Temperature Range 20°C±3° · Humidity Range 30-70% · 12-hour light/dark cycle · 10-12 air changes/hour
Food:	PMI Feeds, Inc.™ Lab Rabbit Diet #5321, in measured amounts
Water:	Municipal water supply analyzed by TNRCC Water Utilities Division; available *ad libitum* from automatic water system

Animal husbandry and housing at STILLMEADOW, Inc. comply with Animal Welfare Act Regulations. No contaminants were expected to have been present in the feed or water which would have interfered with or affected the results of the study.

PROCEDURES

Test Substance Administration

Prior to starting the study, the pH of the test substance was determined to be 7.13. Each animal was prepared on the day prior to treatment by clipping the dorsal area of the trunk free of hair to expose an area at least 8 x 8 cm. Only those animals with exposure areas free of pre-existing skin irritation or defects were selected for testing. A single intact exposure site was selected as the test site while the contralateral intact site served as a control site.

On Day 0, 0.5 mL of the undiluted test substance was applied to each test site and covered with a surgical gauze patch measuring 2.5 x 2.5 cm and four single layers thick. Each patch was secured in place with a strip of non-irritating adhesive tape. The entire trunk of each animal was loosely wrapped with a semi-permeable dressing (orthopedic stockinette) which was secured on both edges with strips of tape to retard evaporation of volatile substances and to prevent possible ingestion of the test substance.

Removal of Test Substance

After four hours, the patches and wrappings were removed. The test sites were gently washed with room temperature tap water and a clean cloth to remove as much residual test substance as possible.

Observations

The test sites were observed for erythema and edema formation, and any other dermal defects or irritation at 1, 24, 48 and 72 hours after unwrap.

Irritation Scoring Method

The scoring scale used to rate dermal irritation is presented in the Legend to Table 1. For each animal, all of the erythema and edema scores through 72 hours were added, and the sum was divided by 4 to obtain an individual irritation score. The primary irritation index was determined by calculating the mean of the irritation scores for all the animals and was used to obtain a rating for the test substance. A Toxicity Category (based only on the observations at 72 hours) was assigned according to the scale presented in the Legend to Table 1.

RESULTS AND DISCUSSION

Evaluation

Signs of dermal irritation or defects are presented in Table 1. Very slight erythema was present in two animals at each observation through 24 hours. Edema was not observed at any time throughout the study. No other signs of irritation were observed during the study.

CONCLUSION

The primary irritation index of 0.3 out of a possible 8.0 was obtained from the 1, 24, 48 and 72 hour observations and was used to give Miller 6064 a descriptive rating of slightly irritating. Based on the 72 hour observations only, Miller 6064 is assigned to Toxicity Category IV.

Study Director: Janice O. Kuhn 26 Mar 01

Janice O. Kuhn, Ph.D., D.A.B.T.
Senior Toxicologist, STILLMEADOW, Inc.

Date

STUDY PERSONNEL

Technical Staff: Carol Morris, B.A.
Hector Fuentes
Michelle Gantt, B.S.

Data Services: Connie Pavatte, Report Preparation

LEGEND TO TABLE 1
ACUTE DERMAL IRRITATION STUDY IN RABBITS
Primary Dermal Irritation Scoring Scale (Draize Technique*)

Evaluation of Skin Reactions

Erythema Formation	Score
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Maximum Possible	4

Edema Formation	Score
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4
Maximum Possible	4

* - Draize, John H., Woodard, Geoffrey, and Calvery, H.O., Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes. J. Pharm & Ther. 82, 377 (1944).

LEGEND TO TABLE 1 (cont.)
ACUTE DERMAL IRRITATION STUDY IN RABBITS
Classification of Test Substance

Descriptive Rating	Primary Irritation Index
Non-irritating	0.0
Slightly Irritating	0.1 - 1.9
Moderately Irritating	2.0 - 5.0
Severely Irritating	5.1 - 8.0

The primary irritation index is calculated using only the observations scheduled through 72 hours.

Dermal Irritation Toxicity Categories (per Proposed Rule, FR Vol. 49, No. 188)

Toxicity Category	Criteria
I	Corrosive
II	Severe irritation at 72 hours
III	Moderate irritation at 72 hours
IV	Non-irritating, mild, or slight irritation at 72 hours

TABLE 1
ACUTE DERMAL IRRITATION STUDY IN RABBITS
Signs of Dermal Irritation
Test Substance: Miller 6064

Animal Number	Erythema							Edema										Primary Irritation Scores
	Hours				Days			Hours				Days						
	1	24	48	72	7	10	14	1	24	48	72	7	10	14				
2280-M	1	1	0	0				0	0	0	0				2	/4	=	0.50
2279-F	0	0	0	0				0	0	0	0				0	/4	=	0.00
2281-F	1	1	0	0				0	0	0	0				2	/4	=	0.50
Primary Irritation Index* =					1.00		/3=	0.3										
Descriptive Rating =								Slightly Irritating										
Toxicity Category** =								IV										

* - Only the first four observation times are used for calculations.

** - Based only on the mean 72 hour score for erythema and edema.

Study Duration - 72 hours

M – Male; F – Female

TABLE 1 (cont.)
ACUTE DERMAL IRRITATION STUDY IN RABBITS
Signs of Dermal Irritation
Test Substance: Miller 6064

Animal Number	Other Observations						
	Hours				Days		
	1	24	48	72	7	10	14
2280-M	-	-	-	-			
2279-F	-	-	-	-			
2281-F	-	-	-	-			

Note: A dash (-) is used if there are no other signs of dermal irritation.
Study Duration - 72 hours
M - Male; F - Female

APPENDIX A

MILLER® CHEMICAL & FERTILIZER CORPORATION

P.O. BOX 333, 120 RADIO ROAD
HANOVER, PENNSYLVANIA 17331 U.S.A.
TELEPHONE: 717-632-8921
FAX NO.: 717-632-4581

1/18/01

CERTIFICATE OF ANALYSIS

Product Name: MILLER 6064
Quantity Produced: 7.4 kilograms
Batch Number: 150386
Date of Formulation: 12/13/00

Appearance: Yellow to Amber Liquid
Odor: Terpenic
Moisture: Negligble (non-aqueous)
Specific Gravity: 0.92 - 0.94 @ 20c
Viscosity @ 20c: 100 to 500 cps
pH(1% solution): 6.0 to 8.0
Solubility in Water: Insoluble (Emulsifies)
Flash Point: over 200 f
Purity: 100%

Signature:

Name: Andrew G. Smith
Title: Compliance Officer

APPENDIX B

STILLMEADOW
INCORPORATED

PROTOCOL FOR STUDY 6210-00

Study Title: ACUTE DERMAL IRRITATION STUDY IN RABBITS (OPPTS 870.2500)

Test Substance: MILLER 6064

Test Facility: STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, Texas 77478

Approved: _____ 26 Dec 00
Janice O. Kuhn, Ph.D., D.A.B.T. Date
Study Director
STILLMEADOW, Inc.

Approved: _____ 6 Dec 00
Mark S. Holbert Date
Vice President
STILLMEADOW, Inc.

Reviewed: _____ 6 Dec 2000
Vicki S. Crutchfield, RQAP Date
Director, Quality Assurance Unit
STILLMEADOW, Inc.

Sponsor: Miller Chemical and Fertilization Corp.
P.O. Box 333
Hanover, PA 17331

Sponsor Representative: Mandava Associates
1730 M Street, N.W., Suite 906
Washington, DC 20036

Approved: _____ December 26, 2000
N. Bhushan Mandava Date
Agent to Miller Chemical and Fertilization Corp.

PROTOCOL FOR STUDY 6210-00

A. GENERAL

1. Study Title: ACUTE DERMAL IRRITATION STUDY IN RABBITS

2. Purpose: To assess the relative level of primary skin irritation produced when rabbits are exposed to the test substance under semioccluded conditions.

3. Regulatory Compliance: This study will be conducted according to OPPTS 870.2500, which is intended to meet testing requirements of both FIFRA (7 U.S.C. 136, *et seq.*) and TSCA (15 U.S.C. 2601).

This study will be conducted in compliance with Good Laboratory Practice Standards:

1. EPA FIFRA: 40 CFR 160
2. EPA TSCA: 40 CFR 792
3. OECD: C(97)186
4. Japanese MAFF

All procedures in this protocol are in compliance with Animal Welfare Act Regulations. All methods can be found in STILLMEADOW, Inc. Standard Operating Procedures (SOPs).

4. Quality Assurance: The Protocol is reviewed by the Quality Assurance Unit (QAU). The study information will be entered into the Master Schedule. The study will be inspected at least once during its progress. Further inspections may be scheduled as needed to ensure the integrity of the study. Any deviations from SOPs, the Protocol, or Good Laboratory Practice Standards will be immediately reported to the Study Director and Management. The report will be audited and a statement prepared and signed which shall specify the dates inspections were made and findings reported to Management and to the Study Director.

5. Test Substance: MILLER 6064. Test substance identification should include the name, batch number and purity. Information regarding safety, stability, storage conditions and disposal should also be provided by the Sponsor. The Sponsor assumes responsibility for purity and stability determinations. Analysis and archiving of reserve samples will be the responsibility of the Sponsor.

6. Proposed Schedule: Testing will begin within approximately three weeks of receipt of the test substance and authorization to conduct the study.

Proposed Start Date: 19 Dec 00
Proposed End Date: 02 Jan 01

If dermal effects are resolved prior to 14 days after treatment, the study may end as early as 72 hours after treatment.

APPENDIX B (cont.)

A. GENERAL (cont.)

7.	Study Director:	Janice O. Kuhn, Ph.D., D.A.B.T.
8.	Experimental Summary:	The test substance will be applied to a single intact skin test site on each of three rabbits. The test substance will be maintained in contact with the skin for 4 hours. The test sites will then be washed as thoroughly as possible with room temperature tap water and/or an appropriate solvent without irritating the skin. The test sites will be scored 30-60 minutes later for signs of skin irritation. The sites will be scored again at 24, 48 and 72 hours after the end of the exposure period (post patch removal) and every 2 - 4 days thereafter until reversible irritation subsides (maximum of 14 days). The Primary Irritation Index will be determined from the scores through 72 hours. Unless otherwise requested by the Sponsor, a Toxicity Category will be assigned based on the scores at 72 hours.
9.	Protocol Amendments:	Any alteration in the Protocol will be justified, approved by the Study Director, and recorded in writing.
10.	Sponsor Audits:	The Sponsor may send an authorized representative to inspect the test system and/or data on the STILLMEADOW, Inc. premises during normal working hours.

B. EXPERIMENTAL DESIGN

1. Animals

a. Species:	Albino rabbit
b. Strain/Source:	New Zealand White (Ray Nichols Rabbitry, Lumberton, Texas or other suitable source)
c. Justification of Species:	The rabbit is conventionally used in primary dermal irritation studies to provide information on which human hazard can be judged, and is preferred by the regulatory agencies.
d. Quantity and Sex:	Three animals; males and/or females may be used
e. Age/Weight:	Young adult (12 weeks - 6 months); approximately 2 - 4 kg
f. Identification:	Ear tag
g. Acclimation and Health Status:	Animals will be acclimated for at least five days prior to testing. Normal weight gain, appearance, and behavior will be factors used to select healthy animals for testing. Only naive animals will be used.

2. Animal Husbandry

a. Cages:	Stainless steel, suspended, wire bottom.
b. Number per Cage:	Animals will be individually housed.
c. Food:	A measured amount of PMI Feeds, Inc.™ Laboratory Rabbit Diet #5321. The food is routinely analyzed by the manufacturer for nutritional components and environmental contaminants.
d. Water:	Tap water; available *ad libitum* (automatic system). Municipal water supply analyzed by Texas Natural Resource Conservation Commission (TNRCC) Water Utilities Division.
e. Contaminants:	There are no known contaminants in the feed or water available to laboratory animals that would be expected to interfere with this study.
f. Environment:	Target temperature: approximately 20°C ± 3°C. Target relative humidity: approximately 30 - 70%. 12-hour light/dark cycle (regulated automatically), and room ventilation of approximately 10 - 12 air changes per hour.

B. EXPERIMENTAL DESIGN (cont.)

3. Test Substance Administration

a. Preparation of Animals: Animals will be prepared on the day prior to treatment by clipping the dorsal area of the trunk of each animal free of hair to expose an area approximately 8 x 8 cm. Animals with exposure areas free from pre-existing skin irritation or defects will be selected for testing. A single intact exposure site will be selected as the test site with the contralateral intact site to remain as a control site.

b. Reason for Route of Administration: Dermal contact is a potential route of human exposure.

c. Stepwise Exposure of Animals: A single rabbit may be used if it is suspected that the test substance might produce severe irritation/corrosion. Three test patches are applied concurrently or sequentially to the animal. The first patch is removed after 3 min. If no serious skin reaction is observed, the second patch is removed after 1 hour. If observations indicate that exposure can be continued humanely, the third patch is removed after 4 hours and the responses graded. If a corrosive effect is observed after an exposure of up to 4 hours, then further animal testing is not required. If no corrosive effect is observed in one animal after a 4-hour exposure, the test is completed using two additional animals, each with one patch only, for an exposure period of 4 hours. If it is expected that the test substance will not produce severe irritancy or corrosion, the test may be started using three animals, each receiving one patch for an exposure period of 4 hours.

d. Application of Test Substance: On Day 0, 0.5 mL in the case of a liquid test substance, or 0.5 g in the case of solid or semi-solid test substance, will be introduced under a surgical gauze patch measuring 2.5 x 2.5 cm and four single layers thick to a single test site on each animal. Solid test substances will be moistened with deionized water or saline to form a thick paste prior to application and may require a 4-ply 5 x 5 cm gauze patch to cover all of the test substance. In some cases, the test substance may be applied to the gauze patch and the patch placed on the skin. If water or saline cannot be used to moisten the substance, acceptable alternatives are corn oil, glycerol, ethanol and water, mineral oil, aqueous carboxymethyl cellulose and gum arabic. The entire trunk will be covered with a semiocclusive dressing.

e. Control Site: A contralateral area of untreated skin will serve as the control against which the reactions of the treated site are evaluated. No separate control group of animals is used.

f. Removal of Test Substance: After the 4-hour exposure period, the patches and wrappings will be removed and the test substance will be removed as thoroughly as possible using water and/or an appropriate solvent (e.g., non-irritating mineral oil) without irritating the skin. The control site will be treated in a similar manner.

B. EXPERIMENTAL DESIGN (cont.)

4. Observations

a. Dermal Irritation: The animals will be observed and scored for erythema, edema and other signs of dermal irritation or defects 30-60 minutes after the removal of the patches and at 24, 48 and 72 hours after the end of the exposure period (patch removal). If irritation persists through 72 hours, observations will be made every 2 - 4 days thereafter until all reversible irritation subsides (maximum of 14 days). The scoring scale for signs of dermal irritation according to the Draize technique is presented in Appendix A.

b. Other Observations: Observations of any other toxic effects will be recorded.

5. Evaluation of Results: For each animal, all of the erythema and edema scores through 72 hours will be added and the sum divided by 4 (the number of observation periods) to obtain an individual irritation score. The Primary Irritation Index will be determined by calculating the mean of the irritation scores for the three animals and will be used to give the test substance a descriptive rating according to the classifications in Appendix B. Unless otherwise requested by the sponsor, a Toxicity Category will be assigned according to the scores at 72 hours only, as described in Appendix B.

6. Test Substance Accountability: A comprehensive inventory of test substance received and used will be kept. The test substance container(s) will be weighed when received at this facility, and a record of all test substance use will be maintained. Test substance and test substance dosing solutions will be stored in the original containers, or in the equivalent thereof, or in glass containers with Teflon-lined caps.

7. Disposal of Unused Test Substance: Unused test substance will be returned or disposed of at the Sponsor's expense after the termination of the study. The Sponsor should specify return or disposal at the time of study authorization. If returned, the recipient of the test substance will be notified in advance of shipping. The sample will be accompanied by a chain of custody letter describing the sample(s) contained in the shipment.

8. Safety Precautions: General safety precautions required by laboratory SOPs will be followed. The Sponsor will supply basic toxicity data on the test substance to be used. However, since the toxicity of test substances is often not well characterized, this laboratory will be conservative in setting safety procedures. The Sponsor or Sponsor's Representative shall be notified of any exposures requiring a physician's examination or care.

C. DATA MANAGEMENT

1. Records:

The following records will be maintained during the study and transferred to the STILLMEADOW, Inc. archives upon study termination.

a. Protocol and Protocol Amendments (if any).
b. Final report and amendments (if any).
c. Study correspondence.
d. Animal receipt/acclimation data.
e. Test substance receipt, identification as supplied by the Sponsor, preparation, administration, and disposition.
f. Test animal information: number, sex, source, strain.
g. All observations and scores for skin irritation for all time periods.
h. Observations of any other toxic effects.
i. Other pertinent data.

2. Data Storage:

All raw data will be retained at STILLMEADOW, Inc.

3. Data Reporting:

The final report will include all data as described in the Good Laboratory Practice Standards, including:

a. Statement from the Quality Assurance Unit.
b. Signature of the Study Director.
c. A GLP Compliance Statement signed by the Study Director.
d. Names of scientific personnel involved in the study.
e. Dates of study initiation and termination.
f. Identification, description, preparation, and storage of the test substance.
g. All pertinent animal data, animal husbandry, dosing information, and observation methods.
h. Description of the test procedures.
i. Identification and compositions of any vehicles used in adminstering the test substance and justification for their use.
j. Individual observations for erythema, eschar, edema, and any other signs of dermal defects or irritation at all observation periods. Tabulation of dermal irritation data, including onset, duration, and reversibility.
k. Primary dermal irritation score.
l. Descriptive rating for the test substance and a Toxicity Category (unless otherwise requested by Sponsor) for the test substance based on the scores at 72 hours.
m. Observations of any toxic effects.
n. A reference to this Protocol.

4. Report Submission:

A report will be submitted after termination of the in-life portion of the study.

APPENDIX B (cont.)

Appendix A
ACUTE DERMAL IRRITATION STUDY IN RABBITS
Evaluation of Skin Reactions

Primary Dermal Irritation Scoring Scale
(Draize Technique*)

Erythema and Eschar Formation	Score
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Maximum Possible	4

Edema Formation	Score
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4
Maximum Possible	4

Other observations may be made when needed, for example: Staining of the test site skin, necrosis, blanching, desquamation, sloughing, eschar, coriaceousness (leathery texture), atonia, etc.

* - Draize, John H., Woodard, Geoffrey, and Calvery, H.O., "Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes." J. Pharm. & Ther. 82, 377 (1944).

Appendix B
ACUTE DERMAL IRRITATION STUDY IN RABBITS
Classification of Test Substance

Descriptive Rating	Primary Irritation Index
Non-irritating	0.0
Slightly Irritating	0.1 - 1.9
Moderately Irritating	2.0 - 5.0
Severely Irritating	5.1 - 8.0

The primary irritation index is calculated using only the observations through 72 hours.

Dermal Irritation Toxicity Categories (per Proposed Rule, FR Vol. 49, No. 188)

Toxicity Category	Criteria
I	Corrosive
II	Severe irritation at 72 hours
III	Moderate irritation at 72 hours
IV	Non-irritating, mild or slight irritation at 72 hours

ATTACHMENT 38

Acute Eye Irritation Study in Rabbits on Miller 6064

STILLMEADOW
INCORPORATED

VOLUME __ OF __ OF SUBMISSION

Miller 6064

FINAL REPORT

ACUTE EYE IRRITATION STUDY IN RABBITS

OPPTS NO. 870.2400

AUTHOR:

Janice O. Kuhn, Ph.D., D.A.B.T.

STUDY INITIATION DATE: 26 December 2000
STUDY COMPLETION DATE: 26 March 2001

CONDUCTED BY:
STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, TX 77478

LABORATORY STUDY NUMBER:

6209-00

VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 29

SUBMITTED TO:
Miller Chemical & Fertilization Corp.
P.O. Box 333
Hanover, PA 17331

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA § 10 (d) (1) (A), (B) or (C).

Company: Miller Chemical & Fertilization Corp.

Company Agent: _____ Date: _____

_____ _____

Title Signature

These data are the property of Miller Chemical & Fertilization Corp., and as such, are considered to be confidential for all purposes other than compliance with FIFRA § 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other statute or in any other country.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was designed and performed in STILLMEADOW, Inc.'s AAALAC accredited laboratory in compliance with:

- United States Environmental Protection Agency FIFRA: GLP Standards, 40 CFR 160 with exception of Sec. 160.31 (d), and 160.105 (b)(e): stability information was not provided
- United States Environmental Protection Agency TSCA 40 CFR 792 with exception of Sec. 792.31 (d), and 792.105 (b)(e): stability information was not provided
- Organization for Economic Cooperation & Dev. Principles of GLP, Annex 2, C(97)186 with exception of Sec. 6.2 (4): stability information was not provided
- Japan Ministry of Agriculture, Forestry & Fisheries, Notification No. 59 Nohsan 3850, Director-General of Agricultural Prod. Bureau, 10 Aug 84 with exception of Art. 5 (2)(9), and 21 (3): stability information was not provided

Janice O. Kuhn, Ph.D., D.A.B.T.
Study Director, STILLMEADOW, Inc.

26 Mar 01
Date

Signature of Agent of Sponsor

Date

Agent Name
Sponsor: Miller Chemical & Fertilization Corp.

Signature of Agent of Submitter

Date

Agent Name
Submitter: Mandava Associates

TABLE OF CONTENTS

QUALITY ASSURANCE STATEMENT

Study Number: 6209-00
Test Substance: Miller 6064
Study Title: Acute Eye Irritation Study in Rabbits

The study report and data have been audited in accordance with STILLMEADOW, Inc. Standard Operating Procedures (SOPs). The findings from the inspection and audit were reported to the Study Director and management as follows:

Phase of Study Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
Dosing	2 Jan 01	2 Jan 01	2 Jan 01
Report/Data Audit	26 Jan 01	26 Jan 01	26 Jan 01

Darrell Zeno

Darrell Zeno, M.S.
Quality Assurance Unit, STILLMEADOW, Inc.

26 Mar 01

Date

SUMMARY

An acute eye irritation study was conducted on six albino rabbits using test substance Miller 6064. The undiluted test substance (0.1 mL) was placed into the conjunctival sac of the right eye of each animal selected for testing. All treated eyes were washed with room temperature deionized water for one minute immediately after recording the 24 hour observation.

The number of animals testing "positive" for each parameter (according to the Legend to Table 1) over the number of animals tested is presented below.

	Time After Treatment Hours			
	1	24	48	72
Cornea				
Opacity	0/6	0/6	0/6	0/6
Iritis	0/6	0/6	0/6	0/6
Conjunctivae				
Redness	0/6	0/6	0/6	0/6
Chemosis	0/6	0/6	0/6	0/6

There were no "positive" effects exhibited in any eyes at any time during the study. The test substance is rated minimally irritating and assigned to Toxicity Category IV.

INTRODUCTION

The objective of this study was to assess the relative level of eye irritation following a single exposure of the test substance to rabbits in accordance with US EPA OPPTS 870.2400, which is intended to meet testing requirements of FIFRA 7 USC 136, et seq, and TSCA 15 USC 2601. This study was conducted for Miller Chemical & Fertilization Corp., according to the approved protocol and STILLMEADOW, Inc. SOPs. There were no deviations from the protocol which affected the quality or outcome of the study. All procedures used in this study are in compliance with Animal Welfare Act Regulations. The protocol, raw data and this report are archived at STILLMEADOW, Inc. The animals were treated with the test substance between 1402 and 1404 on 2 Jan 01. The in-life portion of the study was terminated on 5 Jan 01.

TEST SUBSTANCE

Identification:	Miller 6064
Date & Quantity Received:	19 Dec 00; 2 x 1 gal
Physical Description:	Amber liquid
Storage:	Room temperature
Purity & Composition:	Refer to Certificate of Analysis (Appendix A)
Stability:	Not provided by sponsor

Records pertaining to stability, characterization and verification of test substance identity are the responsibility of the sponsor. A copy of the Certificate of Analysis is included in the report.

TEST SYSTEM

Experimental Animals

Species & Strain:	Albino rabbit; New Zealand White
Justification of Species:	The rabbit is preferred by the various regulatory agencies for use in eye irritation testing.
Source:	Ray Nichols Rabbitry, Lumberton, TX
Date Received:	28 Dec 00
Quarantine Period:	5 days
Quantity & Sex:	3 males and 3 females
Group Identification:	Cage cards
Animal Identification:	Ear tag
Initial Body Weight:	Males: 2.075-2.475 kg; Females: 2.125-2.300 kg
Date of Birth:	8 Oct 00

Animal Husbandry

Cage Type:	Suspended, wire bottom, stainless steel
Housing:	1 per cage
Environmental Controls Set To Maintain:	· Temperature Range 20°C± 3° · Humidity Range 30-70% · 12-hour light/dark cycle · 10-12 air changes/hour
Food:	PMI Feeds, Inc.™ Lab Rabbit Diet #5321, in measured amounts
Water:	Municipal water supply analyzed by TNRCC Water Utilities Division; available *ad libitum* from automatic water system.

Animal husbandry and housing at STILLMEADOW, Inc. comply with Animal Welfare Act Regulations. No contaminants were expected to have been present in the feed or water which would have interfered with or affected the results of the study.

PROCEDURES

Test Substance Administration

Prior to starting the study, the pH of the test substance was determined to be 7.13. Healthy albino rabbits were released from quarantine. Both eyes of each animal were carefully examined within 24 hours prior to treatment with a fluorescein sodium ophthalmic solution, and cobalt-filtered light. Both eyes of each animal were again carefully examined just prior to treatment, but without the fluorescein sodium ophthalmic solution. Only those animals without eye defects or irritation were selected for testing.

On Day 0, a dose of 0.1 mL of the undiluted test substance was placed into the conjunctival sac of the right eye of each animal by gently pulling the lower lid away from the eyeball to form a cup into which the test substance was dropped. The lids were gently held together for one second to prevent loss of material. The untreated left eyes served as comparative controls.

Observations

The treated eyes of all animals were examined without magnification under white room lighting provided by daylight-type fluorescent ceiling fixtures and an additional source of white light present on the examining table. The grades of ocular reaction were recorded at 1, 24, 48 and 72 hours after treatment. The corneas of all treated eyes were examined immediately after the 24-hour observation with a fluorescein sodium ophthalmic solution. A Finoff ocular transilluminator with cobalt blue filter (Welch Allyn, Skaneateles Falls, NY) was utilized to enhance visualization of fluorescein staining. Any of the corneas which exhibited fluorescein staining at the 24-hour observation were re-examined with the fluorescein sodium ophthalmic solution at each consecutive observation until fluorescein staining of the cornea no longer occurred. All treated eyes were washed with room temperature deionized water for one minute immediately after recording the 24-hour observation.

Irritation Scoring Method

Individual irritation scores for each animal at each scheduled observation were determined using the grading scale given in the Legend to Table 1. An average irritation score for each scheduled observation was then determined, based on the number of animals tested. A maximum average irritation score was derived from the observation yielding the highest average irritation score. The maximum average irritation score was used to rate the test substance according to the ratings presented in the Legend to Table 2. The scale used to categorize the test substance is also presented in the Legend to Table 2. Any corneal involvement or iridic irritation with a score of 1 or more is considered positive. Any conjunctival irritation (redness or chemosis) with a score of 2 or more is considered positive.

RESULTS AND DISCUSSION

Evaluation

The number of animals with "positive" findings at each observation period is presented in the summary section of this report. Ocular reactions are presented in Table 1. A summary of irritation scores is presented in Table 2.

The maximum average irritation score of 6.0, obtained at 1 hour after treatment, was used to rate Miller 6064 minimally irritating. Fluorescein staining did not occur in any of the eyes.

Toxicity categories are determined by the presence and duration of corneal involvement, iridic irritation, and "positive" conjunctival irritation. Any corneal involvement or iridic irritation with a score of 1 or more is considered positive. Any conjunctival irritation (redness or chemosis) with a score of 2 or more is considered positive.

CONCLUSION

Based on the Maximum Average Irritation Score of 6.0, the test substance Miller 6064 is rated minimally irritating. Since there were no "positive" effects observed during the study, the test substance is assigned to Toxicity Category IV. No irritation was observed in any eyes at 24 hours.

Study Director: Janice O Kuhn — 26 Mar 01

Janice O. Kuhn, Ph.D., D.A.B.T.
Senior Toxicologist, STILLMEADOW, Inc.

Date

STUDY PERSONNEL

Technical Staff: Carol Morris, B.A.
Hector Fuentes
Michelle Gantt, B.S.

Data Services: Connie Pavatte, Report Preparation

LEGEND TO TABLE 1
ACUTE EYE IRRITATION STUDY IN RABBITS
Grading Scale

I. Cornea

A. Opacity - degree (area most dense taken for reading)

No opacity	0
Slight dulling of normal luster	+
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible	1*
Easily discernible translucent area, details of iris slightly obscured	2*
Nacreous area, no details of iris visible, size of pupil barely discernible	3*
Opaque cornea, iris not discernible through the opacity	4*

B. Area of cornea involved

One quarter (or less), but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4

C. Fluorescein Staining - appearance of yellow-green staining of cornea

Cornea not examined with fluorescein	-
No fluorescein staining	0
Positive fluorescein staining	P
Area of cornea involved	
One quarter (or less), but not zero	A
Greater than one quarter, but less than half	B
Greater than half, but less than three quarters	C
Greater than three quarters, up to whole area	D

D. Stippling - appearance of pinpoint roughening

No stippling	0
Presence of stippling	S
Area of cornea involved	
One quarter (or less), but not zero	A
Greater than one quarter, but less than half	B
Greater than half, but less than three quarters	C
Greater than three quarters, up to whole area	D

A X B X 5 Total Maximum = 80

* - Reaction indicates a positive effect.

Reference: Draize, John H., Woodard, Geoffrey, and Calvery, Herbert O., Journal of Pharmacol. Exp. Ther., 82, 377-390 (1944).

LEGEND TO TABLE 1 (cont.)
ACUTE EYE IRRITATION STUDY IN RABBITS
Grading Scale

II. Iris

A. Grades

Normal	0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperemia or injection (any of these or combination thereof), iris still reacting to light (sluggish reaction is positive)	1*
No reaction to light, hemorrhage, gross destruction (any or all of these)	2*

A X 5 Total Maximum = 10

III. Conjunctivae

A. Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)

Blood vessels normal	0
Some blood vessels definitely hyperemic (injected)	1
Diffuse, crimson color, individual vessels not easily discernible	2*
Diffuse beefy red	3*

B. Chemosis: lids and/or nictitating membrane

No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2*
Swelling with lids about half closed	3*
Swelling with lids more than half closed	4*

C. Discharge

No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3

D. Necrosis or Ulceration of the palpebral and bulbar conjunctivae or nictitating membrane

No necrosis or ulceration	0
Presence of necrosis or ulceration	N

(A + B + C) X 2 Total Maximum = 20

The total score for the eye is the sum of all scores obtained for the cornea, iris, and conjunctivae with the possible maximum total score for the eye being equal to 110.

* - Reaction indicates a positive effect.

LEGEND TO TABLE 2
ACUTE EYE IRRITATION STUDY IN RABBITS
Rating of Test Substance Based on Eye Irritation[1]

Rating	Maximum Average Score	Definition
Non-Irritating	0.0-0.5	To maintain this rating, all scores at the 24-hour reading must be zero; otherwise, increase rating one level.
Practically Non-Irritating	>0.5-2.5	To maintain this rating, all scores at the 24-hour reading must be zero; otherwise, increase rating one level.
Minimally Irritating	>2.5-15.0	To maintain this rating, all scores at the 72-hour reading must be zero; otherwise, increase rating one level.
Mildly Irritating	>15.0-25.0	To maintain this rating, scores at the 7-day reading must be zero; otherwise, increase rating one level.
Moderately Irritating	>25.0-50.0	To maintain this rating, scores at the 7-day reading must be less than or equal to 10 for 60% or more of the animals. Also, the 7-day mean score must be less than or equal to 20. If the 7-day mean score is less than or equal to 20, but less than 60% of the animals show scores less than 10, then no animal among those showing scores greater than 10 can exceed a score of 30 if rating is to be maintained; otherwise, increase rating one level.
Severely Irritating	>50.0-80.0	To maintain this rating, scores at the 7-day reading must be less than or equal to 30 for 60% or more of the animals. Also, the 7-day mean score must be less than or equal to 40. If the 7-day mean score is less than or equal to 40, but less than 60% of the animals show scores less than or equal to 30, then no animal among those showing scores greater than 30 can exceed a score of 60 if rating is to be maintained; otherwise, increase rating one level.
Extremely Irritating	>80.0-110.0	

NOTE: The rating of the test substance is not to be increased more than one level above its maximum average score.

[1]Slightly modified from Kay, J.H. and Calandra, J.C. (1962) Interpretation of Eye Irritation Tests. J. Soc. Cosmetic Chemists 13:281-289

LEGEND TO TABLE 2 (cont.)
ACUTE EYE IRRITATION STUDY IN RABBITS
Criteria of Eye Irritation for Classification into Toxicity Categories [1]

Category	Criteria
I	Corrosive (irreversible destruction of ocular tissue) or corneal involvement or "positive" conjunctival irritation persisting through Day 21.
II	Corneal involvement or "positive" conjunctival irritation clearing in 8-21 days.
III	Corneal involvement or "positive" conjunctival irritation clearing in 7 days or less.
IV	Minimal effects clearing in less than 24 hours. No "positive" effects at 24 hours.

[1] Per Proposed Rule, FR Vol. 49, No. 188

TABLE 1
ACUTE EYE IRRITATION STUDY IN RABBITS
Ocular Reactions
Test Substance: Miller 6064

	Rabbit No. 2290-M										Rabbit No. 2291-F									
	Hrs. After Treatment				Days After Treatment						Hrs. After Treatment				Days After Treatment					
	1	24	48	72	4	7	10	14	17	21	1	24	48	72	4	7	10	14	17	21
I. Cornea																				
A. Opacity	0	0	0	0							0	0	0	0						
B. Area	0	0	0	0							0	0	0	0						
C. Fluorescein Staining	-	0	-	-							-	0	-	-						
D. Stippling	0	0	0	0							0	0	0	0						
SCORE	0	0	0	0							0	0	0	0						
II. Iris																				
A. Grade	0	0	0	0							0	0	0	0						
SCORE	0	0	0	0							0	0	0	0						
III. Conjunctivae																				
A. Redness	1	0	0	0							1	0	0	0						
B. Chemosis	1	0	0	0							1	0	0	0						
C. Discharge	1	0	0	0							1	0	0	0						
D. Necrosis or Ulceration	0	0	0	0							0	0	0	0						
SCORE	6	0	0	0							6	0	0	0						
TOTAL SCORE	6	0	0	0							6	0	0	0						

M – Male; F – Female

Duration of Study: 72 Hours

TABLE 1 (cont.)
ACUTE EYE IRRITATION STUDY IN RABBITS
Ocular Reactions
Test Substance: Miller 6064

	Rabbit No. 2292-M										Rabbit No. 2293-F									
	Hrs. After Treatment				Days After Treatment						Hrs. After Treatment				Days After Treatment					
	1	24	48	72	4	7	10	14	17	21	1	24	48	72	4	7	10	14	17	21
I. Cornea																				
A. Opacity	0	0	0	0							0	0	0	0						
B. Area	0	0	0	0							0	0	0	0						
C. Fluorescein Staining	-	0	-	-							-	0	-	-						
D. Stippling	0	0	0	0							0	0	0	0						
SCORE	0	0	0	0							0	0	0	0						
II. Iris																				
A. Grade	0	0	0	0							0	0	0	0						
SCORE	0	0	0	0							0	0	0	0						
III. Conjunctivae																				
A. Redness	1	0	0	0							1	0	0	0						
B. Chemosis	1	0	0	0							1	0	0	0						
C. Discharge	1	0	0	0							1	0	0	0						
D. Necrosis or Ulceration	0	0	0	0							0	0	0	0						
SCORE	6	0	0	0							6	0	0	0						
TOTAL SCORE	6	0	0	0							6	0	0	0						

M – Male; F – Female
Duration of Study: 72 Hours

TABLE 1 (cont.)
ACUTE EYE IRRITATION STUDY IN RABBITS
Ocular Reactions
Test Substance: Miller 6064

	Rabbit No. 2294-M										Rabbit No. 2295-F									
	Hrs. After Treatment				Days After Treatment						Hrs. After Treatment				Days After Treatment					
	1	24	48	72	4	7	10	14	17	21	1	24	48	72	4	7	10	14	17	21
I. Cornea																				
A. Opacity	0	0	0	0							0	0	0	0						
B. Area	0	0	0	0							0	0	0	0						
C. Fluorescein Staining	-	0	-	-							-	0	-	-						
D. Stippling	0	0	0	0							0	0	0	0						
SCORE	0	0	0	0							0	0	0	0						
II. Iris																				
A. Grade	0	0	0	0							0	0	0	0						
SCORE	0	0	0	0							0	0	0	0						
III. Conjunctivae																				
A. Redness	1	0	0	0							1	0	0	0						
B. Chemosis	1	0	0	0							1	0	0	0						
C. Discharge	1	0	0	0							1	0	0	0						
D. Necrosis or Ulceration	0	0	0	0							0	0	0	0						
SCORE	6	0	0	0							6	0	0	0						
TOTAL SCORE	6	0	0	0							6	0	0	0						

M – Male; F – Female

Duration of Study: 72 Hours

TABLE 2
ACUTE EYE IRRITATION STUDY IN RABBITS
Scores and Score Summary
Test Substance: Miller 6064

Time After Treatment	Rabbit Number						Average Score
	2290-M	2291-F	2292-M	2293-F	2294-M	2295-F	
Hour 1	6	6	6	6	6	6	6.0
Hour 24	0	0	0	0	0	0	0.0
Hour 48	0	0	0	0	0	0	0.0
Hour 72	0	0	0	0	0	0	0.0
Day 4							
Day 7							
Day 10							
Day 14							
Day 17							
Day 21							
Maximum Average Score:				6.0	Minimally Irritating		
Toxicity Category:				IV			
M – Male; F – Female							
Duration of Study: 72 Hours							

APPENDIX A

MILLER CHEMICAL & FERTILIZER CORPORATION

P.O. BOX 333, 120 RADIO ROAD
HANOVER, PENNSYLVANIA 17331 U.S.A.
TELEPHONE: 717-632-8921
FAX NO.: 717-632-4561

1/18/01

CERTIFICATE OF ANALYSIS

Product Name: MILLER 6064
Quantity Produced: 7.4 kilograms
Batch Number: 150386
Date of Formulation: 12/13/00

Appearance: Yellow to Amber Liquid
Odor: Terpenic
Moisture: Negligble (non-aqueous)
Specific Gravity: 0.92 - 0.94 @ 20c
Viscosity @ 20c: 100 to 500 cps
pH(1% solution): 6.0 to 8.0
Solubility in Water: Insoluble (Emulsifies)
Flash Point: over 200 f
Purity: 100%

Signature:

Name: Andrew G. Smith
Title: Compliance Officer

APPENDIX B

STILLMEADOW
INCORPORATED

PROTOCOL FOR STUDY 6209-00

Study Title: ACUTE EYE IRRITATION STUDY IN RABBITS
(OPPTS 870.2400)

Test Substance: MILLER 6064

Test Facility: STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, Texas 77478

Approved: _____ 26 Dec 00
Janice O. Kuhn, Ph.D., D.A.B.T. Date
Study Director
STILLMEADOW, Inc.

Approved: _____ 6 Dec 00
Mark S. Holbert Date
Vice President
STILLMEADOW, Inc.

Reviewed: _____ 6 Dec. 2000
Vicki S. Crutchfield, RQAP Date
Director, Quality Assurance Unit
STILLMEADOW, Inc.

Sponsor: Miller Chemical and Fertilization Corp.
P.O. Box 333
Hanover, PA 17331

Sponsor Representative: Mandava Associates
1730 M Street, N.W., Suite 906
Washington, DC 20036

Approved: _____ December 26, 2000
N. Bhushan Mandava Date
Agent to Miller Chemical and Fertilization Corp.

PROTOCOL FOR STUDY 6209-00

A. GENERAL

1. Study Title: ACUTE EYE IRRITATION STUDY IN RABBITS

2. Purpose: To assess the relative level of irritation produced following a single exposure of a test substance to one eye of albino rabbits.

3. Regulatory Compliance: This study will be conducted according to OPPTS 870.2400, which is intended to meet testing requirements of both FIFRA (7 U.S.C. 136, *et seq.*) and TSCA (15 U.S.C. 2601).

This study will be conducted in compliance with Good Laboratory Practice Standards:
1. EPA FIFRA: 40 CFR 160
2. EPA TSCA: 40 CFR 792
3. OECD: C(97)186
4. Japanese MAFF

All procedures in this protocol are in compliance with Animal Welfare Act Regulations. All methods can be found in STILLMEADOW, Inc. Standard Operating Procedures (SOPs).

4. Quality Assurance: The Protocol is reviewed by the Quality Assurance Unit (QAU). The study information will be entered into the Master Schedule. The study will be inspected at least once during its progress. Further inspections may be scheduled as needed to ensure the integrity of the study. Any deviations from SOPs, the Protocol, or Good Laboratory Practice Standards will be immediately reported to the Study Director and Management. The report will be audited and a statement prepared and signed which shall specify the dates inspections were made and findings reported to Management and to the Study Director.

5. Test Substance: MILLER 6064. Test substance identification should include the name, batch number, and purity. Information regarding safety, stability, storage conditions, and disposal should also be provided by the Sponsor. The Sponsor assumes responsibility for purity and stability determinations. Analysis and archiving of reserve samples will be the responsibility of the Sponsor.

6. Proposed Schedule: Testing will begin within approximately three weeks of receipt of the test substance and authorization to conduct the study.

Proposed Start Date: 18 Dec 00
Proposed End Date: 08 Jan 01

If ocular effects are resolved prior to 21 days after treatment, the study may end as early as 72 hours after treatment. The period of observation will not exceed 21 days.

A. GENERAL (cont.)

7. Study Director:	Janice O. Kuhn, Ph.D., D.A.B.T.
8. Experimental Summary:	One eye of each of six rabbits will be treated with the test substance. Eye irritation scores will be determined at 1, 24, 48 and 72 hours after treatment. If irritation persists at the 72-hour reading, observations will be made at 4 and 7 days and every 2 - 4 days thereafter until the eyes are clear or for a maximum of 21 days. These scores will be used to determine a descriptive rating for the test substance.
9. Protocol Amendments:	Any alteration in the Protocol will be justified, approved by the Study Director, and recorded in writing.
10. Sponsor Audits:	The Sponsor may send an authorized representative to inspect the test system and/or data on the STILLMEADOW, Inc. premises during normal working hours.

B. EXPERIMENTAL DESIGN

1. Animals

a.	Species:	Albino rabbit
b.	Strain/Source:	New Zealand White; Ray Nichols Rabbitry, Lumberton, TX (or other suitable source)
c.	Justification of Species:	The rabbit is conventionally used in primary eye irritation studies to furnish information on which human hazard can be judged, and is preferred by the regulatory agencies.
d.	Quantity and Sex:	Six rabbits. Both sexes will be represented on test.
e.	Age/Weight at Initiation:	Young adult (12 weeks - 6 months); approximately 2 - 4 kg
f.	Identification:	Ear tag
g.	Acclimation and Health Status:	Animals will be acclimated for at least five days prior to testing. Normal weight gain, appearance, behavior, and a negative pretest eye examination will be factors used to select healthy animals for testing. Only naive animals will be selected.

2. Animal Husbandry

a.	Cages:	Stainless steel, suspended, wire bottom. The housing will be maintained to exclude sawdust, woodchips, and other extraneous substances that might produce eye irritation.
b.	Number per Cage:	Animals will be individually housed.
c.	Food:	A measured amount of PMI Feeds, Inc.™ Laboratory Rabbit Diet #5321. The food is routinely analyzed by the manufacturer for nutritional components and environmental contaminants.
d.	Water:	Tap water; available *ad libitum* (automatic system). Municipal water supply analyzed by Texas Natural Resource Conservation Commission (TNRCC) Water Utilities Division.
e.	Contaminants:	There are no known contaminants in the feed or water available to laboratory animals that would be expected to interfere with this study.
f.	Environment:	Target temperature: approximately 20°C ± 3°C Target relative humidity: approximately 30 - 70% 12-hour light/dark cycle (regulated automatically) Room ventilation of approximately 10 - 12 air changes per hour.

B. EXPERIMENTAL DESIGN (cont.)

3. Test Substance Administration

a. Pretest Considerations: Ideally, the primary skin irritation potential of the test substance will be determined prior to the eye irritation test; however, this is not a requirement of this Protocol.

Any test substance with a pH of ≤2 or ≥11.5 will not be tested in the rabbit eye for irritation without consulting with the Sponsor's Representative. pH will be measured on powders (placed in aqueous solution) only if requested. pH measurement may not be appropriate for non-aqueous liquids.

Any test substance with a known Primary Irritation Index (PII) ≥5.0 will not be tested in the rabbit eye without consulting with the Sponsor's Representative.

b. Preparation of Animals: Both eyes of each animal will be examined using a fluorescein sodium ophthalmic solution within 24 hours prior to treatment. Only eyes without defects or irritation will be selected for testing.

c. Reason for Route of Administration: Ocular contact is a potential route of human exposure.

d. Application of Test Substance: On Day 0, a dose of 0.1 mL in the case of liquids or 0.1 mL by volume (with a weight of not more than 100 mg) in the case of solids or pastes, flakes, granules, powders, or other particulate forms, will be applied at room temperature to each test eye. If the test substance is solid or granular, it will be ground to a fine dust. If it is believed that the test substance may cause extreme pain, a local anesthetic may be used in both test and control eyes prior to the instillation of the test substance. The test substance will be placed in the selected eye of each animal by gently pulling the lower eyelid away from the eyeball to form a cup into which the test substance will be dropped. To prevent loss of material the eyelids will then be gently held together for approximately one second before releasing. The other eye remains untreated and serves as a control.

If the test substance is contained in a pressurized aerosol container, the eye will be held open and the test substance administered in a single burst of about one second from a distance of 10 cm directly in front of the eye.

After the twenty-four hour observation, the treated eye of each animal will be washed for one minute with room temperature deionized water.

B. EXPERIMENTAL DESIGN (cont.)

4. Ocular Observations

The treated eyes of all animals will be examined (magnification may be used as an aid) and the grades for ocular reactions will be recorded at 1, 24, 48 and 72 hours after treatment. If irritation or injury persists at the 72-hour observations, observations will be made on Days 4 and 7 and every 2 - 4 days thereafter until the eyes are clear, or for a maximum of 21 days. The study will be terminated when all animals on the study are clear of eye irritation. Fluorescein sodium ophthalmic solution will be used as an aid at the 24-hour observation. Any of the corneas which exhibit positive fluorescein staining at the 24-hour observation will be re-examined at each successive observation time until fluorescein staining is no longer present. The visualization of fluorescein staining will be aided by using a Finoff ocular transilluminator with cobalt blue filter (Wellch-Allyn, Skaneateles Falls, N.Y.).

Irritation will be graded and scored using the Draize technique. The grading scale is presented in Appendix A. All animals that have a damaged eye producing undue stress or discomfort will be sacrificed for humane reasons after consulting with the Sponsor.

5. Non-ocular Effects

Any non-ocular effects observed following treatment will be recorded.

6. Evaluation of Results:

An average irritation score will be determined for each observation time based on the number of animals scored. A maximum average irritation score will be derived from the observation period yielding the highest average irritation score. The maximum average irritation scores will be used to rate the test substance according to the ratings in Appendix B. In addition, the number of eyes with positive findings at each time period will be noted, and a determination of irritation reversibility will be made (no positive findings). Unless otherwise requested by the Sponsor, a determination of the toxicity category will be made according to the criteria in Appendix C.

7. Test Substance Accountability:

A comprehensive inventory of test substance received and used will be kept. The test substance container(s) will be weighed when received at this facility, and a record of all test substance use will be maintained. Test substance and test substance dosing solutions will be stored in the original containers, or in the equivalent thereof, or in glass containers with Teflon-lined caps.

8. Disposal of Unused Test Substance:

Unused test substance will be returned or disposed of at the Sponsor's expense after the termination of the study. The Sponsor should specify return or disposal at the time of study authorization. If returned, the recipient of the test substance will be notified in advance of shipping. The sample will be accompanied by a chain of custody letter describing the sample(s) contained in the shipment.

9. Safety Precautions:

General safety precautions required by laboratory SOPs will be followed. The Sponsor will supply basic toxicity data on the test substance to be used. However, since the toxicity of test substances is often not well characterized, this laboratory will be conservative in setting safety procedures. The Sponsor or Sponsor's Representative shall be notified of any exposures requiring a physician's examination or care.

C. DATA MANAGEMENT

1. Records:

The following records will be maintained during the study and transferred to the STILLMEADOW, Inc. archives upon study termination.

a. Protocol and Protocol Amendments (if any).
b. Final report and amendments (if any).
c. Study correspondence.
d. Animal receipt/acclimation data.
e. Test substance receipt, identification as supplied by Sponsor, preparation, administration, and disposition.
f. Test animal information: number, sex, source, strain.
g. Observation data for ocular irritation or injury.
h. Other pertinent data.

2. Data Storage:

All raw data will be retained at STILLMEADOW, Inc.

3. Data Reporting:

The final report will include all data as described in the Good Laboratory Practice Standards, including:

a. Statement from the Quality Assurance Unit.
b. Signature of the Study Director.
c. A GLP Compliance Statement signed by the Study Director.
d. Names of scientific personnel involved in the study.
e. Dates of study initiation and termination.
f. Identification, description, preparation, and storage of the test substance.
g. Identification and description of any vehicles, anesthetics or other materials used in the study.
h. All pertinent animal data, animal husbandry, acclimation information, dosing information.
i. Description of the method used to score irritation.
j. Description of any non-ocular effects noted.
k. Individual observations for treated eyes.
l. The number of eyes with positive findings at each time period; determination when irritation was reversible (no positive findings).
m. Individual eye irritation scores for each time period for each animal.
n. Maximum average irritation scores.
o. Rating and Toxicity Category (unless otherwise requested by the Sponsor) of the test substance.
p. A reference to this Protocol.

4. Report Submission:

A report will be submitted after termination of the in-life portion of the study.

Appendix A
ACUTE EYE IRRITATION STUDY IN RABBITS
Grading Scale

I. Cornea

A. Opacity - degree (area most dense taken for reading)

No opacity 0
Slight dulling of normal luster ..... +
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible 1*
Easily discernible translucent areas, details of iris slightly obscured 2*
Opalescent area, no details of iris visible, size of pupil barely discernible 3*
Opaque cornea, iris not discernible through the opacity 4*

B. Area of cornea involved

One quarter (or less), but not zero 1
Greater than one quarter, but less than half 2
Greater than half, but less than three quarters 3
Greater than three quarters, up to whole area 4

C. Fluorescein Staining - appearance of yellow-green staining of cornea

Cornea not examined with fluorescein ..... -
No fluorescein staining 0
Positive fluorescein staining ..... P
Area of cornea involved
One quarter (or less), but not zero ..... A
Greater than one quarter, but less than half ..... B
Greater than half, but less than three quarters C
Greater than three quarters, up to whole area ..... D

D. Stippling - appearance of pinpoint roughening

No stippling 0
Presence of stippling ..... S
Area of cornea involved
One quarter (or less), but not zero ..... A
Greater than one quarter, but less than half ..... B
Greater than half, but less than three quarters C
Greater than three quarters, up to whole area ..... D

A X B X 5 Total Maximum = 80

* - Reaction indicates a positive effect.

Reference: Draize, John H., Woodard, Geoffrey, and Calvery, Herbert O., Journal of Pharmacol. Exp. Ther., 82, 377-390 (1944).

Appendix A (cont.)
ACUTE EYE IRRITATION STUDY IN RABBITS
Grading Scale

II. Iris
A. Grades
Normal 0
Folds above normal, congestion, swelling, moderate circumcorneal hyperemia or injection (any of these or combination thereof), iris still reacting to light (sluggish reaction is positive) 1*
No reaction to light, hemorrhage, gross destruction (any or all of these) 2*

A X 5 Total Maximum = 10

III. Conjunctivae
A. Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)
Blood vessels normal 0
Some blood vessels definitely hyperemic (injected) 1
Diffuse, crimson color, individual vessels not easily discernible 2*
Diffuse beefy red 3*

B. Chemosis: lids and/or nictitating membrane
No swelling 0
Any swelling above normal (includes nictitating membrane) 1
Obvious swelling with partial eversion of lids 2*
Swelling with lids about half closed 3*
Swelling with lids more than half closed 4*

C. Discharge
No discharge 0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals) 1
Discharge with moistening of the lids and hairs just adjacent to lids 2
Discharge with moistening of the lids and hairs, and considerable area around the eye 3

D. Necrosis or Ulceration of the palpebral and bulbar conjunctivae or nictitating membrane
No necrosis or ulceration 0
Presence of necrosis or ulceration ..... N

(A + B + C) X 2 Total Maximum = 20

The total score for the eye is the sum of all scores obtained for the cornea, iris, and conjunctivae with the possible maximum total score for the eye being equal to 110.

* - Reaction indicates a positive effect.

Appendix B
ACUTE EYE IRRITATION STUDY IN RABBITS
Rating of Test Substance Based on Eye Irritation

Rating	Maximum Average Score	Definition
Non-Irritating	0.0 - 0.5	To maintain this rating, all scores at the 24-hour reading must be zero; otherwise, increase rating one level.
Practically Non-Irritating	> 0.5 - 2.5	To maintain this rating, all scores at the 24-hour reading must be zero; otherwise, increase rating one level.
Minimally Irritating	> 2.5 - 15.0	To maintain this rating, all scores at the 72-hour reading must be zero; otherwise, increase rating one level.
Mildly Irritating	> 15.0 - 25.0	To maintain this rating, scores at the 7-day reading must be zero; otherwise, increase rating one level.
Moderately Irritating	> 25.0 - 50.0	To maintain this rating, scores at the 7-day reading must be less than or equal to 10 for 60% or more of the animals. Also, the 7-day mean score must be less than or equal to 20. If the 7-day mean score is less than or equal to 20, but less than 60% of the animals show scores less than 10, then no animal among those showing scores greater than 10 can exceed a score of 30 if rating is to be maintained; otherwise, increase rating one level.
Severely Irritating	> 50.0 - 80.0	To maintain this rating, scores at the 7-day reading must be less than or equal to 30 for 60% or more of the animals. Also, the 7-day mean score must be less than or equal to 40. If the 7-day mean score is less than or equal to 40, but less than 60% of the animals show scores less than or equal to 30, then no animal among those showing scores greater than 30 can exceed a score of 60 if rating is to be maintained; otherwise, increase rating one level.
Extremely Irritating	> 80.0 - 110.0	

NOTE: The rating of the test material is not to be increased more than one level above its maximum average score.

Reference: Modification of Classification System of John H. Kay, Ph.D., and Joseph C. Calandra, Ph.D., Interpretation of Eye Irritation Tests, Journal of the Society of Cosmetic Chemists, p. 286.

Appendix C
ACUTE EYE IRRITATION STUDY IN RABBITS
Criteria of Eye Irritation for Classification into Toxicity Categories

Category	Criteria
I	Corrosive (irreversible destruction of ocular tissue) or corneal involvement or "positive" conjunctival irritation persisting through Day 21.
II	Corneal involvement or "positive" conjunctival irritation clearing in 8-21 days.
III	Corneal involvement or "positive" conjunctival irritation clearing in 7 days or less.
IV	Minimal effects clearing in less than 24 hours. No "positive" effects at 24 hours.

ATTACHMENT 39

Skin Sensitization Study
(Guinea Pig Maximization Test for Topically Applied Test Substance)
on Miller 6064

STILLMEADOW
INCORPORATED

VOLUME __ OF __ OF SUBMISSION

Miller 6064

FINAL REPORT

GUINEA PIG MAXIMIZATION TEST FOR TOPICALLY APPLIED TEST SUBSTANCE

OPPTS NO. 870.2600

AUTHOR - Janice O. Kuhn, Ph.D., D.A.B.T.

STUDY INITIATION DATE: 26 December 2000
STUDY COMPLETION DATE: 27 March 2001

CONDUCTED BY:
STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, TX 77478

LABORATORY STUDY NUMBER

6211-00

VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 29

SUBMITTED TO:
Miller Chemical & Fertilizer Corp.
P.O. Box 333, Radio Road
Hanover, PA 17331

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA § 10 (d) (1) (A), (B) or (C).

Company: Miller Chemical & Fertilizer Corp.

Company Agent: _____ Date: _____

_____ _____
Title Signature

These data are the property of Miller Chemical & Fertilizer Corp., and as such, are considered to be confidential for all purposes other than compliance with FIFRA § 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other statute or in any other country.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was designed and performed in STILLMEADOW, Inc.'s AAALAC accredited laboratory in compliance with:

- United States Environmental Protection Agency FIFRA: GLP Standards, 40 CFR 160 with exception of Sec. 160.31 (d) and 160.105 (b)(e): stability information was not provided
- United States Environmental Protection Agency TSCA: 40 CFR 792, with exception of Sec. 792.31 (d) and 792.105 (b)(e): stability information was not provided
- Organization for Economic Cooperation & Dev. Principles of GLP, Annex 2, C(97)186, with exception of Sec. 6.2 (4): stability information was not provided
- Japan Ministry of Agriculture, Forestry & Fisheries, Notification No. 59 Nohsan 3850, Director-General of Agricultural Prod. Bureau, 10 Aug 84, with exception of Art. 5 (2)(9) and 22 (3): stability information was not provided

Janice O. Kuhn, Ph.D., D.A.B.T.
Study Director, STILLMEADOW, Inc.

27 Mar 01
Date

Signature of Agent of Sponsor

Date

Agent Name
Sponsor: Miller Chemical & Fertilizer Corp.

Signature of Agent of Submitter

Date

Agent Name
Submitter: Mandava Associates

TABLE OF CONTENTS

QUALITY ASSURANCE STATEMENT

Study Number: 6211-00
Test Substance: Miller 6064
Study Title: Guinea Pig Maximization Test For Topically Applied Test Substance

The study report and data have been audited in accordance with STILLMEADOW, Inc. Standard Operating Procedures (SOP). The findings from inspection and audit were reported to study Director and management as follows:

Phase of Study Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
Dosing	30 Jan 01	31 Jan 01	31 Jan 01
Report/Data Audit	7 Mar 01	7 Mar 01	7 Mar 01

B. Lynn Murphy, B.S.
Quality Assurance Unit, STILLMEADOW, Inc.

27 Mar 01
Date

SUMMARY

A maximization test for topically applied test substances was conducted on 30 short-haired male and female albino guinea pigs to determine if the test substance, Miller 6064, produced a sensitizing reaction. Group I animals, the test group (10/sex), each received three pairs of intradermal injections (adjuvant, a solution of test substance in deionized water, and a 50:50 mixture of adjuvant and the test substance solution) followed one week later by a single topical application of undiluted test substance. Ten additional animals (5/sex) served as a control group (Group II). Control animals were treated at the same time periods and locations but with the vehicle used in place of the test substance/solution. Two weeks after the topical application, the test animals were challenged with a second topical application of undiluted test substance at a virgin test site. Control animals were also given a topical application of undiluted test substance. The percentage of animals exhibiting erythema with or without edema after the challenge treatment was used to assign the test substance a sensitization potency rating. Since 0% of the test animals exhibited scores greater than zero, Miller 6064 was given a sensitization potency rating of non-sensitizer.

INTRODUCTION

The objective of this study was to determine the sensitizing potential of the test substance, using the methods of Magnusson and Kligman (J. Invest. Dermat 52: 268-276 (1969)). This study was conducted for Miller Chemical & Fertilizer Corp., according to the approved protocol and STILLMEADOW, Inc. SOP's. There were no deviations from the protocol that affected the quality or outcome of the study. All procedures used in this study are in compliance with Animal Welfare Act Regulations. The protocol, raw data and this report are archived at STILLMEADOW, Inc. The treatment schedule was as follows, and the study was terminated on 2 Feb 01:

	Treatments		
	Intradermal Injections	Topical Applications	
Groups	Initial Treatment	Second Insult	Challenge
Test and Control	9 Jan 01	16 Jan 01	30 Jan 01

TEST SUBSTANCE

Label:	Miller 6064
Quantity & Date Received:	19 Dec 00; 2 x 1 gal
Physical Description:	Amber liquid
Storage:	Room temperature
Purity & Composition:	See Certificate of Analysis (App. B)
Stability:	Not provided by sponsor
Concentrations Administered:	3% v/v solution of test substance in vehicle with and without adjuvant (50:50 v/v) for intradermal injections; 0.5 mL undiluted for topical and challenge applications
Vehicle:	Deionized water

Records pertaining to stability, characterization, and verification of test substance identity are the responsibility of the sponsor. A copy of the Certificate of Analysis is included in the report.

ADJUVANT

Label:	FREUND'S ADJUVANT Complete Lot # 20K8933
Manufacturer:	Sigma Chemical
Physical Description:	Clear liquid
Storage:	Store in a cool, dark place at 0 to 5°C, do not freeze
Concentration Administered:	Diluted at 50% v/v in 0.9% saline
Purity & Composition:	Available from manufacturer
Stability:	Expiration – Mar 03

VEHICLE AND/OR OTHER MATERIALS

Label:	0.9% Sodium Chloride Lot # 50-164-JT
Manufacturer:	Abbott Labs
Expiration Date:	Mar 01

TEST SYSTEM

Experimental Animals

Species & Strain:	Guinea Pig; Hartley-Albino
Justification of Species:	The guinea pig is conventionally used in skin sensitization studies to provide information on which human hazard can be judged.
Source:	Charles River Laboratories, Wilmington, MA
Quantity & Sex:	15 males and 15 females
Quarantine Period:	5 days
Date Received:	2 Jan 01
Animal Identification:	Ear punch
Weight When Tested:	Males (328-406 g); Females (323-368 g)

Animal Husbandry

Cage Type:	Suspended, wire bottom, stainless steel
Housing:	Housed individually for 3 days (during wrapping) 1-4 animals per cage (males separate from females)
Environmental Controls Set to Maintain:	·Temperature Range 20°C ± 3° ·Humidity Range 30-80% ·12-hour light/dark cycle ·10-12 air changes/hour
Food:	PMI Feeds, Inc.™ Guinea Pig Diet #5025 available *ad libitum*
Water:	Municipal water supply analyzed by TNRCC Water Utilities Division; available *ad libitum* from water bowls or automatic system

Animal husbandry and housing at STILLMEADOW, Inc. comply with Animal Welfare Act Regulations. No contaminants were expected to have been present in the feed or water which would have interfered with or affected the results of the study.

POSITIVE CONTROL INFORMATION

Positive Control Material

Label:	1-Chloro-2,4-Dinitro-Benzene Min. 98% [97-00-7] EEC No. 202-551-4 C-6396 Lot 87H0799
Manufacturer:	Sigma Chemical
Physical Description:	Light yellow crystals
Concentrations Administered:	0.5% w/v solution of test substance in cottonseed oil with and without adjuvant (50:50 v/v) for intradermal injections; 2.0% w/w concentration of test substance in petrolatum for topical insult and challenge application
Purity, Composition & Stability:	Available from manufacturer

POSITIVE CONTROL INFORMATION (cont.)

Positive Control Testing
The sensitivity of guinea pigs to a positive control material is confirmed in this laboratory periodically. The positive control animals used to conduct this study were supplied by Charles River Laboratory, and were tested according to the Magnusson and Kligman (J. Invest. Dermat 52: 268-276 (1969)).

STILLMEADOW, Inc. Study No. 5782-00
In-life start: 18 Apr 00; In-life completed: 12 May 00

Results: Data from this study are presented in Appendix A. Since all 20 animals of the test group exhibited patchy to intense erythema after the challenge treatment, and only three of the 10 animals of the naive control group exhibited patchy to moderate erythema after the challenge treatment, the test substance is considered an extreme sensitizer and confirmed the sensitivity of guinea pigs to the positive control material.

PROCEDURES

Test Substance Preparation
A 3% v/v solution of test substance in deionized water was selected for intradermal injection, and 0.5 mL of undiluted test substance was selected for the topical applications (induction and challenge).

Healthy, short-haired, albino guinea pigs (males and females) were released from quarantine prior to testing. Five animals per sex were assigned to a control group (Group II). Ten animals per sex were assigned to the test group (Group I). On the day prior to each treatment, the animals were prepared by clipping the appropriate exposure areas free of hair. Individual body weights were recorded on Days -1 and 24. The animals were treated on Days 0, 7, and 21.

Test Substance Application
Induction: Intradermal Injections: The animals were treated on Day 0 by making three pairs of symmetrical intradermal injections on the upper back of each animal within a 4 x 6 cm exposure area running laterally across the shoulders. For the test animals (Group I), the first pair of injections (one on each side of the spinal column and approximately 3.5 cm apart), consisting of Freund's Complete Adjuvant diluted to 50% v/v in saline, was made at the anterior edge of the exposure area. The second pair of injections, consisting of 3% v/v test substance in deionized water, was made approximately 0.5 cm behind the first pair. The third pair of injections, consisting of a 50:50 mixture of Freund's Complete Adjuvant (diluted to 50% v/v in saline) and a solution of 3% v/v test substance in deionized water was made approximately 0.5 cm behind the second pair of injections. Group II control animals received the same injections with the vehicle substituted for the test substance in the second and third pairs of injections. All injections were within a 2 x 4 cm area of the 4 x 6 cm exposure area. A volume of 0.1 mL was administered at each site.

PROCEDURES (cont.)

Induction: Topical Applications: On Day 7, 0.5 mL of undiluted test substance was applied to the exposure area of each test group animal (Group I) to cover the intradermal injection sites. A 5 cm round patch of filter paper was used to cover the dose site. The patch was then occluded with an adhesive masking tape and secured in place with an elastic adhesive wrap wound around the torso of the animal. Control group animals (Group II) received 0.5 mL of vehicle and a 5 cm round patch of filter paper was placed over the dose sites. The wrappings and patches were removed after 48 hours. Test sites 3 and 4 were observed for dermal irritation on Day 10.

Challenge: Test and Control Animals: On Day 21, a 5 x 5 cm area was clipped on both the left and right flanks of each test and control animal. For the challenge treatment, 0.5 mL of undiluted test substance was applied topically to the right flank of each animal in a manner identical to the Day 7 treatment. A 5 cm round patch of filter paper was used to cover the dose site. A dry 5 cm round patch of filter paper was applied topically to the left flank of each animal. Patches were secured as above.

Observations and Scoring Methods

On Day 22 (24 hours after challenge), the wrappings and patches were removed. On Day 23 (24 hours after unwrapping), the test sites were observed for skin reactions, and again observed on Day 24 (48 hours after unwrapping). Observations were made of right and left flanks of each animal in Groups I and II. The scoring scale used for grading skin reactions after both intradermal and topical exposures is presented in the Legend to Table 1. After both induction treatments (intradermal injections and topical applications) and the challenge exposure, average skin reaction scores were calculated. These data appear in Table 1.

Any Group I animals which exhibited scores greater than 0 for erythema with or without edema for the treated right flank after the challenge treatment were considered possibly sensitized. However, the skin reactions of the left flank treated with patch alone, and the skin reactions of the naive controls were also evaluated. The test substance was graded and rated for sensitization potency based upon the percentage of animals sensitized using the scoring scale presented below:

% Sensitized	Grade	Rating
0	0	Non-sensitizer
1-8	I	Weak
9-28	II	Mild
29-64	III	Moderate
65-80	IV	Strong
81-100	V	Extreme

RESULTS AND DISCUSSION

Skin reaction scores and average skin reaction scores are presented in Table 1. Body weights are presented in Table 2. Body weight gain was unaffected by the administration of the test substance. The challenge treatments with either patch alone or test substance produced no erythema in any test or control group animals.

CONCLUSION

Since 0% of the test animals exhibited scores greater than zero, Miller 6064 was given a sensitization potency rating of non-sensitizer.

Study Director: Janice O. Kuhn — 27 Mar 01
Janice O. Kuhn, Ph.D., D.A.B.T. — Date
Senior Toxicologist, STILLMEADOW, Inc.

STUDY PERSONNEL

Technical Staff: Carol Morris, B.A.
Hector Fuentes
Michelle Gantt, B.S.

Data Services: Connie Pavatte, Report Preparation

TABLE 1
GUINEA PIG MAXIMIZATION TEST FOR TOPICALLY APPLIED TEST SUBSTANCE
Skin Reaction Scores and Averages
Test Substance: Miller 6064
Group I – Test

	Intradermal Induction						Topical Induction		Challenge			
Animal Number	Scores for each site on Day 6						Day 10 Scores		Day 23		Day 24	
	1	2	3	4	5	6	Site 3	Site 4	LF	RF	LF	RF
1-M	2	2	1	1	1	1	1	1	0	0	0	0
2-M	2	2	1	2	1	2	1	1	0	0	0	0
3-M	2	2	1	1	1	1	1	1	0	0	0	0
* 4-M	2	2	1	1	1	2	0	1	0	0	0	0
5-M	2	2	1	1	1	1	0	0	0	0	0	0
6-M	2	2	1	1	1	2	1	1	0	0	0	0
7-M	2	2	1	1	1	1	1	1	0	0	0	0
8-M	1	1	1	1	1	1	1	1	0	0	0	0
9-M	2	2	0	0	1	1	1	1	0	0	0	0
10-M	2	2	1	1	1	2	1	1	0	0	0	0
11-F	2	2	1	1	2	2	1	1	0	0	0	0
12-F	2	2	1	1	2	2	1	1	0	0	0	0
13-F	1	2	1	1	1	2	0	1	0	0	0	0
14-F	2	1	1	1	2	1	0	1	0	0	0	0
15-F	2	1	1	1	1	1	0	0	0	0	0	0
16-F	1	1	1	1	1	1	1	1	0	0	0	0
17-F	2	2	1	1	1	2	1	1	0	0	0	0
18-F	1	1	1	1	1	1	1	1	0	0	0	0
19-F	1	1	1	1	1	1	1	1	0	0	0	0
20-F	1	1	1	0	1	1	0	0	0	0	0	0
Mean:	1.7	1.7	1.0	1.0	1.2	1.4	0.8		0.0	0.0	0.0	0.0
	1.3						Incidence of Reactions:		0/20		0/20	

% Sensitized: 0% Sensitization Potency Rating: Non-sensitizer

Injection Sites:
Sites 1 & 2 - Freund's Complete Adjuvant - 50% v/v in saline
Sites 3 & 4 - Test Substance in deionized water
Sites 5 & 6 - Mixture (50:50) of Freund's Complete Adjuvant in saline and Test Substance in deionized water

Induction topical and Challenge Treatments: 100% Test Substance
LF - Left Flank (dosed w/dry patch); RF - Right Flank (dosed w/Test Substance)

M - Male; F - Female

TABLE 1 (cont.)
GUINEA PIG MAXIMIZATION TEST FOR TOPICALLY APPLIED TEST SUBSTANCE
Skin Reaction Scores and Averages
Test Substance: Miller 6064
Group II - Control

	Intradermal Induction						Topical Induction		Challenge			
Animal Number	Scores for each site on Day 6						Day 10 Scores		Day 23		Day 24	
	1	2	3	4	5	6	Site 3	Site 4	LF	RF	LF	RF
21-M	2	2	0	0	0	0	0	0	0	0	0	0
22-M	2	2	0	0	0	0	0	0	0	0	0	0
23-M	2	2	0	0	0	0	0	0	0	0	0	0
24-M	2	2	0	0	0	0	0	0	0	0	0	0
25-M	2	2	0	0	0	0	0	0	0	0	0	0
26-F	1	1	0	0	0	0	0	0	0	0	0	0
27-F	2	2	0	0	0	0	0	0	0	0	0	0
28-F	2	2	0	0	0	0	0	0	0	0	0	0
29-F	1	1	0	0	0	0	0	0	0	0	0	0
30-F	1	1	0	0	0	0	0	0	0	0	0	0
Mean:	1.7	1.7	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0
	0.6						Incidence of Reactions:		0/10		0/10	

Injection Sites:
Sites 1 & 2 - Freund's Complete Adjuvant - 50% v/v in saline
Sites 3 & 4 – Vehicle (deionized water)
Sites 5 & 6 - Mixture (50:50) of Freund's Complete Adjuvant in saline and vehicle

Induction Topical Treatments: 0.5 mL of vehicle

Challenge Treatments: 100% Test Substance
LF - Left Flank (dosed w/dry patch); RF - Right Flank (dosed w/Test Substance)

M - Male; F - Female

LEGEND TO TABLE 1
GUINEA PIG MAXIMIZATION TEST FOR TOPICALLY APPLIED TEST SUBSTANCE
Evaluation of Skin Reactions

Magnusson and Kligman Grading Scale for the Evaluation of Challenge Patch Test Reactions*

Erythema Formation	Score
No visible change	0
Slightly patchy erythema	±
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

* - OECD Guidelines for the Testing of Chemicals, Volume 2, Section 4, Number 406, Skin Sensitization, Paragraph 23, page 4/9, Adopted 17 Jul 92

APPENDIX A
GUINEA PIG MAXIMIZATION TEST FOR TOPICALLY APPLIED TEST SUBSTANCE
Positive Control Table 1
Skin Reaction Scores and Averages
Positive Control Substance: DNCB C-6396 Lot 87H0799
Study Number: 5782-00
Group I – Test

	Intradermal Induction						Topical Induction		Challenge			
Animal Number	Scores for each site on Day 6						Day 10 Scores		Day 23		Day 24	
	1	2	3	4	5	6	Site 3	Site 4	LF	RF	LF	RF
261-M	0	0	0	0	0	0	1	2	0	2	0	2
262-M	0	±	0	0	0	0	3	3	0	2	0	2
263-M	0	0	±	±	0	0	2	2	0	3	0	2
264-M	0	0	±	0	0	0	2	3	0	3	0	2
265-M	0	0	0	1	0	0	2	2	0	3	0	2
266-M	0	0	0	±	1	0	3	2	0	3	0	2
267-M	±	±	0	0	0	0	3	3	0	3	0	1
268-M	0	0	0	0	±	±	1	1	0	3	0	2
269-M	±	±	0	0	0	±	3	3	0	1	0	2
270-M	±	±	0	±	0	0	3	3	0	2	0	1
271-F	±	±	0	0	0	0	3	3	0	1	0	1
272-F	±	±	0	±	±	0	3	3	0	3	0	2
273-F	0	0	0	0	0	±	3	3	0	3	0	3
274-F	±	±	0	0	0	±	3	3	0	3	0	1
275-F	±	±	0	0	0	0	2	3	0	1	0	1
276-F	1	±	0	0	0	0	3	3	0	3	0	2
277-F	0	0	0	0	0	0	2	3	0	1	0	1
278-F	0	±	0	0	0	0	3	3	0	3	0	2
279-F	1	±	0	0	0	0	2	3	0	1	0	3
280-F	0	±	0	0	0	0	3	2	0	2	0	2
Mean:	0.1	0.0	0.0	0.1	0.1	0.0	2.6		0.0	2.3	0.0	1.8
	0.1						Incidence of Reactions:		20/20		20/20	

% Sensitized: 100% Sensitization Potency Rating: Extreme Sensitizer

Injection Sites:
Sites 1 & 2 - Freund's Complete Adjuvant - 50% v/v in saline
Sites 3 & 4 - Test Substance 0.5% w/v in cottonseed oil
Sites 5 & 6 - Mixture (50:50) of Freund's Complete Adjuvant in saline and Test Substance (0.5% w/v)

Induction Topical and Challenge Treatments: 2% w/w concentration of Test Substance in petrolatum
LF - Left Flank (dosed w/vehicle); RF - Right Flank (dosed w/Test Substance in vehicle)

M - Male; F - Female

APPENDIX A (cont.)
GUINEA PIG MAXIMIZATION TEST FOR TOPICALLY APPLIED TEST SUBSTANCE
Positive Control Table 1 (cont.)
Skin Reaction Scores and Averages
Positive Control Substance: DNCB C-6396 Lot 87H0799
Study Number: 5782-00
Group II - Control

	Intradermal Induction						Topical Induction		Challenge			
Animal Number	Scores for each site on Day 6						Day 10 Scores		Day 23		Day 24	
	1	2	3	4	5	6	Site 3	Site 4	LF	RF	LF	RF
281-M	±	±	0	0	0	0	0	2	0	2	0	1
282-M	±	±	0	0	0	0	0	0	0	0	0	0
283-M	±	±	0	0	0	0	0	0	0	1	0	1
284-M	±	±	0	0	0	0	0	0	0	0	0	0
285-M	±	±	0	0	0	0	0	0	0	0	0	0
286-F	±	±	0	0	±	0	0	0	0	0	0	0
287-F	±	±	0	0	0	0	0	0	0	1	0	1
288-F	±	±	0	0	0	±	0	0	0	0	0	0
289-F	±	±	0	0	0	0	0	0	0	0	0	0
290-F	0	±	0	0	0	0	0	0	0	0	0	0
Mean:	0.0	0.0	0.0	0.0	0.0	0.0	0.1		0.0	0.4	0.0	0.3
	0.0						Incidence of Reactions:		3/10		3/10	

Injection Sites:
Sites 1 & 2 - Freund's Complete Adjuvant - 50% v/v in saline
Sites 3 & 4 – Cottonseed oil
Sites 5 & 6 - Mixture (50:50) of Freund's Complete Adjuvant in saline and Cottonseed oil

Induction Topical Treatments: 0.5 g of petrolatum

Challenge Treatments: 2% w/w concentration of Test Substance in petrolatum
LF - Left Flank (dosed w/vehicle); RF - Right Flank (dosed w/Test Substance in vehicle)

APPENDIX B

MILLER® CHEMICAL & FERTILIZER CORPORATION

P.O. BOX 333, 120 RADIO ROAD
HANOVER, PENNSYLVANIA 17331 U.S.A.
TELEPHONE: 717-632-8921
FAX NO.: 717-632-4561

1/18/01

CERTIFICATE OF ANALYSIS

Product Name: MILLER 6064
Quantity Produced: 7.4 kilograms
Batch Number: 150386
Date of Formulation: 12/13/00

Appearance: Yellow to Amber Liquid
Odor: Terpenic
Moisture: Negligible (non-aqueous)
Specific Gravity: 0.92 - 0.94 @ 20c
Viscosity @ 20c: 100 to 500 cps
pH(1% solution): 6.0 to 8.0
Solubility in Water: Insoluble (Emulsifies)
Flash Point: over 200 f
Purity: 100%

Signature:

Name: Andrew G. Smith
Title: Compliance Officer

APPENDIX C

STILLMEADOW
INCORPORATED

PROTOCOL FOR STUDY 6211-00

Study Title: GUINEA PIG MAXIMIZATION TEST FOR TOPICALLY APPLIED TEST SUBSTANCE (OPPTS 870.2600)

Test Substance: MILLER 6064

Test Facility: STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, Texas 77478

Approved: _____ 26 Dec 00
Janice O. Kuhn, Ph.D., D.A.B.T. Date
Study Director
STILLMEADOW, Inc.

Approved: _____ 6 Dec 00
Mark S. Holbert Date
Vice President
STILLMEADOW, Inc.

Reviewed: _____ 6 Dec. 2000
Vicki S. Crutchfield, RQAP Date
Director, Quality Assurance Unit
STILLMEADOW, Inc.

Sponsor: Miller Chemical & Fertilization Corp.
P.O. Box 333
Hanover, PA 17331

Sponsor Representative: Mandava Associates
1730 M Street, N.W., Suite 906
Washington, DC 20036

Approved: _____ December 26, 2000
N. Bhushan Mandava Date
Agent to Miller Chemical & Fertilization Corp.

APPENDIX C (cont.)

PROTOCOL FOR STUDY 6211-00

A. GENERAL

1. Study Title: GUINEA PIG MAXIMIZATION TEST FOR TOPICALLY APPLIED TEST SUBSTANCE

2. Purpose: To determine the skin sensitization potential of the test substance in guinea pigs. This protocol follows the recommendations of the "maximization" method of Magnusson B. and Kligman A.M. (*Journal of Investigative Dermatology*. 52: 268-276 (1969)) concerning the evaluation of the dermal sensitizing potential in guinea pigs.

3. Regulatory Compliance: This study meets or exceeds the requirements of OECD Guideline 406 and EPA OPPTS Health Effects Test Guidelines 870.2600.

This study will be conducted in compliance with Good Laboratory Practice Standards:

1. EPA FIFRA: 40 CFR 160
2. EPA TSCA: 40 CFR 792
3. OECD: C(97)186
4. Japanese MAFF

All procedures in this protocol are in compliance with Animal Welfare Act Regulations. All methods can be found in STILLMEADOW, Inc. Standard Operating Procedures (SOPs).

4. Quality Assurance: The protocol is reviewed by the Quality Assurance Unit (QAU). The study information will be entered into the Master Schedule. The study will be inspected at least once during its progress. Further inspections may be scheduled as needed to ensure the integrity of the study. Any deviations for SOPs, the Protocol, or Good Laboratory Practice Standards will be immediately reported to the Study Director and Management. The report will be audited and a statement prepared and signed which shall specify the dates inspections were made and findings reported to Management and to the Study Director.

5. Test Substance: MILLER 6064. Test substance identification should include the name, batch number and purity. Information regarding safety, stability, storage conditions and disposal should also be provided by the Sponsor. The Sponsor assumes responsibility for purity and stability determinations. Analysis and archiving of reserve samples will be the responsibility of the Sponsor.

6. Positive Control Substance: 2,4-Dinitrochlorobenzene (DNCB - CAS No. 97-00-7) or other suitable positive control substance such as α-hexylcinnamaldehyde (CAS No. 101-86-0); tested periodically in this laboratory to confirm sensitization potential of the animals used and validate procedures. Results of a separate positive control study will be referenced in the final report.

APPENDIX C (cont.)

A. GENERAL (cont.)

7. Proposed Schedule:	Testing will begin within approximately three weeks of receipt of test substance and authorization to conduct the study. Proposed Start Date: 20 Dec 00 Proposed End Date: 26 Jan 01 In-life portion of the study: 25 days; if equivocal results are obtained, a rechallenge will be conducted 7 days later.
8. Study Director:	Janice O. Kuhn, Ph.D., D.A.B.T.
9. Experimental Summary:	Test group guinea pigs will be given three pairs of intradermal injections (adjuvant, test substance, and a mixture of adjuvant and test substance) followed one week later by a single topical application of the test substance in the same exposure area. (The intradermal and topical concentrations of the test substance that produce no more than moderate irritation will be determined from a range-finding test and used for the induction treatments.) Two weeks after the topical application, the animals will be challenged by a second topical application of the test substance at a virgin site using the maximum non-irritating concentration as determined from the range-finding. Control group guinea pigs will be treated at the same times and exposure areas, but with no test substance. If equivocal results are obtained at the challenge, the animals will be rechallenged after seven days. The percentage of animals exhibiting erythema with or without edema after the challenge treatment will be used to assign the test substance a sensitization rating.
10. Protocol Amendments:	Any alteration in the Protocol will be justified, approved by the Study Director, and recorded in writing.
11. Sponsor Audits:	The Sponsor may send an authorized representative to inspect the test system and/or data on the STILLMEADOW, Inc. premises during normal working hours.

APPENDIX C (cont.)

B. EXPERIMENTAL DESIGN

1. Animals

a. Species:	Guinea Pig
b. Strain/Source:	Hartley Albino (Harlan Sprague Dawley, Inc., Houston, Texas or other suitable supplier)
c. Justification of Species:	The guinea pig is conventionally used in skin sensitization studies to provide information on which human hazard can be judged, and is the preferred species in the Guidelines.
d. Quantity and Sex:	Test Group: 20 (10/sex) (females nulliparous and non-pregnant) Control Group: 10 (both sexes will be represented) (females nulliparous and non-pregnant) Several additional animals will be used for a preliminary range-finding study. Additional animals may be required if a rechallenge is necessary.
e. Age/Weight:	Young adult; approximately 300 - 500 grams
f. Identification:	Ear punch
g. Acclimation and Health Status:	Animals will be acclimated for at least five days prior to testing. Range-finding may be conducted during the acclimation period. Normal weight gain, appearance, and behavior will be factors used to select healthy animals for testing.

2. Animal Husbandry

a. Cages:	Stainless steel, suspended, wire bottom
b. Number per Cage:	Animals will be housed 1 - 4 per cage (and individually during the topical application exposure periods).
c. Food:	PMI Feeds, Inc.™ Guinea Pig Diet #5025; available *ad libitum*. Analyzed by manufacturer.
d. Water:	Tap water; available *ad libitum*. Water bowl or automatic system. Municipal water supply analyzed by Texas Natural Resource Conservation Commission (TNRCC) Water Utilities Division.
e. Contaminants:	There are no known contaminants in the feed or water available to laboratory animals that would be expected to interfere with this study.
f. Environment:	Environmental controls for the animal room will be set to maintain a temperature of 20°C ± 3°C, a relative humidity range of 30 - 80%, a 12-hour light/dark cycle (regulated automatically), and room ventilation of approximately 10 - 12 air changes per hour.

B. EXPERIMENTAL DESIGN (cont.)

3. Test Substance Administration

a. Route of Administration: Intradermal injection and application of a topical patch will be employed.

b. Justification for Route of Administration: Dermal exposure is a potential route of human exposure; intradermal injection in the presence of an adjuvant is intended to maximally stimulate the immune response.

c. Positive Control Substance: 2,4-Dinitrochlorobenzene (DNCB - CAS No. 97-00-7) or other suitable positive control substance such as α-hexylcinnamaldehyde (CAS No. 101-86-0); tested periodically in this laboratory by this method. Test is done within six months of the definitive study to confirm sensitization potential of the animals used and validate procedures. The date the test was performed and the results will be reported in the final report.

d. Range-finding: Determination of the Maximum Irritating Concentration (MIC) by the Intradermal Route:

The day before treatment, the dorsal region of the animals will be clipped. The test substance will be diluted or suspended in an appropriate vehicle as necessary. Intradermal injection of the test substance at a volume of 0.1 mL at increasing concentrations (4 concentrations per animal) will be made in order to determine the maximum concentration which causes no more than moderate irritation without necrosis or ulceration. Typically, concentrations will range from 1 - 5% w/v or v/v. Cutaneous reactions will be evaluated 24 and 48 hours after the injections. Observations will be made using the scoring scale presented in Appendix A.

Determination of the Maximum Irritating Concentration (MIC) and the Maximum Non-Irritating Concentration (MNIC) by the External cutaneous route:

The day before treatment, the dorsal region of the animals will be clipped. Liquids will be tested undiluted, if possible, as well as diluted in an appropriate vehicle as necessary. Solids will be finely pulverized and mixed with petrolatum to a maximum concentration of 25% w/v. 500 mg or 0.5 mL of each concentration (2 concentrations per animal) will be applied under a 2 x 2 cm filter paper patch and held in place by an occlusive dressing for 24 hours. Cutaneous reactions will be evaluated 24 hours after removal of the patches to determine the topical MIC (maximum topical concentration that produces no more than moderate irritation without necrosis or ulceration) and topical MNIC (maximum non-irritating concentration).

Further screening tests may be necessary if results obtained do not adequately define the MIC and MNIC for the external cutaneous treatments for the induction on Day 7 and the challenge on Day 21, respectively.

B. EXPERIMENTAL DESIGN (cont.)

3. Test Substance Administration (cont.)

e. Preparation of Animals: The animals will be prepared on the day prior to each treatment by clipping the exposure area on the back (4 x 6 cm across the shoulders or 5 x 5 cm on each flank) with animal clippers. This procedure may be repeated as necessary.

f. Sensitization by Intradermal and Cutaneous Routes:

Induction: Intradermal Injections

On Day -1, each animal will be clipped to expose a 4 x 6 cm area running laterally across the shoulders. On Day 0, three pairs of symmetrical intradermal injections of 0.1 mL will be made in a 2 x 4 cm area within the larger exposed area. Injections 1 & 2 will be given close together and nearest the head. The third pair of injections will be given towards the caudal part of the test area.

1) Treated Group

- *Injection 1* - Freund's Complete Adjuvant (FCA) diluted at 50% in injectable isotonic saline (NaCl 0.9%) (hereafter referred to as FCA/saline)
- *Injection 2* - Test substance in the vehicle, diluted as indicated by the range-finding study to the maximum concentration to obtain no more than moderate irritation.
- *Injection 3* - Test substance at the selected concentration and FCA/saline, 50/50 (v/v). If the test substance is water soluble, it is first dissolved in the saline and then mixed thoroughly with the adjuvant. If the test substance is not water soluble, it is first mixed with FCA and then diluted with saline.

2) Control Group

- *Injection 1* - FCA/saline
- *Injection 2* - Vehicle alone
- *Injection 3* - A mixture of FCA/saline and vehicle, 50/50 (v/v)

Observations for reactions to the intradermal injections will be made on Day 6. Prior to observations, each animal will be reclipped, if necessary, to expose a 4 x 6 cm area running laterally across the shoulders.

If the Day 6 observations indicate that the test substance was not a skin irritant, the exposure area of each animal in both treated and control groups will be treated (Day 6) with 0.5 mL of a 10% w/w mixture of sodium lauryl sulfate in petrolatum in order to create a local irritation. The mixture will be gently massaged into the skin using a glass rod. The area will not be occluded. If the Day 6 observations indicate that the test substance was a skin irritant, there will be no further treatment on Day 6.

APPENDIX C (cont.)

B. EXPERIMENTAL DESIGN (cont.)

3. Test Substance Administration (cont.)

f. Sensitization by Intradermal and Cutaneous Routes (cont.):

Induction: Topical Application

On Day 7, one week after the intradermal induction treatment, the test substance will be applied topically to the same exposure area on the shoulders at the maximum topical concentration that produced no more than moderate irritation in the range-finding test (topical MIC).

Treated Group - On Day 7, a 4.5 cm circle of filter paper will be saturated with the liquid test substance (0.5 mL of the topical MIC as indicated by the range-finding study) and placed over the exposure area to cover the three initial pairs of injections. Alternatively, a solid test substance (topical MIC as indicated by the range-finding study) will be suspended in petrolatum, and 500 mg of the mixture will be spread thickly on the patch before it is applied to the exposure area. The patch will then be occluded with non-irritating adhesive tape and secured in place with an elastic adhesive bandage wound around the torso of the animal. The exposure will last for 48 hours.

Control Group - On Day 7, application of 0.5 mL of the vehicle alone on a filter paper patch, or a dry patch of filter paper if no vehicle is used, will be made as above. The exposure will last for 48 hours.

All animals - On Day 10, 24 hours after unwrapping, observations for dermal irritation will be made.

g. Challenge Treatment (both Treated and Control Groups): For the challenge treatment, 5 x 5 cm areas will be clipped on both the left and right flanks on Day 20. On Day 21, a 2 x 2 cm patch of filter paper, dry, or saturated with 0.5 mL of the vehicle (if applicable) will be applied to the left flank as a control. As determined from the range-finding test, the maximum non-irritating concentration of the test substance (topical MNIC) will be applied topically as for the topical induction, but application will be to the right flank. The patches will be sealed with non-irritating adhesive tape and secured in place with an elastic adhesive bandage wound around the torso of the animal. After a 24-hour exposure period, the wrappings and patches will be removed. Animals will be clipped 21 hours after patch removal.

4. Observations for Dermal Irritation: Observations for erythema with or without edema and other signs of dermal irritation will be made on Days 6, 10, 23, and 24. Observations will be made using the scoring scale presented in Appendix A.

5. Other Observations: Any unusual systemic reactions or any other unusual findings will be observed and recorded.

APPENDIX C (cont.)

B. EXPERIMENTAL DESIGN (cont.)

6. Body Weights: Body weights will be taken on Days -1 and 24. If rechallenge is conducted, body weights will be taken at study termination.

7. Study Design:: The following schedule will be followed during the study.

Day -1: A 4 x 6 cm exposure area will be clipped free of hair on each animal. The exposure area will run laterally across the shoulders. Body weights will be taken.

Day 0: Each animal will be treated with three pairs of symmetrical intradermal injections.

Day 6: Exposure areas clipped, if necessary. Observations for dermal irritation after intradermal injections. If no skin irritation observed, dermal administration of sodium lauryl sulfate.

Day 7: Each animal will be treated with the test substance or vehicle by topical application, and the exposure area will be occluded for 48 hours.

Day 9: All wrappings and patches will be removed.

Day 10: Observations for dermal irritation after topical application.

Day 20: A 5 x 5 cm exposure area will be clipped free of hair on the right and left flanks of each animal.

Day 21: Each animal will be treated with the test substance on the right flank and vehicle on the left flank, by topical application, and the exposure areas will be occluded for 24 hours.

Day 22: All wrappings and patches will be removed.

Day 23: The exposure areas will be observed for skin reactions 24 hours after removing the patches. If necessary, animals will be clipped at least two hours prior to observations.

Day 24: The exposure areas will be observed for skin reactions 24 hours after the Day 23 observations. Body weights will be recorded.

8. Rating of Sensitization: Any test group animal that exhibits scores greater than zero for erythema or edema and/or greater than control group animals' reactions after the challenge treatment will be considered sensitized.

% Sensitized	Grade	Rating
0	0	Non-Sensitizer
1-8	I	Weak
9-28	II	Mild
29-64	III	Moderate
65-80	IV	Strong
81-100	V	Extreme

B. EXPERIMENTAL DESIGN (cont.)

9. Rechallenge:	If it is necessary to clarify the results obtained in the first challenge, a second challenge (a rechallenge), where appropriate with a new control group, should be conducted approximately one week after the first one. A rechallenge may also be performed on the original control group. Body weights will be recorded at study termination.
10. Necropsy:	At the end of the study, the animals will be weighed and sacrificed by CO_2 inhalation in excess. For animals found dead during the study, a macroscopic examination of the main organs will be performed and abnormalities recorded.
11. Histopathology:	No histopathologic examination will be performed routinely. Cutaneous samples showing "doubtful" macroscopic reactions will be examined microscopically only after an agreement from the Sponsor and the Study Director. Increased vascularity, edema, and accumulations of plasma cells, mast cells and/or lymphocytes will be considered indicative of a sensitization reaction.
12. Test Substance Accountability:	A comprehensive inventory of test substance received and used will be kept. The test substance container(s) will be weighed when received at this facility, and a record of all test substance use will be maintained. Test substance and test substance dosing solutions will be stored in the original containers, or in the equivalent thereof, or in glass containers with Teflon-lined caps.
13. Disposal of Unused Test Substance:	Unused test substance will be returned after the termination of the study to the Sponsor or Sponsor's Representative. The Sponsor should specify return or disposal at the time of study authorization. If returned, the recipient of the test substance will be notified in advance of shipping. The sample will be accompanied by a chain of custody letter describing the sample(s) contained in the shipment.
14. Safety Precautions:	General safety precautions required by laboratory SOPs will be followed. The Sponsor will supply basic toxicity data on the test substance to be used. However, since the toxicity of test substances is often not well characterized, this laboratory will be conservative in setting safety procedures. The Sponsor or Sponsor's Representative shall be notified of any exposures requiring a physician's examination or care.

APPENDIX C (cont.)

C. DATA MANAGEMENT

1. Records:

The following records will be maintained during the study and transferred to the STILLMEADOW, Inc. archives upon study termination.

a. Protocol and Protocol Amendments (if any).
b. Final report and amendments (if any).
c. Study correspondence.
d. Animal receipt/acclimation data.
e. Test substance receipt, identification as provided by the Sponsor, preparation, administration, and disposition. Data on the vehicle used in dilutions, range-finding, or administration.
f. Test animal information: number, species, strain, age, source and sex.
g. Body weight data.
h. Range-finding study information.
i. Individual scores for dermal reactions and any other irritation.
j. Observations for unusual systemic reactions, if any.
k. Records from an appropriate positive control study conducted within six months of the definitive study.
l. Other pertinent data.

2. Data Storage:

All raw data will be retained at STILLMEADOW, Inc.

3. Data Reporting:

The final report will include all data as described in the Good Laboratory Practice Standards, including:

a. Statement from the Quality Assurance Unit.
b. Signature of the Study Director.
c. A GLP Compliance Statement signed by the Study Director.
d. Names of scientific personnel involved in the study.
e. Dates of study initiation and termination.
f. Identification, description, and storage of the test substance, and identification of the vehicle used in dilutions.
g. All pertinent animal data, animal husbandry, dosing information, and observation methods.
h. Description of the test procedures.
i. Determination of whether or not the test substance was a sensitizing agent.
j. Individual observations for dermal reactions and any other irritation.
k. Mean and individual skin irritation scores for each group for each time period.
l. Individual body weight data.
m. Results of pretest screening.
n. Observations of unusual systemic reactions or any other unusual findings.
o. Results from an appropriate positive control study conducted within six months of the definitive study.
p. A reference to this Protocol.

4. Report Submission:

A report will be submitted after termination of the in-life portion of the study.

APPENDIX C (cont.)

Appendix A
GUINEA PIG MAXIMIZATION TEST FOR TOPICALLY
APPLIED NON-IRRITATING TEST SUBSTANCE
Evaluation of Skin Reactions

MAGNUSSON AND KLIGMAN GRADING SCALE FOR THE EVALUATION OF CHALLENGE PATCH TEST REACTIONS*

Observation	Score
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

* - OECD Guidelines for the Testing of Chemicals, Volume 2, Section 4, Number 406, Skin Sensitization, Paragraph 23, page 4/9, Adopted 17 Jul 92

ATTACHMENT 40

Bluegill Sunfish (*Lepomis macrochirus*)
Static 96-Hour Acute Toxicity Test
on Miller 6064

STILLMEADOW
INCORPORATED

VOLUME __ OF __ OF SUBMISSION

Miller 6064

AMENDED FINAL REPORT

BLUEGILL SUNFISH (*Lepomis macrochirus*) STATIC 96-HOUR ACUTE TOXICITY TEST

OPPTS No. 850.1075

AUTHOR:

Neil A. Rodrigue, M.S.

STUDY INITIATION DATE: 22 June 2001
STUDY COMPLETION DATE: 17 Oct 2001
AMENDED STUDY DATE: 9 May 02

CONDUCTED BY:
STILLMEADOW, Inc.
10161 Harwin Drive, Suite 150
Houston, Texas 77036

LABORATORY STUDY NUMBER:

6419-01

VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 24

SUBMITTED TO:
Miller Chemical & Fertilizer Corporation
P.O. Box 333
Radio Road
Hanover, PA 17331

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA § 10(d) (1) (A), (B), or (C).

Company: Miller Chemical and Fertilizer Corporation

Company Agent:_____ Date: _____

Title_____ Signature _____

These data are the property of Miller Chemical and Fertilizer Corporation and as such, are considered to be confidential for all purposes other than compliance with FIFRA § 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality that may exist under any other statute or in any other country.

GLP COMPLIANCE STATEMENT

This study was designed and performed in STILLMEADOW, Inc.'s laboratory in compliance with the following:

· United States Environmental Protection Agency (USEPA) FIFRA; Good Laboratory Practice Standards 40 CFR 160 with exception of sections 160.105 (b)(e) and 160.31 (d), stability information was not provided; 160.105 (b) solubility not determined; and 40 CFR 160.113 (a) mixture analysis not performed.

· Organization for Economic Cooperation and Development's Principles of Good Laboratory Practice, Annex 2, C(97)186 with exception of section 6.2 (4), stability information was not provided, and section 6.2 (5), mixture analysis was not conducted.

· Japan Ministry of Agriculture, Forestry and Fisheries, Notification No. 59 Nohsan 3850, Director-General of Agricultural Production Bureau, 10 August 1984 with the exception of Article 5 (2) (9) and Article 21 (3), stability information was not provided, and Article 23 (1), mixture analysis was not conducted.

Neil A. Rodrigue, M.S.
Study Director
STILLMEADOW, Inc.

09 May 02
Date
Original Date: 17 Oct 01

Signature of Agent of Sponsor

Date

Agent Name
Sponsor: Miller Chemical & Fertilizer Corporation
P.O. Box 333
Radio Road
Hanover, PA 17331

Signature of Agent of Submitter

Date

Agent Name
Submitter: Mandava Associates

TABLE OF CONTENTS

QUALITY ASSURANCE STATEMENT

Study Title: Bluegill Sunfish (*Lepomis macrochirus*) Static 96-Hour Acute Toxicity Test
Test Substance: Miller 6064

The study has been inspected and the report and data have been audited in accordance with STILLMEADOW, Inc. Standard Operating Procedures (SOPs). The findings from the inspection and audit were reported to the Study Director and Management as follows:

Phase of Study Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
Observation	10 Aug 01	10 Aug 01	10 Aug 01
Report/Data Audit	10 Oct 01	11 Oct 01	11 Oct 01
Amended Report Audit	06 Mar 02	07 Mar 02	07 Mar 02

B. Lynn Murphy

B. Lynn Murphy, B.S.
Quality Assurance Unit
STILLMEADOW, Inc.

09 May 2002
Date

SUMMARY

This study was conducted to assess the toxicity of the test substance (Miller 6064) to *Lepomis macrochirus* in a 96-hour static, non-renewal test.

Test considerations were determined by preliminary range-finding tests. The test substance concentrations chosen (25, 43, 71, 118 and 197 mg/L) were administered to the test system, *Lepomis macrochirus*, in reconstituted water. For each test concentration, two replicates of ten organisms each were treated with the appropriate concentration of the test substance. A control group containing twenty organisms was not exposed to test substance. Dissolved oxygen, temperature, conductivity, and pH measurements were recorded at dosing and daily throughout the study. Observations of mortality were made at 24, 48, 72, and 96 hours after treatment. The test was terminated after 96 ± 2 hours of exposure.

Survival rates of 100, 100, 100, 30 and 0% were observed in fish treated with 25, 43, 71, 118, and 197 mg/L of the test substance (target concentrations), respectively. A 100% survival rate was observed in both control and solvent control groups. Based on this data, the median lethal concentration (LC_{50}) was determined to be 106.67 mg/L with a 95% confidence interval of 96.08 to 118.43 mg/L, and the NOEC was determined to be 71 mg/L.

INTRODUCTION

The objective of this study was to assess the toxicity of the test substance to *Lepomis macrochirus* in a 96-hour test. This study was conducted for Miller Chemical & Fertilizer Corporation according to the approved protocol, STILLMEADOW, Inc. SOPs, and Product Properties Test Guidelines, Series 850, Section 1075 of the United States Environmental Protection Agency Office of Prevention, Pesticides, and Toxic Substances. This study was initiated on 22 June 2001. The laboratory portion of the study was conducted between 19 Jul 01 to 23 Jul 01 and 25 Jul 01 to 28 Jul 01 for the range-finding tests, and 09 Aug 01 and 13 Aug 01 for the definitive test. The original protocol, raw data, and report are on file in the STILLMEADOW, Inc. archives.

TEST SUBSTANCE

Identification:	MILLER 6064
Date and Quantity Received:	19 Dec 00; 2 x 1 gal
Physical Description:	Amber liquid
Storage:	Room temperature
Purity and Composition:	Refer to Certificate of Analysis (Appendix D)
Stability:	Not provided by the Sponsor

Records pertaining to stability, characterization, and verification of test substance identity are the responsibility of the Sponsor. A copy of the Certificate of Analysis is included in the report.

TEST SYSTEM

Experimental Organism	
Species:	*Lepomis macrochirus*
Source:	Osage Cat Fisheries (Lake Ozark, Missouri) 03 Jul 01
Age:	Juvenile
Size:	Less than 3.0 g at dosing; the longest fish was not more than twice the length of the shortest.
Quantity:	Range-finding: 2 per test concentration Definitive: 20 per test concentration
Organism Husbandry	
Test Room:	Environmentally controlled chamber (Chamber C)
Test Chambers:	1 liter glass beaker (range-finding) and 2.5 gallon glass aquaria (definitive)
Test Medium:	Reconstituted water with total hardness between 40 and 180 mg $CaCO_3$ and with a pH between 6.0 and 8.0.
Loading:	Maximum loading of 0.8 g fish/liter.
Holding:	All fish were held in the laboratory at least 14 days before they were used for testing. The fish were held in water of the quality used in the test for at least seven days immediately before testing.
Environmental Controls	
Set to Maintain:	Temperature Range of 22±2°C 16-hours light / 8-hours dark cycle Dissolved oxygen concentration of at least 60 percent saturation
Food:	Fish were fed daily until 48 hours before the test was started. Fish were not fed during the test.

Animal husbandry and housing at STILLMEADOW, Inc. comply with Animal Welfare Act Regulations. No contaminants were expected to have been present in the feed or water which would have interfered with or affected the results of the study.

PROCEDURES

Range-finding Test

Two preliminary range-finding tests were conducted using five concentrations of the test substance (1, 5, 10, 50, and 100 mg/L). Since the test substance was insoluble in water, the test substance was administered using N,N-Dimethylformamide as a solvent at a rate of 1 mL N,N-Dimethylformamide per liter of solution volume. Following randomization, two organisms per each range finding were placed into each beaker containing the appropriate concentration of test substance. Two organisms per each range finding, which were not exposed to test substance, served as controls to demonstrate the condition of the test population. Additionally, two organisms per each range finding which were not exposed to test substance were exposed to the solvent (N,N-Dimethylformamide). This solvent control was used to demonstrate artifactual toxicity produced by the solvent. At 24, 48, 72, and 96 hours following dosing, each beaker was examined for mortality and the number of live fish was recorded.

PROCEDURES (cont.)

Definitive Test

Based on the results of the range-finding tests, test substance concentrations were chosen for definitive testing. Five target concentrations of the test substance were used (25, 43, 71, 118, and 197 mg/L). Since the test substance was insoluble in water, the test substance was administered using N,N-Dimethylformamide as a solvent in the same method used for the range-finder. Each test concentration consisted of two replicates of ten fish per replicate. Two replicates containing ten fish each were not exposed to test substance and served as controls to demonstrate the condition of the test population. Additionally, two replicates containing ten fish each which were not exposed to test substance were exposed to the solvent (N,N-Dimethylformamide). The solvent control was used to demonstrate artifactual toxicity produced by the solvent. At 24, 48, 72, and 96 hours following dosing, each of the aquaria was examined for mortality and the number of live fish was recorded. Fish were considered dead when there was no visible movement (e.g. gill movements) and if touching of the caudal peduncle produced no reaction. Dead fish were removed when observed. Visible abnormalities were also recorded (e.g. loss of reflex, erratic swimming, increased excitability, lethargy, and changes in appearance or physiology such as discoloration, excess mucous production, hyperventilation, opaque eyes, curved spine, or hemorrhaging, etc.).

Chemical and Physical Monitoring

The following measurements were recorded daily during definitive testing: dissolved oxygen, temperature, conductivity, and pH of control and treated containers.

RESULTS AND DISCUSSION

Test Validity

The test was considered valid if control mortality did not exceed 10 percent. Since control mortality was zero percent, the definitive test was considered valid.

Range-finding

A 0% survival rate was observed in fish treated at concentration of 100 mg/L. A 50% survival rate was observed in fish treated at concentrations of 0 and 5mg/L. A 75% survival rate was observed in fish treated with solvent only and with fish treated at concentrations of 1 and 10 mg/L. A 100% survival rate was observed in fish treated at concentrations of 50 mg/L. Mortality was observed in fish treated with 0, 1, 5, 10 and 100 mg/L of test substance.

Target Concentration (mg/L)	Range Finding Test	Number of Surviving Organisms				
		0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	A	2	2	1[a]	1	1
	B	2	1[b]	1	1	1
1	A	2	2	2	2	2
	B	2	2	2	2[c]	1[b]
5	A	2	2	2[d]	1[b]	1
	B	2	2	1[b]	1	1
10	A	2	2	2	2	2
	B	2	2	1[b]	1	1
50	A	2	2	2	2	2
	B	2	2	2	2	2
100	A	2	0[e]	0	0	0
	B	2	0	0[f]	0	0
Solvent Control	A	2	2	1[b]	1	1
	B	2	2	2	2	2

A – Range-finding test of 19 Jul 01 to 23 Jul 01

B – Range-finding test of 25 Jul 01 to 28 Jul 01

[a] - One fish floating on top, dead no movement

[b] – One fish dead lying on side at the bottom of the tank

[c] – One fish lying on side at bottom of tank still breathing, trying to swim

[d] – One fish dark in color staying at bottom of tank, has gill movement but appears weak

[e] – Fish white in color with curved spines

[f] - Both fish dead lying on side with curved spine

RESULTS AND DISCUSSION (cont.)

Definitive

Survival rates of 0 and 30% were observed in fish treated with 197and 118 mg/L of the test substance (target concentrations), respectively. A 100% survival rate was observed in fish treated with solvent only, and 0, 25, 43 and 71 mg/L of the test substance. Chemical and physical monitoring data (dissolved oxygen, temperature, conductivity, and pH) of control and treated containers for the definitive test are presented in Appendix A.

Target Concentration (mg/L)	Replicate	Number of Surviving Organisms				
		0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	A	10	10	10	10	10
	B	10	10	10	10	10
25	A	10	10	10	10	10
	B	10	10	10	10	10
43	A	10	†	†	†	10
	B	10	†	†	†	10
71	A	10	10[a]	10[a]	10	10
	B	10	10[a]	10[a]	10	10
118	A	10	6†[b]	6[c]	5[d]	5
	B	10	2†[e]	1[f]	1	1
197	A	10	†[g]	0[h]	0	0
	B	10	†[e]	0[h]	0	0
C2	A	10	10	10	10	10
	B	10	10	10	10	10

† – Too turbid to count
[a] – Fish swimming irregularly on side and vertically; hemorrhage around gills
[b] – Four dead animals on bottom; some fish swimming on side
[c] – Fish floating on top with gill movement
[d] – One fish dead floating on top of water
[e] – Eight animals dead; pale in color
[f] – One fish dead at bottom
[g] – Ten dead animals; pale in color; curved dorsal fin
[h] – All fish dead lying on side at the bottom of tank

RESULTS AND DISCUSSION (cont.)

Evaluation of Results

The median lethal concentration (LC_{50}) for the test substance, Miller 6064, was determined to be 106.67 mg/L with a 95% confidence interval of 96.08 to 118.43 mg/L using the Trimmed Spearman-Karber statistical method, and the NOEC was determined to be 71 mg/L.

CONCLUSION

The test substance, Miller 6064, was evaluated for toxicity to *Lepomis macrochirus* in a 96-hour static, non-renewal test. The median lethal concentration (LC_{50}) was determined to be 106.67 mg/L with a 95% confidence interval of 96.08 to 118.43 mg/L, and the NOEC was determined to be 71 mg/L

Neil A. Rodrigue

Neil A. Rodrigue, M.S.
Study Director
STILLMEADOW, Inc.

09 May 02

Date
Original Date: 17 Oct 01

STUDY PERSONNEL

Technical Staff
Mel Rivera, B.S.
Rob Stowe. B.S.
Abigail Campbell, B.S.
Brandy Goffinet

Technical Writer
Diana W. Cook, B.S.

Appendix A: Chemical and Physical Monitoring Data
Bluegill Sunfish (*Lepomis macrochirus*) Static 96-Hour Acute Toxicity Test
Test Substance: Miller 6064

Table 1. Temperature (°C)

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	21	21	21	21	21
25	21	21	21	21	21
43	21	21	21	21	21
71	21	21	21	21	21
118	21	21	21	21	21
197	21	21	21	-	-
Solvent Cntrl	21	21	21	21	21

Table 2. pH

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	7.9	7.2	7.4	7.4	7.5
25	7.9	7.2	7.4	7.5	7.5
43	7.9	7.2	7.4	7.5	7.5
71	7.9	7.2	7.5	7.5	7.6
118	8.0	7.2	7.5	7.5	7.5
197	7.9	7.2	7.5	-	-
Solvent Cntrl	7.9	7.2	7.4	7.4	7.5

Table 3. Dissolved Oxygen (mg/L)

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	7.9	5.8	6.0	6.0	4.6
25	8.0	6.2	6.2	6.0	4.7
43	8.0	6.0	6.2	5.6	5.0
71	7.9	6.2	6.2	5.6	5.0
118	7.8	6.2	6.4	5.6	4.8
197	7.8	6.8	6.4	-	-
Solvent Cntrl	7.8	6.0	6.2	5.8	5.2

Appendix A: Chemical and Physical Monitoring Data (cont.)
Bluegill Sunfish (*Lepomis macrochirus*) Static 96-Hour Acute Toxicity Test
Test Substance: Miller 6064

Table 4. Conductivity (µmhos/cm)

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	315	290	290	290	290
25	320	290	290	300	290
43	320	295	290	300	290
71	320	290	290	300	295
118	320	295	295	300	300
197	320	295	295	-	-
Solvent Cntrl	315	285	285	300	295

Appendix B: Statistics
Bluegill Sunfish (*Lepomis macrochirus*) Static 96-Hour Acute Toxicity Test
Test Substance: Miller 6064

Concentration (mg/L)	Number Exposed	Mortalities
0.00	20	0
25	20	0
43	20	0
71	20	0
118	20	14
197	20	20

LC_{50}:	106.67
95% Lower Confidence:	96.08
95% Upper Confidence	118.43

Appendix C: Protocol and Amendment

STILLMEADOW
INCORPORATED

PROTOCOL AMENDMENT #1
STILLMEADOW, Inc. Study Number 6419-01

Sponsor: Miller Chemical & Fertilizer Corporation
PO Box 333
Radio Road
Hanover PA 17331

Effective Date: 17 Oct 2001

Test Substance: Miller 6064

Study Title: BLUEGILL SUNFISH (*Lepomis macrochirus*) STATIC 96-HOUR ACUTE TOXICITY TEST

The following alteration is being made to the cover and Section A.7 of the protocol.

To Change:	Abigail Campbell, B.S.
To Read:	Neil Rodrigue, M.S.
Justification:	The study director is being changed because Abigail Campbell is no longer with the company.
Impact:	There will be no impact on the study.

This amendment has been reviewed and/or approved by the following:

Approved: Neil A. Rodrigue — 17OCT01 — Date
Neil Rodrigue, M.S.
Study Director
STILLMEADOW, Inc.

Approved: [signature] — 17Oct01 — Date
Mark S. Holbert
Vice President
STILLMEADOW, Inc.

Reviewed:: [signature] — 17Oct01 — Date
Vicki S. Crutchfield, R.Q.A.P.
Director, Quality Assurance Unit
STILLMEADOW, Inc.

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281 240-8828 • Fax 281 240-8448
www.stillmeadow.com
10161 Harwin, Suite 150 • Houston, Texas 7703
713 272-7444 • Fax 713 272-7501

Appendix C: Protocol and Amendment (cont.)

STILLMEADOW
INCORPORATED

PROTOCOL FOR STUDY 6419-01

Study Title: BLUEGILL SUNFISH (*Lepomis macrochirus*) STATIC 96-HOUR ACUTE TOXICITY TEST

Test Substance: Miller 6064

Test Facility: STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, Texas 77479

Approved: _____ 23 June 01
Abigail Campbell, B.S.
Study Director
STILLMEADOW, Inc.
Date

Approved: _____ 5 June 2001
Elizabeth D. Sabol, B.A., B.S.Ed
Vice President
STILLMEADOW, Inc.
Date

Reviewed: _____ 5 June 2001
Vicki Crutchfield, R.Q.A.P.
Director, Quality Assurance Unit
STILLMEADOW, Inc.
Date

Sponsor: Miller Chemical & Fertilizer Corporation
PO Box 333
Radio Road
Hanover PA 17331

Sponsor Representative
Mandava Associates
1730 M Street, Suite 906
Washington, D.C. 20036-4510

Approved: _____ 15 JUNE 2001
N. Bhushan Mandava, Ph.D.
Date

Appendix C: Protocol and Amendment (cont.)

PROTOCOL FOR STUDY 6419-01

A. GENERAL

1. Study Title:	BLUEGILL SUNFISH (*Lepomis macrochirus*) STATIC 96-HOUR ACUTE TOXICITY TEST
2. Purpose:	To assess the toxicity of the test substance to bluegill sunfish (*Lepomis macrochirus*) in a static 96-hour test.
3. Regulatory Compliance:	This study will be conducted according to OPPTS 850.1075, which is intended to meet testing requirements of both FIFRA (7 U.S.C. 136. *et seq.*) and TSCA (15 U.S.C. 2601). This study will be conducted in compliance with Good Laboratory Practice Standards: 1. EPA FIFRA: 40 CFR 160 2. OECD: C(81)30 (Final) 3. Japanese MAFF All methods can be found in STILLMEADOW, Inc. Standard Operating Procedures (SOPs).
4. Quality Assurance:	The Protocol is reviewed by the Quality Assurance Unit (QAU). The study information will be entered into the Master Schedule. The study will be inspected at least once during its progress. Further inspections may be scheduled as needed to ensure the integrity of the study. Any deviations from SOPs, the Protocol, or Good Laboratory Practice Standards will be immediately reported to the Study Director and Management. The report will be audited and a statement prepared and signed which shall specify the dates inspections were made and findings reported to Management and to the Study Director.
5. Test Substance:	Miller 6064. Test substance identification should include the name, batch number, and purity. Information regarding safety, stability, storage conditions, and disposal should also be provided by the Sponsor. The Sponsor assumes responsibility for purity and stability determinations.
6. Proposed Schedule:	Testing will begin within approximately three weeks of receipt of the test substance and authorization to conduct the study. Proposed Start Date: 13 Jun 01 Proposed End Date: 11 Jul 01
7. Study Director:	Abigail Campbell, B.S.

Appendix C: Protocol and Amendment (cont.)

A. GENERAL (cont.)

8. Experimental Summary:

Definitive test concentrations will be determined by a preliminary range finder. The test substance concentrations chosen will be administered to the test system, bluegill sunfish (*Lepomis macrochirus)*, in reconstituted water. For each test concentration, 20 organisms will be treated with the appropriate concentration of the test substance. Two control groups which will not contain test substance will be used in this test. One group will have solvent added at the highest volume used for any test concentration preparation and will represent the solvent control. The other control group will remain untreated and will demonstrate the condition of the test population. Dissolved oxygen, temperature, conductivity, and pH will be measured and recorded in each treatment and the control at test initiation and daily throughout the study. Observations of mortality in each test chamber will be made at 24, 48, 72, and 96 hours. The test will be terminated after 96 ± 2 hours of exposure.

The test will be considered valid if control mortality does not exceed 10 percent.

9. Protocol Amendments:

Any alteration in the Protocol will be justified, approved by the Study Director, and recorded in writing.

10. Sponsor Audits:

The Sponsor may send an authorized representative to inspect the test system and/or data on the STILLMEADOW, Inc. premises during normal working hours.

Appendix C: Protocol and Amendment (cont.)

B. EXPERIMENTAL DESIGN

1. Organism

a. Species:	Bluegill sunfish (*Lepomis macrochirus*)
b. Justification of Species:	Specified in the OPPTS regulations.
c. Age/Size:	Juvenile fish, less than 3.0 g at test initiation. The longest fish will not be more than twice the length of the shortest.
d. Number:	The rangefinder will use 2 fish for each concentration and the controls. The definitive test will use 20 sunfish for each concentration and each control group (2 replicates each containing 10 fish).
e. Source:	*Lepomis macrochirus* will be obtained from Aquatic Research Organisms, Inc. (Hampton, New Hampshire) or another suitable supplier.
f. Identification:	Organisms will be labeled by study number, lot number, date of receipt, and number of organisms.

2. Animal Husbandry

a. Test Medium:	Reconstituted water with total hardness between 40 and 180 mg $CaCO_3$ and with a pH between 6.0 and 8.0.
b. Acclimation:	All fish will be held in the laboratory for at least 14 days before they are used for testing. They will be held in water of the quality to be used in the test for at least seven days immediately before testing. Pretest mortality must be less than 5% during acclimation or the organisms will be held for an additional seven days. If pretest mortality is greater than 10%, then the entire lot will be rejected and a new lot of fish will be obtained to begin acclimation.
b. Test Chamber:	Test containers will be 2½ gallon aquaria. Test containers will be loosely covered to reduce the loss of test solution or dilution water due to evaporation and to minimize the entry of dust or other particles into the solutions.
c. Temperature:	Test temperature will be 22±2°C.
d. Photoperiod:	16 hours light, 8 hours dark
e. Dissolved Oxygen Concentrations:	At least 60 percent air saturation value.
f. Food:	Fish will be fed daily until 48 hours prior to test initiation. Fish will not be fed during test.
g. Loading:	Maximum loading of 0.8 g fish/liter.

Appendix C: Protocol and Amendment (cont.)

B. EXPERIMENTAL DESIGN (cont.)

3. Pre-test Preparation

a. Test Substance Receipt:	Test substance will be supplied by the Sponsor in appropriately sized glass containers sealed and delivered to STILLMEADOW, Inc. Samples will be stored according to the Sponsor's instructions until prepared for testing.
b. Test Substance Preparation:	The test substance is insoluble in water and will be administered using an appropriate solvent (DMF, ethanol, methanol, etc.) as weight/volume concentrations. A solvent control will be included in the test design. The test substance dilutions will be prepared on the day of treatment.
c. Route of Administration:	The test substance will be administered to the test system at test initiation by introduction to the test containers containing the test system.
d. Reason for Route of Administration:	Specified by the cited guidelines for evaluation of the toxicity potential of a test substance.
e. Preparation of Test System:	The organisms will be randomized into aquaria containing the appropriate concentration of test substance. Each test concentration will consist of 20 fish. Each aquaria will house of a maximum of 10 fish.
f. Control Groups:	Twenty fish will not have test substance added and will be considered the control. This control will be used to demonstrate the condition of the test population. An additional 20 fish will not have test substance added but will contain the solvent at the highest volume used for any test concentration preparation. The solvent control will be used to demonstrate artifactual toxicity produced by the solvent.

4. Test Substance Administration

a. Dosing Concentrations:	A range finder will be conducted with at least five concentrations of the test substance to obtain an approximate LC_{50} value for the test substance. The test concentrations will be at least 50% greater than the lowest test concentrations (not to exceed 120%). Five test concentrations chosen from the range-finding data and the controls will be prepared on the day of test initiation.
b. Initial Measurements:	Dissolved oxygen, temperature, conductivity, and pH of the control and treated containers will be measured and recorded at test initiation.

Appendix C: Protocol and Amendment (cont.)

B. EXPERIMENTAL DESIGN (cont.)

5. Observations

a. Biological Monitoring: Containers will be inspected at 24, 48, 72, and 96 hours for mortality. Fish are considered dead if there is no visible movement (e.g. gill movements) and if touching of the caudal peduncle produces no reaction. Dead fish will be removed when observed, and mortalities will be recorded. Visible abnormalities will be recorded (e.g. loss of reflex, erratic swimming, increased excitability, lethargy, and changes in appearance or physiology such as discoloration, excess mucous production, hyperventilation, opaque eyes, curved spine, or hemorrhaging, etc.)

b. Chemical and Physical Monitoring: At a minimum, the following measurements will be made daily: dissolved oxygen, temperature, conductivity, and pH of the controls and treated containers.

6. Test Duration: The test will be terminated after 96 ± 2 hours.

7. Quality Criterion: The test will be considered valid if the control mortality does not exceed 10 percent.

8. Evaluation of Results: The survival in the test concentrations will be statistically compared to survival in the control to determine the highest concentration of test substance that demonstrates no significant reduction in survival. This concentration will be the No Observed Effect Level (NOEL) for survival. The NOEL will be determined by using a commercially available statistical program (Toxstat®).

The median lethal concentration (LC_{50}) will be estimated using a linear regression model. Several models are available for LC_{50} determination: Probit, Trimmed Spearman-Karber, and Binomial. The most appropriate model will be selected for estimating the LC_{50} if a dose response is exhibited in the study.

9. Test Substance Accountability: A comprehensive inventory of test substance received and used will be kept. The test substance container(s) will be weighed when received at this facility, and a record of all test substance use will be maintained. Test substance and test substance dosing solutions will be stored in the original containers or in the equivalent thereof, or in glass containers with Teflon-lined caps.

Appendix C: Protocol and Amendment (cont.)

B. EXPERIMENTAL DESIGN (cont.)

10. Disposal of Unused Test Substance:

Unused test substance will be disposed of at the Sponsor's expense after the termination of the study. STILLMEADOW, Inc. will retain a reserve sample.

11. Safety Precautions:

General safety precautions required by laboratory SOPs will be followed. The Sponsor will supply basic toxicity data on the test substance to be used. However, since the toxicity of test substances is often not well characterized, STILLMEADOW, Inc. will be conservative in setting safety procedures. The Sponsor or Sponsor's Representative shall be notified of any exposures requiring a physician's examination or care.

C. DATA MANAGEMENT

1. Records:

The following records will be maintained during the study and transferred to the STILLMEADOW, Inc. archives upon study termination.

a. Protocol and Protocol Amendments (if any).
b. Final report and amendments (if any).
c. Study correspondence.
d. Test culture data.
e. Test substance receipt, identification as supplied by Sponsor, preparation, administration, and disposition.
f. Range finder data and results.
g. Initial and daily measurements for dissolved oxygen, temperature, and pH of the control and treated containers.
h. Cumulative mortality at each concentration at each observation time.
i. Determination of the validity of the study.
j. Other pertinent data.

2. Data Storage:

All raw data and a reserve sample of the test substance will be retained at STILLMEADOW, Inc. for a period of at least five years.

Appendix C: Protocol and Amendment (cont.)

C. DATA MANAGEMENT (cont.)

3. Data Reporting:

The final report will include all data as described in the Good Laboratory Practice Standards, including:

a. Statement from the Quality Assurance Unit.
b. Signature of the Study Director.
c. A GLP Compliance Statement signed by the Study Director.
d. Names of scientific personnel involved in the study.
e. Dates of study initiation and termination.
f. Identification, description, preparation, and storage of the test substance.
g. All pertinent culture information, preparation of test medium, test conditions, dosing information, and observation methods.
h. Initial and daily data for dissolved oxygen, temperature, and pH of the control and treated containers.
i. Cumulative mortality at each concentration at each observation time.
j. Graph of the concentration-mortality curve at the end of the test.
k. Statistical procedures used for determining the LC_{50} and NOEL values.
l. Determination of the validity of the test based on the control data.
m. Abnormalities observed in test and control animals.
n. Any protocol deviations or occurrences which may have influenced the final results of the test.
o. Evaluation of results.
p. A reference to this Protocol.

4. Report Submission:

A report will be submitted after termination of the laboratory portion of the study.

Appendix D: Certificate of Analysis

MILLER CHEMICAL & FERTILIZER CORPORATION
P O. BOX 333, 120 RADIO ROAD
HANOVER, PENNSYLVANIA 17331 U.S.A.
TELEPHONE: 717-632-8921
FAX NO.: 717-632-1561

1/18/01

CERTIFICATE OF ANALYSIS

Product Name: MILLER 6064
Quantity Produced: 7.4 kilograms
Batch Number: 150386
Date of Formulation: 12/13/00

Appearance: Yellow to Amber Liquid
Odor: Terpenic
Moisture: Negligible (non-aqueous)
Specific Gravity: 0.92 - 0.94 @ 20c
Viscosity @ 20c: 100 to 500 cps
pH(1% solution): 6.0 to 8.0
Solubility in Water: Insoluble (Emulsifies)
Flash Point: over 200 f
Purity: 100%

Signature:

Name: Andrew G. Smith
Title: Compliance Officer

Appendix E: Amendment

Miller 6064

Bluegill Sunfish (*Lepomis macrochirus*) Static 96-Hour Acute Toxicity Test
(OPPTS 850. 1075)

Study Number 6419-01

Sponsor: **Miller Chemical & Fertilizer Corporation**

Final Report Amendment

This amendment makes the following changes in the final report:

To change: Page 6, Test Substance; Purity and Composition: Certificate of Analysis not provided by sponsor
To: Purity and Composition: Refer to Certificate of Analysis (Appendix D)

Reason: The Certificate of Analysis with composition information was not included in the original report.

To Add: Pages 6 and 11, "and the NOEC was determined to be 71 mg/L."

Reason: The NOEC was not included in the original report.

To Change: Page 6, Summary
From: Survival rates of 100, 100, 100, 100, 30 and 0% were observed in fish treated with 25, 43, 71, 118 and 197 mg/L of the test substance (target concentrations), respectively.

To: Survival rates of 100, 100, 100, 30 and 0% were observed in fish treated with 25, 43, 71, 118 and 197 mg/L of the test substance (target concentrations), respectively.

Reason: Typographical error.

To add: Appendix C, D and E to the Table of Contents and the report.

Reason: The protocol and Certificate of Analysis were not included in the original report.

Amendment Approval:

Neil A. Rodrigue

Neil A. Rodrigue, M.S.
Study Director
STILLMEADOW, Inc.

09 May 02
Date

ATTACHMENT 41

Rainbow Trout (*Oncorhynchus mykiss*)
Static 96-Hour Acute Toxicity Study
on Miller 6064

STILLMEADOW
INCORPORATED

VOLUME __ OF __ OF SUBMISSION

Miller 6064

FINAL REPORT

RAINBOW TROUT (*Oncorhynchus mykiss*) STATIC 96-HOUR ACUTE TOXICITY TEST

AUTHOR:

Neil A. Rodrigue, M.S.

STUDY INITIATION DATE: 22 Jun 2001
STUDY COMPLETION DATE: 9 May 2002

CONDUCTED BY:
STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, Texas 77048

LABORATORY STUDY NUMBER:

6420-01

VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 26

SUBMITTED TO:
Miller Chemical and Fertilizer Corporation
P. O. Box 333
Radio Road
Hanover, PA 17331

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA § 10(d) (1) (A), (B), or (C).

Company: Miller Chemical and Fertilizer Corporation

Company Agent_____ Date_____

Title_____ Signature_____

These data are the property of Miller Chemical and Fertilizer Corporation and as such, are considered to be confidential for all purposes other than compliance with FIFRA § 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality that may exist under any other statute or in any other country.

GLP COMPLIANCE STATEMENT

This study was designed and performed in STILLMEADOW, Inc.'s laboratory in compliance with the following:

· United States Environmental Protection Agency (USEPA) FIFRA; Good Laboratory Practice Standards 40 CFR 160 with exception of sections 160.105 (b) (e) and 160.31 (d), stability information was not provided; 160.105 (b) solubility not determined; and 160.113 (a) mixture was not performed.

· Organization for Economic Cooperation and Development's Principles of Good Laboratory Practice, Annex 2, C(97)186 with the exception of section 6.2 (4), stability information was not provided and section 6.2 (5), mixture analysis was not performed.

· Japan Ministry of Agriculture, Forestry and Fisheries, Notification No. 59 Nohsan 3850, Director-General of Agricultural Production Bureau, 10 August 1984 with the exception of Article 5 (2) (9) and Article 21 (3), stability information was not provided and Article 23 (1), mixture analysis was not performed.

Neil A. Rodrigue

Neil A. Rodrigue, M.S.
Study Director
STILLMEADOW, Inc.

09 May 02
Date
Original Date: 17 Oct 01

Signature of Agent of Sponsor

Date

Agent Name
Sponsor: Miller Chemical & Fertilizer Corporation
P.O. Box 333
Radio Road
Hanover, PA 17331

Signature of Agent of Submitter

Date

Agent Name
Submitter: Mandava Associates

TABLE OF CONTENTS

QUALITY ASSURANCE STATEMENT

Study Title: Rainbow Trout (*Oncorhynchus mykiss*) Static 96-Hour Acute Toxicity Test
Test Substance: Miller 6064

The study has been inspected and the report and data have been audited in accordance with STILLMEADOW, Inc. Standard Operating Procedures (SOPs). The findings from the inspection and audit were reported to the Study Director and Management as follows:

Phase of Study Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
Randomization/Dosing	12 Jul 01	13 Jul 01	13 Jul 01
Report/Data Audit	16 Nov 01	16 Nov 01	16 Nov 01
Final Report Audit	25 Mar 02	25 Mar 02	25 Mar 02

B. Lynn Murphy

B. Lynn Murphy, B.S.
Quality Assurance Unit
STILLMEADOW, Inc.

09 May 2002
Date

SUMMARY

This study was conducted to assess the toxicity of the test substance Miller 6064 to *Oncorhynchus mykiss* in a 96-hour static, non-renewal test.

Test considerations were determined by a preliminary range-finding test. The test substance concentrations chosen by the range-finding test (5, 8, 13, 21 and 35 mg/L) were administered to the test system, *Oncorhynchus mykiss*, in laboratory fresh water. There were two control groups without the test substance, one to determine the condition of the test population, and another, a solvent control, to demonstrate artifactual toxicity produced by the solvent N,N-Dimethylformamide. For each test concentration, two replicates of ten organisms each were treated with the appropriate concentration of the test substance. Dissolved oxygen, temperature, conductivity, and pH measurements were recorded at dosing and daily throughout definitive portion of the study. Observations of mortality were made at 24, 48, 72 and 96 hours after treatment. The test was terminated after 96 ± 2 hours of exposure.

Since there was mortality only at the 35-mg/L concentration for the first definitive test, a second definitive test was conducted in the same manner with concentrations of 13, 21, 35, and 58 mg/L. An even higher concentration was not used due to the potential toxicity of the solvent. A 100% survival rate was observed in fish treated with 0, 13, 21 and 35 mg/L of the test substance and the solvent control. A 55% survival rate was observed in fish treated with 58 mg/L of the test substance.

It was determined that the solvent did not have a high enough toxicity level to interfere with using higher concentrations of the test substance, so a third definitive level was conducted at concentrations of 20, 40, 60, 80 and 100 mg/L, as well as another control and solvent control. There was 100% survival in both controls as well as the 20 mg/L concentration, and a 35% survival rate in the 40 mg/L level. All fish died at the 60, 80 and 100 mg/L concentrations. The LC_{50} level, based on the three definitive tests conducted, is 46.90 mg/L with 95% confidence limits of 38.21 – 58.88 mg/L. The NOEC level is 35 mg/L.

INTRODUCTION

The objective of this study was to assess the toxicity of the test substance, Miller 6064, to *Oncorhynchus mykiss* in a 96-hour test. This study was conducted for Miller Chemical and Fertilizer Corporation according to the approved protocol, STILLMEADOW, Inc. SOPs, and OPPTS 850.1075. This study was initiated on 22 Jun 01. The laboratory portions of the study were conducted between 06 Jul 01 and 25 Feb 02. The original protocol, raw data, and report are on file in the STILLMEADOW, Inc. archives. A reserve sample of the test substance will be retained at STILLMEADOW, Inc. for a period of at least five years.

TEST SUBSTANCE AND SOLVENT

Identification:	Miller 6064
Date and Quantity Received:	19 Dec 00; 2 X 1 gal.
Physical Description:	Amber liquid
Storage:	Room temperature
Purity and Composition:	Certificate of Analysis not provided by Sponsor
Stability:	Not provided by the Sponsor
Solvent:	N,N-Dimethylformamide, Fisher Lot No. 001380, Exp. 19 Jul 05

Records pertaining to stability, characterization, and verification of test substance identity are the responsibility of the Sponsor.

TEST SYSTEM

Experimental Organism

Species:	Rainbow Trout, *Oncorhynchus mykiss*
Source and Receive Date:	Lost River Trout Farm (Mackay, Idaho), 12 Jun, 23 Aug 01 and 28 Jan 02
Age:	Juvenile (DOB 28 Mar 01, 21 Jul 01 and 26 Nov 02)
Size:	Less than 3.0 g at test initiation; the longest fish was not more than twice the length of the shortest.
Quantity:	Range-finding: 2 per test concentration Definitives: 20 per test concentration

Organism Husbandry

Test Room:	Environmentally controlled chamber (Chambers B, D and E)
Test Chambers:	2 ½-gallon glass aquaria
Test Medium:	Reconstituted water with total hardness between 40 and 180 mg/L $CaCO_3$ and with a pH between 6.0 and 8.0.
Loading:	Maximum loading of 0.8 g fish/liter.
Holding:	All fish were held in the laboratory at least 14 days before they were used for testing. The fish were held in water of the quality used in the test for at least seven days immediately before testing.

Environmental Controls

Set to Maintain:	Temperature Range of 12 ±2°C 16-hours light/8-hours dark cycle Dissolved oxygen concentration of at least 60 percent saturation
Food:	Fish were fed daily until 48 hours before the test was started. Fish were not fed during the test.

Animal husbandry and housing at STILLMEADOW, Inc. comply with Animal Welfare Act Regulations. No contaminants were expected to have been present in the feed or water which would have interfered with or affected the results of the study.

PROCEDURES

Range-finding Test

A range-finding test was conducted using five concentrations (1, 5, 10, 50, and 100 mg/L) of the test substance Miller 6064. Organisms were randomly placed individually into each beaker containing the appropriate concentration of test substance. Each beaker contained two organisms. Two organisms that were not exposed to test substance served as controls to demonstrate the condition of the test population. Two organisms were exposed to the solvent N,N-dimethylformamide only in order to demonstrate any artifactual toxicity produced. At 24, 48, 72, and 96 hours following dosing, each beaker was examined for mortality and the number of live fish was recorded. Final parameters were recorded for all test concentrations either at study termination or when there were no survivors in a replicate.

Definitive Test

Based on the results of the range-finding test, concentrations of Miller 6064 chosen for definitive testing were 5, 8, 13, 21, and 35 mg/L. Each test concentration consisted of two replicates of ten fish per replicate. Two replicates containing ten fish each were not exposed to test substance and served as controls to demonstrate the condition of the test population and two replicates containing ten fish each were exposed to the solvent only. Small groups of fish were randomly placed in the test vessels until each aquarium contained the appropriate number of organisms. At 24, 48, 72, and 96 hours following dosing, each aquarium was examined for mortality and the number of live fish was recorded. Fish were considered dead when there was no visible movement (e.g. gill movements) and if touching of the caudal peduncle produced no reaction. Dead fish were removed when observed. Visible abnormalities were also recorded (e.g. loss of reflex, erratic swimming, increased excitability, lethargy, and changes in appearance or physiology such as discoloration, excess mucous production, hyperventilation, opaque eyes, curved spine, or hemorrhaging, etc.). Since there was only a 5% mortality rate at the highest concentration, a second definitive test was set in the same manner using concentrations of 13, 21, 35 and 58 mg/L. Once it was determined that the solvent was not toxic at the higher concentrations necessary for higher concentrations of the test substance, a third definitive test was conducted at concentrations of 20, 40, 60, 80 and 100 mg/L.

Chemical and Physical Monitoring

The following measurements of control and treated containers were recorded daily during definitive testing: dissolved oxygen, temperature, conductivity, and pH.

RESULTS AND DISCUSSION

Test Validity

The test was considered valid if control mortality did not exceed 10 percent. Since control and solvent control mortality were zero percent, the range-finding test was considered valid. Control data were compared with the mortality endpoints of the test concentrations.

RESULTS AND DISCUSSION (cont.)

Range-finding

A 100% survival rate was observed in fish treated at concentrations of 0, 1 and 10 mg/L. 100% mortality was observed in all fish treated with 50 and 100 mg/L of test substance.

Concentration (mg/L)	Number of Surviving Organisms				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	2	2	2	2	2
1	2	2	2	2	2
5	2	2	2	1[d]	1
10	2	2	2	2	2
50	2	2[a]	0[c]	-	-
100	2	0[b]	-	-	-
C2	2	2	2	2	2

C2 – Solvent control
[a] - Fish laying on the bottom of the tank in curved position. Gills still moving, appear dead but jerk when stimulated.
[b] - Fish all dead. No movement, with curved spines.
[c] - All fish dead, white in color with curved spines.
[d] - Dead fish has very extended gills and mouth.

Definitive

In the first definitive test conducted, a 100% survival rate was observed in fish treated with 0, 5, 8, 13 and 21 mg/L of the test substance and in the solvent control. A 95% survival rate was observed in fish treated at the 35mg/L level. Since there was only a 5% mortality rate at the highest concentration, a second definitive test was set in the same manner using concentrations of 13, 21, 35, 58 and 97 mg/L. The 97 mg/L concentration subsequently had to be dropped from the test since it was feared that the amount of solvent needed to dissolve the test substance would exceed the toxicity level for the fish. In this second definitive test, a 100% survival rate was seen in both controls and in the 13, 21 and 35 mg/L levels. A 55% survival rate was seen at the 58-mg/L level. Once it was determined that the higher concentrations of solvent could be used, a third definitive test was run at higher concentrations. The concentrations used were 20, 40, 60, 80 and 100 mg/L. There were 100% survival rates observed in both controls as well as the 20-mg/L concentration. The 40-mg/L level had only a 35% survival rate, and the 60, 80 and 100 mg/L levels had 100% mortality. Results of the definitive tests follow.

Chemical and physical monitoring data (dissolved oxygen, temperature, conductivity, and pH) of control and treated containers for the three definitive tests are presented in Appendix A.

RESULTS AND DISCUSSION (cont.)

First Definitive Test (12 to 16 Jul 01)

Target Concentration (mg/L)	Replicate	Number of Surviving Organisms				
		0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	A	10	10	10	10	10
	B	10	10	10	10	10
5	A	10	10	10	10	10
	B	10	10	10	10	10
8	A	10	10	10	10	10
	B	10	10	10	10	10
13	A	10	10	10	10	10
	B	10	10	10	10	10
21	A	10	10	10	10	10
	B	10	10	10	10	10
35	A	10	10[a]	10	10[d]	9[e]
	B	10	10[b]	10[c]	10[c]	10[c]
C2	A	10	10	10	10	10
	B	10	10	10	10	10

C2 – Solvent control
[a] - All fish dark in color, swimming on bottom of tank. Three fish laying on side with curved spine and gill movement.
[b] - All fish dark in color laying on bottom of tank with curved spine and gill movement.
[c] - Four fish laying on side with curved spine and gill movement.
[d] - One fish laying on side with curved spine and gill movement.
[e] - One fish dead with curved spine.

RESULTS AND DISCUSSION (cont.)

Second Definitive Test (04 to 08 Oct 01)

Target Concentration (mg/L)	Replicate	Number of Surviving Organisms				
		0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	A	10	10	10	10	10
	B	10	10	10	10	10
13	A	10	10	10	10	10
	B	10	10	10	10	10
21	A	10	10	10	10	10
	B	10	10	10	10	10
35	A	10	10	10	10	10
	B	10	10	10	10	10
58	A	10	10[a]	10[a]	7[b]	5[d]
	B	10	10[a]	10[a]	6[c]	6[a]
C2	A	10	10	10	10	10
	B	10	10	10	10	10

C2 – Solvent control
[a] - All fish laying on side at botom of tank with gill movement.
[b] - Three fish dead laying on side at bottom, no gill movement.
[c] - Four fish dead laying on side at bottom.
[d] - Two fish dead laying on side with curved spine, white in color.

Third Definitive Test (21 to 25 Feb 02)

Target Concentration (mg/L)	Replicate	Number of Surviving Organisms				
		0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	A	10	10	10	10	10
	B	10	10	10	10	10
20	A	10	10	10	10	10
	B	10	10	10	10	10
40	A	10	4[a]	4	4	4
	B	10	3[a]	3	3	3
60	A	10	2[a]	0[a]	0	0
	B	10	2[a]	0[a]	0	0
80	A	10	0[a]	0	0	0
	B	10	0[a]	0	0	0
100	A	10	0[a]	0	0	0
	B	10	0[a]	0	0	0
C2	A	10	10	10	10	10

C2 – Solvent control. There was insufficient solvent to run two replicates of the solvent control.
[a] - Fish dead, laying on bottom.

RESULTS AND DISCUSSION (cont.)

Evaluation of Results

The LC_{50} level for the test substance, Miller 6064, based on the three definitive tests conducted, is 46.90 mg/L with 95% confidence limits of 38.21 – 58.88 mg/L. The NOEC level is 35 mg/L.

CONCLUSION

The test substance, Miller 6064, was evaluated for toxicity to *Oncorhynchus mykiss* in a 96-hour static, non-renewal test. The LC_{50} level, based on the three definitive tests conducted, is 46.90 mg/L with 95% confidence limits of 38.21 – 58.88 mg/L. The NOEC level is 35 mg/L.

Neil A. Rodrigue

09 May 02

Neil A. Rodrigue, M.S.
Study Director
STILLMEADOW, Inc.

Date

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Appendix A: Chemical and Physical Monitoring Data (First Definitive Test – 12 to 16 Jul 01)

Table 1. Temperature (°C)

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	14	12[a]	12[a]	12[a]	12[a]
5	14	12[a]	12[a]	12[a]	12[a]
8	14	12[a]	12[a]	12[a]	12[a]
13	14	12[a]	12[a]	12[a]	12[a]
21	14	12[a]	12[a]	12[a]	12[a]
35	14	12[a]	12[a]	12[a]	12[a]
C2	14	12[a]	12[a]	12[a]	12[a]

[a] – Temperature taken from chamber thermometer

Table 2. pH

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	7.7	7.5	7.3	7.3	7.4
5	7.8	7.5	7.3	7.3	7.3
8	7.9	7.4	7.3	7.3	7.3
13	7.9	7.4	7.3	7.4	7.3
21	7.9	7.4	7.3	7.4	7.3
35	7.9	7.4	7.2	7.4	7.2
C2	7.8	7.4	7.3	7.4	7.3

Table 3. Dissolved Oxygen (mg/L)

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	10.2	8.2	8.0	8.0	8.0
5	9.8	8.2	8.0	8.0	7.8
8	10.0	7.6	8.0	8.0	7.8
13	10.0	7.6	8.0	7.8	7.8
21	10.2	7.0	7.4	7.8	7.0
35	10.2	6.6	7.0	5.8	6.4
C2	10.0	8.2	8.0	8.1	7.7

Table 4. Conductivity (µmhos/cm)

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	250	245	250	250	250
5	265	250	250	250	250
8	270	250	245	250	250
13	265	250	245	245	250
21	265	245	245	245	245
35	260	245	230	245	245
C2	260	245	245	245	245

Appendix A: Chemical and Physical Monitoring Data (Second Definitive Test – 04 to 08 Oct 01)

Table 1. Temperature (°C)

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	13	11[a]	11[a]	11[a]	11[a]
13	13	11[a]	11[a]	11[a]	11[a]
21	13	11[a]	11[a]	11[a]	11[a]
35	13	11[a]	11[a]	11[a]	11[a]
58	13	11[a]	11[a]	11[a]	11[a]
C2	13	11[a]	11[a]	11[a]	11[a]

[a] – Temperature taken from chamber thermometer

Table 2. pH

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	7.6	7.4	7.8	7.8	7.6
13	7.5	7.4	7.8	7.7	7.6
21	7.6	7.4	7.8	7.7	7.6
35	7.6	7.4	7.7	7.7	7.6
58	7.6	7.4	7.7	7.7	7.5
C2	7.5	7.4	7.6	7.7	7.6

Table 3. Dissolved Oxygen (mg/L)

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	8.4	6.4	7.2	6.6	6.0
13	8.6	6.7	7.4	6.4	5.4
21	8.6	6.4	7.4	6.4	5.8
35	8.6	6.5	7.2	5.8	4.4
58	8.6	6.5	7.2	3.9	4.6
C2	8.6	6.6	7.2	6.2	5.4

Table 4. Conductivity (µmhos/cm)

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	260	230	230	240	240
13	260	235	230	235	240
21	260	235	230	230	235
35	260	230	230	230	235
58	255	230	235	235	230
C2	260	230	230	230	235

Appendix A: Chemical and Physical Monitoring Data (Third Definitive Test – 21 to 25 Feb 02)

Table 1. Temperature (°C)

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	12	13[a]	13[a]	13[a]	13[a]
20	12	13[a]	13[a]	13[a]	13[a]
40	12	13[a]	13[a]	13[a]	13[a]
60	12	13[a]	13[a]	13[a]	13[a]
80	12	13[a]	13[a]	13[a]	13[a]
100	12	13[a]	13[a]	13[a]	13[a]
C2	13	13[a]	13[a]	13[a]	13[a]

[a] – Temperature taken from chamber thermometer

Table 2. pH

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	8.0	7.7	7.5	7.2	7.7
20	8.2	7.7	7.5	7.2	7.7
40	8.2	7.7	7.5	7.2	7.7
60	8.2	7.7	7.6	7.2	-
80	8.2	7.7	-	-	-
100	8.2	7.7	-	-	-
C2	8.1	7.7	7.5	7.2	7.6

Table 3. Dissolved Oxygen (mg/L)

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	9.8	9.6	9.4	9.0	8.8
20	10.0	9.4	9.6	9.2	8.8
40	10.0	9.4	9.6	9.2	8.8
60	10.2	10.2	9.6	-	-
80	10.3	10.0	-	-	-
100	10.2	10.0	-	-	-
C2	10.2	9.6	9.5	9.0	9.0

Table 4. Conductivity (µmhos/cm)

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	210	210	210	210	210
20	205	210	210	210	210
40	205	205	210	210	210
60	205	205	210	-	-
80	205	210	-	-	-
100	205	210	-	-	-
C2	215	210	210	210	210

Appendix B: Statistics

96 hour Static, Non-Renewal Acute Definitive Toxicity Test-96 Hr Survival

Start Date:	2/21/02 17:16	Test ID:	6420-01	Sample ID:	Miller 6064
End Date:	2/25/02 16:42	Lab ID:	Miller 6064	Sample Type:	Product
Sample Date:		Protocol:	OPPTS 850.1075	Test Species:	OM-Oncorhynchus mykiss
Comments:	Data from 3 Definitive tests				

Conc-mg/L	1	2	3	4	5	6
Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
t Control (C2)	1.0000	1.0000	1.0000	1.0000	1.0000	
5	1.0000	1.0000				
8	1.0000	1.0000				
13	1.0000	1.0000	1.0000	1.0000		
20	1.0000	1.0000				
21	1.0000	1.0000	1.0000	1.0000		
35	0.9000	1.0000	1.0000	1.0000		
40	0.4000	0.3000				
58	0.5000	0.6000				
60	0.0000	0.0000				
80	0.0000	0.0000				
100	0.0000	0.0000				

			Transform: Arcsin Square Root					1-Tailed			Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	6				0	60
t Control (C2)	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	5					
5	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.861	0.0870	0	20
8	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.861	0.0870	0	20
13	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	0.000	2.861	0.0688	0	40
20	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.861	0.0870	0	20
21	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	0.000	2.861	0.0688	0	40
35	0.9750	0.9750	1.3713	1.2490	1.4120	5.942	4	1.695	2.861	0.0688	1	40
*40	0.3500	0.3500	0.6322	0.5796	0.6847	11.753	2	25.648	2.861	0.0870	13	20
*58	0.5500	0.5500	0.8357	0.7854	0.8861	8.518	2	18.953	2.861	0.0870	9	20
*60	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	41.217	2.861	0.0870	20	20
*80	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	41.217	2.861	0.0870	20	20
*100	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	41.217	2.861	0.0870	20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.64297	0.908	-1.8591	7.74529
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 1.00)	0	2.26216		

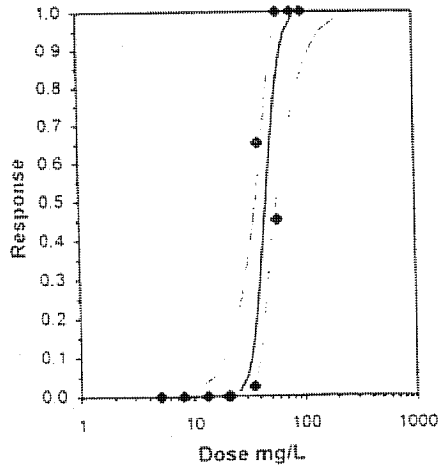
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	35	40	37.4166		0.03419	0.03507	0.73935	0.00139	4.9E-24	11, 22

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	8.8605	2.38084	3.47465	14.2463	0	37.5254	16.919	2.1E-05	1.67115	0.11286	5
Intercept	-9.8072	3.96924	-18.786	-0.8281							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	25.6209	9.94312	33.2567
EC05	3.355	30.5849	15.3809	37.7044
EC10	3.718	33.6132	19.3003	40.5385
EC15	3.964	35.824	22.4031	42.7438
EC20	4.158	37.6843	25.1296	44.7442
EC25	4.326	39.3571	27.6311	46.7043
EC40	4.747	43.909	34.3001	53.2385
EC50	5.000	46.8972	38.2191	58.8759
EC60	5.253	50.0887	41.8204	66.3018
EC75	5.674	55.8817	47.0753	83.3463
EC80	5.842	58.3623	48.9853	91.9275
EC85	6.036	61.393	51.1426	103.388
EC90	6.282	65.4309	53.8014	120.284
EC95	6.645	71.9094	57.724	151.254
EC99	7.326	85.8419	65.3165	234.428

Significant heterogeneity detected (p = 2.12E-05)



Appendix C: Protocol and Amendment

STILLMEADOW
INCORPORATED

PROTOCOL AMENDMENT #1
STILLMEADOW, Inc. Study Number 6420-01

Sponsor: Miller Chemical & Fertilizer Corporation
PO Box 333
Radio Road
Hanover PA 17331

Effective Date: 22 Oct 2001

Test Substance: Miller 6064

Study Title: RAINBOW TROUT (*Oncorhynchus mykiss*) STATIC 96-HOUR ACUTE TOXICITY TEST

The following alteration is being made to the cover and Section A.7 of the protocol.

To Change:	Abigail Campbell, B.S.
To Read:	Neil Rodrigue, M.S.
Justification:	The study director is being changed because Abigail Campbell is no longer with the company.
Impact:	There will be no impact on the study.

This amendment has been reviewed and/or approved by the following:

Approved: Neil A. Rodrigue — 08 Nov 01
Neil Rodrigue, M.S.
Study Director
STILLMEADOW, Inc.
Date

Approved: — 6 Nov 01
Mark S. Holbert
Vice President
STILLMEADOW, Inc.
Date

Reviewed:: — 6 Nov 2001
Vicki S. Crutchfield, R.Q.A.P.
Director, Quality Assurance Unit
STILLMEADOW, Inc.
Date

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Appendix C: Protocol and Amendment (cont.)

STILLMEADOW
INCORPORATED

PROTOCOL FOR STUDY 6420-01

Study Title: RAINBOW TROUT (*Oncorhynchus mykiss*) STATIC 96-HOUR ACUTE TOXICITY TEST

Test Substance: Miller 6064

Test Facility: STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, Texas 77479

Approved: Abigail Campbell — 22 Jun 01
Abigail Campbell, B.S.
Study Director
STILLMEADOW, Inc.
Date

Approved: Elizabeth L. Sabol — 5 June 2001
Elizabeth L. Sabol, B.A., B.S.Ed
Vice President
STILLMEADOW, Inc.
Date

Reviewed: Vicki Crutchfield — 5 June 2001
Vicki Crutchfield, R.Q.A.P.
Director, Quality Assurance Unit
STILLMEADOW, Inc.
Date

Sponsor: Miller Chemical & Fertilizer Corporation
PO Box 333
Radio Road
Hanover PA 17331

Sponsor Representative
Mandava Associates
1730 M Street, Suite 906
Washington, D.C. 20036-4510

Approved: N. Bhushan Mandava — 15 June 2001
N. Bhushan Mandava, Ph.D.
Date

Appendix C: Protocol and Amendment (cont.)

PROTOCOL FOR STUDY 6420-01

A. GENERAL

1. Study Title:	RAINBOW TROUT (*Oncorhynchus mykiss*) STATIC 96-HOUR ACUTE TOXICITY TEST
2. Purpose:	To assess the toxicity of the test substance to rainbow trout (*Oncorhynchus mykiss*) in a static 96-hour test.
3. Regulatory Compliance:	This study will be conducted according to OPPTS §50.1075, which is intended to meet testing requirements of both FIFRA (7 U.S.C. 136, *et seq.*) and TSCA (15 U.S.C. 2601). This study will be conducted in compliance with Good Laboratory Practice Standards: 1. EPA FIFRA: 40 CFR 160 2. OECD: C(81)30 (Final) 3. Japanese MAFF All methods can be found in STILLMEADOW, Inc. Standard Operating Procedures (SOPs).
4. Quality Assurance:	The Protocol is reviewed by the Quality Assurance Unit (QAU). The study information will be entered into the Master Schedule. The study will be inspected at least once during its progress. Further inspections may be scheduled as needed to ensure the integrity of the study. Any deviations from SOPs, the Protocol, or Good Laboratory Practice Standards will be immediately reported to the Study Director and Management. The report will be audited and a statement prepared and signed which shall specify the dates inspections were made and findings reported to Management and to the Study Director.
5. Test Substance:	Miller 6064. Test substance identification should include the name, batch number, and purity. Information regarding safety, stability, storage conditions, and disposal should also be provided by the Sponsor
6. Proposed Schedule:	Testing will begin within approximately three weeks of receipt of the test substance and authorization to conduct the study. Proposed Start Date: 13 Jun 01 Proposed End Date: 11 Jul 01
7. Study Director:	Abigail Campbell, B.S.

Appendix C: Protocol and Amendment (cont.)

A. GENERAL (cont.)

8. Experimental Summary:	Definitive test concentrations will be determined by a preliminary range finder. The test substance concentrations chosen will be administered to the test system, rainbow trout (*Oncorhynchus mykiss)*, in reconstituted water. For each test concentration, 20 organisms will be treated with the appropriate concentration of the test substance. Two control groups, which will not contain test substance, will be used in this test. One group will have solvent added at the highest volume used for any test concentration preparation and will represent the solvent control. The other control group will remain untreated and will demonstrate the condition of the test population. Dissolved oxygen, temperature, conductivity, and pH will be measured and recorded in each treatment and the control at test initiation and daily throughout the study. Observations of mortality in each test chamber will be made at 24, 48, 72, and 96 hours. The test will be terminated after 96 ± 2 hours of exposure. The test will be considered valid if control mortality does not exceed 10 percent.
9. Protocol Amendments:	Any alteration in the Protocol will be justified, approved by the Study Director, and recorded in writing.
10. Sponsor Audits:	The Sponsor may send an authorized representative to inspect the test system and/or data on the STILLMEADOW, Inc. premises during normal working hours.

Appendix C: Protocol and Amendment (cont.)

B. EXPERIMENTAL DESIGN

1. Organism

a. Species:	Rainbow trout (*Oncorhynchus mykiss*)
b. Justification of Species:	Specified in the OPPTS regulations.
c. Age/Size:	Juvenile fish, less than 3.0 g at test initiation. The longest fish will not be more than twice the length of the shortest.
d. Number:	The range finder will use 2 fish for each concentration and the controls. The definitive test will use 20 fish for each concentration and each control group (2 replicates each containing 10 fish).
e. Source:	*Oncorhynchus mykiss* will be obtained from Aquatic Research Organisms, Inc. (Hampton, New Hampshire) or another suitable supplier.
f. Identification:	Organisms will be labeled by study number, lot number, date of receipt, and number of organisms.

2. Animal Husbandry

a. Test Medium:	Reconstituted water with total hardness between 40 and 180 mg $CaCO_3$ and with a pH between 6.0 and 8.0.
b. Acclimation:	All fish will be held in the laboratory for at least 14 days before they are used for testing. They will be held in water of the quality to be used in the test for at least seven days immediately before testing. Pretest mortality must be less than 5% during acclimation or the organisms will be held for an additional seven days. If pretest mortality is greater than 10%, then the entire lot will be rejected and a new lot of fish will be obtained to begin acclimation.
b. Test Chamber:	Test containers will be 2½ gallon aquaria. Test containers will be loosely covered to reduce the loss of test solution or dilution water due to evaporation and to minimize the entry of dust or other particles into the solutions.
c. Temperature:	Test temperature will be 12±2°C.
d. Photoperiod:	16 hours light, 8 hours dark
e. Dissolved Oxygen Concentrations:	At least 60 percent air saturation value.
f. Food:	Fish will be fed daily until 48 hours prior to test initiation. Fish will not be fed during test.
g. Loading:	Maximum loading of 0.8 g fish/liter.

Appendix C: Protocol and Amendment (cont.)

B. EXPERIMENTAL DESIGN (cont.)

3. Pre-test Preparation

a. Test Substance Receipt: Test substance will be supplied by the Sponsor in appropriately sized glass containers, sealed and delivered to STILLMEADOW, Inc. Samples will be stored according to the Sponsor's instructions until prepared for testing.

b. Test Substance Preparation: The test substance is insoluble in water and will be administered using an appropriate solvent (DMF, ethanol, methanol, etc.) as weight/volume concentrations. A solvent control will be included in the test design. The test substance dilutions will be prepared on the day of treatment.

c. Route of Administration: The test substance will be administered to the test system at test initiation by introduction to the test containers containing the test system.

d. Reason for Route of Administration: Specified by the cited guidelines for evaluation of the toxicity potential of a test substance.

e. Preparation of Test System: The organisms will be randomized into aquaria containing the appropriate concentration of test substance. Each test concentration will consist of 20 fish. Each aquarium will house of a maximum of 10 fish.

f. Control Groups: Twenty fish will not have test substance added and will be considered the control. This control will be used to demonstrate the condition of the test population. An additional 20 fish will not have test substance added but will contain the solvent at the highest volume used for any test concentration preparation. The solvent control will be used to demonstrate artifactual toxicity produced by the solvent.

4. Test Substance Administration

a. Dosing Concentrations: A range finder will be conducted with at least five concentrations of the test substance to obtain an approximate LC_{50} value for the test substance. The test concentrations will be at least 50% greater than the lowest test concentrations (not to exceed 120%).

Five test concentrations chosen from the range-finding data and the controls will be prepared on the day of test initiation.

b. Initial Measurements: Dissolved oxygen, temperature, conductivity, and pH of the control and treated containers will be measured and recorded at test initiation.

Appendix C: Protocol and Amendment (cont.)

B. EXPERIMENTAL DESIGN (cont.)

5. Observations

a. Biological Monitoring: Containers will be inspected at 24, 48, 72, and 96 hours for mortality. Fish are considered dead if there is no visible movement (e.g. gill movements) and if touching of the caudal peduncle produces no reaction. Dead fish will be removed when observed, and mortalities will be recorded. Visible abnormalities will be recorded (e.g. loss of reflex, erratic swimming, increased excitability, lethargy, and changes in appearance or physiology such as discoloration, excess mucous production, hyperventilation, opaque eyes, curved spine, or hemorrhaging, etc.)

b. Chemical and Physical Monitoring: At a minimum, the following measurements will be made daily: dissolved oxygen, temperature, conductivity, and pH of the controls and treated containers.

6. Test Duration: The test will be terminated after 96 ± 2 hours.

7. Quality Criterion: The test will be considered valid if the control mortality does not exceed 10 percent.

8. Evaluation of Results: The survival in the test concentrations will be statistically compared to survival in the control to determine the highest concentration of test substance that demonstrates no significant reduction in survival. This concentration will be the No Observed Effect Level (NOEL) for survival. The NOEL will be determined by using a commercially available statistical program (Toxstat®).

The median lethal concentration (LC_{50}) will be estimated using a linear regression model. Several models are available for LC_{50} determination: Probit, Trimmed Spearman-Karber, and Binomial. The most appropriate model will be selected for estimating the LC_{50} if a dose response is exhibited in the study.

9. Test Substance Accountability: A comprehensive inventory of test substance received and used will be kept. The test substance container(s) will be weighed when received at this facility, and a record of all test substance use will be maintained. Test substance and test substance dosing solutions will be stored in the original containers, or in the equivalent thereof, or in glass containers with Teflon-lined caps.

Appendix C: Protocol and Amendment (cont.)

B. EXPERIMENTAL DESIGN (cont.)

10. Disposal of Unused Test Substance:

Unused test substance will be disposed of at the Sponsor's expense after the termination of the study. STILLMEADOW, Inc. will retain a reserve sample.

11. Safety Precautions:

General safety precautions required by laboratory SOPs will be followed. The Sponsor will supply basic toxicity data on the test substance to be used. However, since the toxicity of test substances is often not well characterized, STILLMEADOW, Inc. will be conservative in setting safety procedures. The Sponsor or Sponsor's Representative shall be notified of any exposures requiring a physician's examination or care.

C. DATA MANAGEMENT

1. Records:

The following records will be maintained during the study and transferred to the STILLMEADOW, Inc. archives upon study termination.

a. Protocol and Protocol Amendments (if any).
b. Final report and amendments (if any).
c. Study correspondence.
d. Test culture data.
e. Test substance receipt, identification as supplied by Sponsor, preparation, administration, and disposition.
f. Range finder data and results.
g. Initial and daily measurements for dissolved oxygen, temperature, and pH of the control and treated containers.
h. Cumulative mortality at each concentration at each observation time.
i. Determination of the validity of the study.
j. Other pertinent data.

2. Data Storage:

All raw data and a reserve sample of the test substance will be retained at STILLMEADOW, Inc. for a period of at least five years.

Appendix C: Protocol and Amendment (cont.)

C. DATA MANAGEMENT (cont.)

3. Data Reporting:

The final report will include all data as described in the Good Laboratory Practice Standards, including:

a. Statement from the Quality Assurance Unit.
b. Signature of the Study Director.
c. A GLP Compliance Statement signed by the Study Director.
d. Names of scientific personnel involved in the study.
e. Dates of study initiation and termination.
f. Identification, description, preparation, and storage of the test substance.
g. All pertinent culture information, preparation of test medium, test conditions, dosing information, and observation methods.
h. Initial and daily data for dissolved oxygen, temperature, and pH of the control and treated containers.
i. Cumulative mortality at each concentration at each observation time.
j. Graph of the concentration-mortality curve at the end of the test.
k. Statistical procedures used for determining the LC_{50} and NOEL values.
l. Determination of the validity of the test based on the control data.
m. Abnormalities observed in test and control animals.
n. Any protocol deviations or occurrences which may have influenced the final results of the test.
o. Evaluation of results.
p. A reference to this Protocol.

4. Report Submission:

A report will be submitted after termination of the laboratory portion of the study.

Appendix D: Certificate of Analysis

MILLER CHEMICAL & FERTILIZER CORPORATION

P O. BOX 333, 120 RADIO ROAD
HANOVER, PENNSYLVANIA 17331 U.S.A.
TELEPHONE 717-632-8921
FAX NO.: 717-632-4561

1/18/01

CERTIFICATE OF ANALYSIS

Product Name: MILLER 6064
Quantity Produced: 7.4 kilograms
Batch Number: 150386
Date of Formulation: 12/13/00

Appearance: Yellow to Amber Liquid
Odor: Terpenic
Moisture: Negligble (non-aqueous)
Specific Gravity: 0.92 - 0.94 @ 20c
Viscosity @ 20c: 100 to 500 cps
pH(1% solution): 6.0 to 8.0
Solubility in Water: Insoluble (Emulsifies)
Flash Point: over 200 f
Purity: 100%

Signature:

Name: Andrew G. Smith
Title: Compliance Officer

ATTACHMENT 42

Daphnia magna
Static 48-Hour Acute Toxicity Test
on Miller 6064

STILLMEADOW
INCORPORATED

VOLUME __ OF __ OF SUBMISSION

Miller 6064

FINAL REPORT

***Daphnia magna* STATIC 48-Hour ACUTE TOXICITY TEST**

OPPTS No. 850.1010

AUTHOR:

Neil A. Rodrigue, M.S.

STUDY INITIATION DATE: 29 June 2001
STUDY COMPLETION DATE: 9 May 2002

CONDUCTED BY:
STILLMEADOW, Inc.
10161 Harwin Drive, Suite 150
Houston, Texas 77036

LABORATORY STUDY NUMBER:

6421-01

VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 23

SUBMITTED TO:
Miller Chemical & Fertilizer Corporation
P.O. Box 333
Radio Road
Hanover, PA 17331

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA § 10(d) (1) (A), (B), or (C).

Company: Miller Chemical and Fertilizer Corporation

Company Agent:_____ Date: _____

Title_____ Signature _____

These data are the property of Miller Chemical and Fertilizer Corporation and as such, are considered to be confidential for all purposes other than compliance with FIFRA § 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality that may exist under any other statute or in any other country.

GLP COMPLIANCE STATEMENT

This study was designed and performed in STILLMEADOW, Inc.'s laboratory in compliance with the following:

· United States Environmental Protection Agency (USEPA) FIFRA; Good Laboratory Practice Standards 40 CFR 160 with exception of sections 160.105 (b) (e) and 160.31 (d), stability information was not provided; 160.105 (b) solubility not determined; and 160.113 (a) mixture was not performed.

· Organization for Economic Cooperation and Development's Principles of Good Laboratory Practice, Annex 2, C(97)186 with the exception of section 6.2 (4), stability information was not provided and section 6.2 (5), mixture analysis was not performed.

· Japan Ministry of Agriculture, Forestry and Fisheries, Notification No. 59 Nohsan 3850, Director-General of Agricultural Production Bureau, 10 August 1984 with the exception of Article 5 (2) (9) and Article 21 (3), stability information was not provided and Article 23 (1), mixture analysis was not performed.

Neil A. Rodrigue — 09 May 02

Neil A. Rodrigue, M.S.
Study Director
STILLMEADOW, Inc.

Date

Signature of Agent of Sponsor

Date

Agent Name
Sponsor: Miller Chemical & Fertilizer Corporation
P.O. Box 333
Radio Road
Hanover, PA 17331

Signature of Agent of Submitter

Date

Agent Name
Submitter: Mandava Associates

TABLE OF CONTENTS

QUALITY ASSURANCE STATEMENT

Study Title: *Daphnia magna* Static 48-Hour Acute Toxicity Test
Test Substance: Miller 6064

The study has been inspected and the report and data have been audited in accordance with STILLMEADOW, Inc. Standard Operating Procedures (SOPs). The findings from the inspection and audit were reported to the Study Director and Management as follows:

Phase of Study Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
Randomization and Dosing	21 Aug 01	22 Aug 01	22 Aug 01
Counts	12 Oct 01	15 Oct 01	15 Oct 01
Report/Data Audit	14 Dec 01	15 Dec 01	15 Dec 01
Final Report/Data Audit	06 Mar 02	07 Mar 02	07 Mar 02

B. Lynn Murphy
Quality Assurance Unit
STILLMEADOW, Inc.

09 May 2002
Date

SUMMARY

This study was conducted to assess the toxicity of the test substance (Miller 6064) to *Daphnia magna* in a 48-hour static, non-renewal test.

Test considerations were determined by a preliminary range-finding test. The test substance concentrations chosen (0, 0.5, 0.9, 1.5, 2.5 and 5 mg/L) were administered to the test system, *Daphnia magna*, in reconstituted water. For each target test concentration, two replicates of ten organisms each were treated with the appropriate concentration of the test substance. Two control containers each contained 10 daphnids in reconstituted water and no test substance. Because the test substance was insoluble in water, N,N-Dimethylformamide was used as a solvent. Two control containers each contained 10 daphnids in the solvent as solvent controls. Dissolved oxygen, temperature, conductivity and pH measurements were recorded at dosing and termination. Observations for immobilization in each test chamber were made daily. The test was terminated after 48 ± 1 hours of exposure.

100% survival rates were seen in the daphnids treated with 0 mg/L of Miller 6064 and in the solvent control, and mortality was observed in daphnids treated with 0.5, 0.9, 1.5, 2.5 and 5 mg/L of the test substance.

Because of the high mortality rate, it was determined to run another definitive test. The test substance concentrations chosen (0, 0.1, 0.3, 0.5, 0.9 and 1.5mg/L) were administered to the test system using identical criteria as the first definitive test. The median lethal concentration (LC_{50}) was determined to be 0.54 mg/L with 95% confidence limits of 0.39 to 0.78 mg/L and a NOEC of less that 0.1 mg/L.

INTRODUCTION

The objective of this study was to assess the toxicity of the test substance to *Daphnia magna* in a 48-hour test. This study was conducted for Miller Chemical & Fertilizer Corporation according to the approved protocol, STILLMEADOW, Inc. SOPs, and Product Properties Test Guidelines, Series 850, Section 1010 of the United States Environmental Protection Agency Office of Prevention, Pesticides, and Toxic Substances. This study was initiated on 29 Jun 01. The laboratory portion of the study was conducted between 19 Jul 01 to 21 Jul 01 for the range-finding test, 21 Aug 01 to 23 Aug 01 for the first definitive test, and 11 Oct 01 to 13 Oct 01 for the second definitive test. The original protocol, raw data, and report are on file in the STILLMEADOW, Inc. archives. A reserve sample of the test substance will be retained at STILLMEADOW, Inc. for a period of at least five years.

TEST SUBSTANCE

Identification:	MILLER 6064
Date and Quantity Received:	19 Dec 00; 2 X 1 gal
Physical Description:	Amber liquid
Storage:	Room temperature
Purity and Composition:	Certificate of Analysis not provided by Sponsor
Stability:	Not provided by the Sponsor

Records pertaining to stability, characterization, and verification of test substance identity are the responsibility of the Sponsor.

TEST SYSTEM

Experimental Organism

Species:	*Daphnia magna*
Source:	STILLMEADOW, Inc. culture laboratory
Age at dosing:	Less than 24 hours old at dosing
Quantity:	Range-finding: 5 per test concentration Definitive: 20 per target test concentration

Organism Husbandry

Test Room:	Environmentally controlled chambers (Chamber D for the range-finding test, and Chamber C for the definitive tests)
Test Chambers:	250 mL glass beaker (range-finding and definitive)
Test Medium:	Reconstituted water
Holding:	The daphnids were held in water of the quality used in the tests for at least 48 hours immediately before testing.

Environmental Controls

Set to Maintain:	Temperature Range of 20±2°C 16-hours light / 8-hours dark cycle Dissolved oxygen concentration of at least 60 percent saturation at dosing
Food:	Daphnids were not fed during the tests

Animal husbandry and housing at STILLMEADOW, Inc. comply with Animal Welfare Act Regulations. No contaminants were expected to have been present in the feed or water which would have interfered with or affected the results of the study.

PROCEDURES

Range-finding Test

A preliminary range-finding test was conducted using five concentrations of the test substance (1, 5, 10, 50, and 100 mg/L). Following randomization, five organisms were placed into each beaker containing the appropriate concentration of test substance. Five organisms, which were not exposed to test substance, served as controls to demonstrate the condition of the test population. Additionally, five organisms, which were not exposed to test substance were exposed to the solvent (N,N-Dimethylformamide). This solvent control was used to demonstrate artifactual toxicity produced by the solvent. At 24 and 48 hours following dosing, each beaker was examined for mortality and the number of live daphnids was recorded.

PROCEDURES (cont.)

Definitive Test

Based on the results of the range-finding test, test substance concentrations were chosen for definitive testing. Five target concentrations of the test substance were used (0.5, 0.9, 1.5, 2.5 and 5 mg/L). Each target test concentration consisted of two replicates of ten daphnids per replicate. Two replicates containing ten daphnids each were not exposed to test substance and served as controls to demonstrate the condition of the test population. Additionally, two replicates of ten organisms, which were not exposed to test substance were exposed to the solvent (N,N-Dimethylformamide). This solvent control was used to demonstrate artifactual toxicity produced by the solvent. At 24 and 48 hours following dosing, each beaker was examined for mortality and the number of live daphnids was recorded.

Because of high mortality rates, it was determined to run another definitive test. Five target concentrations of the test substance were used (0.1, 0.3, 0.5, 0.9 and 1.5 mg/L). Identical criteria were used for the second test.

Chemical and Physical Monitoring

The following measurements were recorded during definitive testing: dissolved oxygen, temperature, conductivity, and pH of control and treated containers.

RESULTS AND DISCUSSION

Test Validity

The test was considered valid if control mortality did not exceed 10 percent. Since control mortality was zero percent, the range-finding and definitive tests were considered valid.

Range-finding

A 100% survival rate was observed in daphnids treated at a concentration of 0 mg/L of the test substance and the solvent control. Mortality was observed in all daphnids treated with 1, 5, 10, 50 and100 mg/L of test substance.

Concentration (mg/L)	Number of Surviving Organisms		
	0 Hours	24 Hours	48 Hours
0	5	5	5
1	5	5[a]	3
5	5	5[b]	1
10	5	5[c]	0
50	5	5	1
100	5	5	0
C2	5	5	5

[a] – Daphnids floating on surface of water, still alive with gill movement.
[b] – Daphnids floating on surface of water, still alive with gill movement. Two stuck together.
[c] – Daphnids collected on surface of water, alive with gill movement.

RESULTS AND DISCUSSION (cont.)

Definitive

In the definitive study of 21 Aug 01 to 23 Aug 01, 100% survival rates were seen in the daphnids treated with 0 mg/L of Miller 6064 and the solvent control. Mortality was observed in daphnids treated with 0.5, 0.9, 1.5, 2.5 and 5 mg/L of the test substance. In the definitive study of 11 Oct 01 to 13 Oct 01, 100% survival was observed in daphnids treated with 0 mg/L of the test substance and in the solvent control. Mortality was observed in daphnids treated with 0.1, 0.3, 0.5, 0.9 and 1.5 mg/L of the test substance. Chemical and physical monitoring data (dissolved oxygen, temperature, conductivity, and pH) of control and treated containers for the definitive tests are presented in Appendix A.

Definitive Test of 21 Aug 01 to 23 Aug 01

Target Concentration (mg/L)	Replicate	Number of Surviving Organisms		
		0 Hours	24 Hours	48 Hours
0	A	10	10	10
	B	10	10	10
0.5	A	10	10	4
	B	10	9	1
0.9	A	10	10	4
	B	10	10	3
1.5	A	10	10	2
	B	10	10	4
2.5	A	10	10	2
	B	10	10	3
5	A	10	10	1
	B	10	10	3
C2	A	10	10	10
	B	10	10	10

RESULTS AND DISCUSSION (cont.)

Definitive Test of 11 Oct 01 to 13 Oct 01

Target Concentration (mg/L)	Replicate	Number of Surviving Organisms		
		0 Hours	24 Hours	48 Hours
0	A	10	10	10
	B	10	10	10
0.1	A	10	9	9
	B	10	10	8
0.3	A	10	10[a]	8
	B	10	10[a]	9
0.5	A	10	10[a]	5
	B	10	10[a]	3
0.9	A	10	9[ab]	2
	B	10	9[ab]	4
1.5	A	10	10[a]	3
	B	10	10[ab]	1
C2	A	10	10	10
	B	10	10	10

[a] – Surviving animals floating on surface.

[b] – Two pair of animals stuck together but alive.

Evaluation of Results

Using the Trimmed Spearman-Karber Statistical Method, the median lethal concentration (LC_{50}) of Miller 6064 was determined to be 0.54 mg/L with 95% confidence limits of 0.39 0.78 mg/L, and a NOEC of less than 0.1 mg.l

CONCLUSION

The test substance, Miller 6064, was evaluated for toxicity to *Daphnia magna* in a 48-hour static, non-renewal test. The median lethal concentration (LC_{50}) of Miller 6064 was determined to 0.54 mg/L with 95% confidence limits of 0.39 to 0.78 mg/L and a NOEC of less than 0.1 mg/L

Neil A. Rodrigue

Neil A. Rodrigue, M.S.
Study Director
STILLMEADOW, Inc.

09 May 02
Date

STUDY PERSONNEL

Technical Staff

Mel S. Rivera, B.S.
Richard Sankar, B.S.
Rob Stowe, B.S.
Abigail Campbell, B.S.
Brandy Goffinet

Technical Writer

Diana W. Cook, B.S.

Appendix A: Chemical and Physical Monitoring Data
Definitive Test of 21 Aug 01 to 23 Aug 01

Table 1. Temperature (°C)

Target Concentration (mg/L)	0 Hours	48 Hours
0	21	21
0.5	21	21
0.9	21	21
1.5	21	21
2.5	21	21
5	21	21
C2	21	21

Table 2. pH

Target Concentration (mg/L)	0 Hours	48 Hours
0	8.1	7.8
0.5	8.2	7.8
0.9	8.2	7.8
1.5	8.2	7.8
2.5	8.2	7.9
5	8.2	7.9
C2	8.1	7.9

Table 3. Dissolved Oxygen (mg/L)

Target Concentration (mg/L)	0 Hours	48 Hours
0	6.8	8.4
0.5	6.6	8.4
0.9	6.6	8.4
1.5	6.6	8.4
2.5	6.4	8.4
5	6.4	8.4
C2	6.8	8.4

Table 4. Conductivity (µmhos/cm)

Target Concentration (mg/L)	0 Hours	48 Hours
0	290	265
0.5	290	270
0.9	290	270
1.5	290	270
2.5	290	270
5	290	270
C2	290	260

Appendix A: Chemical and Physical Monitoring Data (cont.)
Definitive Test of 11 Oct 01 to 13 Oct 01

Table 1. Temperature (°C)

Target Concentration (mg/L)	0 Hours	48 Hours
0	21	21
0.1	21	21
0.3	21	21
0.5	21	21
0.9	21	21
1.5	21	21
C2	21	21

Table 2. pH

Target Concentration (mg/L)	0 Hours	48 Hours
0	8.0	8.4
0.1	8.0	8.3
0.3	8.1	8.3
0.5	8.1	8.3
0.9	8.1	8.3
1.5	8.1	8.3
C2	*	*

* - pH not recorded

Table 3. Dissolved Oxygen (mg/L)

Target Concentration (mg/L)	0 Hours	48 Hours
0	7.6	7.4
0.1	7.8	7.4
0.3	7.9	7.5
0.5	7.9	7.4
0.9	7.9	7.4
1.5	7.9	7.4
C2	8.0	7.6

Table 4. Conductivity (µmhos/cm)

Target Concentration (mg/L)	0 Hours	48 Hours
0	295	290
0.1	300	290
0.3	300	290
0.5	300	290
0.9	300	290
1.5	300	290
C2	290	295

Appendix B: Trimmed Spearman-Karber Method Statistics

Concentration (mg/L)	Number Exposed	Mortalities
0	40	0
0.10	20	3
0.30	20	3
0.50	20	12
0.54	20	15
0.90	40	27
1.50	40	30
2.50	20	15
5.00	20	16

Spearman-Karber Estimates:

LC_{50}:	0.54
95% Lower Confidence:	0.39
95% Upper Confidence:	0.78

Fisher's Exact Test: NOEC: <0.1

Appendix C: Protocol and Amendment

STILLMEADOW
INCORPORATED

PROTOCOL AMENDMENT #1
STILLMEADOW, Inc. Study Number 6421-01

Sponsor: Miller Chemical & Fertilizer Corporation
PO Box 333
Radio Road
Hanover PA 17331

Effective Date: 22 Oct 2001

Test Substance: Miller 6064

Study Title: *Daphnia magna* STATIC 48-HOUR ACUTE TOXICITY TEST

The following alteration is being made to the cover and Section A.7 of the protocol.

To Change:	Abigail Campbell, B.S.
To Read:	Neil Rodrigue, M.S.
Justification:	The study director is being changed because Abigail Campbell is no longer with the company.
Impact:	There will be no impact on the study.

This amendment has been reviewed and/or approved by the following:

Approved: Neil A. Rodrigue — 08 Nov 01
Neil Rodrigue, M.S.
Study Director
STILLMEADOW, Inc.
Date

Approved: [signature] — 6 Nov 01
Mark S. Holbert
Vice President
STILLMEADOW, Inc.
Date

Reviewed:: [signature] — 6 Nov 2001
Vicki S. Crutchfield, R.Q.A.P.
Director, Quality Assurance Unit
STILLMEADOW, Inc.
Date

12852 Park One Drive • Sugar Land, Texas 77478
281 240-8828 • Fax 281 240-8448
www.stillmeadow.com
10161 Harwin, Suite 150 • Houston, Texas 7703
713 272-7444 • Fax 713 272-7501

STILLMEADOW
INCORPORATED

PROTOCOL FOR STUDY 6421-01

Study Title: *Daphnia magna* STATIC 48-HOUR ACUTE TOXICITY TEST

Test Substance: Miller 6064

Test Facility: STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, Texas 77479

Approved: _____ 29 June 01
Abigail Campbell, B.S.
Study Director
STILLMEADOW, Inc.
Date

Approved: _____ 5 June 2001
Elizabeth J. Sabol, B.A., B.S.Ed
Vice President
STILLMEADOW, Inc.
Date

Reviewed: _____ 5 June 2001
Vicki Crutchfield, R.Q.A.P.
Director, Quality Assurance Unit
STILLMEADOW, Inc.
Date

Sponsor: Miller Chemical & Fertilizer Corporation
PO Box 333
Radio Road
Hanover PA 17331

Sponsor Representative
Mandava Associates
1730 M Street, Suite 906
Washington, D.C. 20036-4510

Approved: _____ 27 June 01
N. Bhushan Mandava, Ph.D.
Date

Appendix C: Protocol and Amendment

PROTOCOL FOR STUDY 6421-01

A. GENERAL

1. Study Title: *Daphnia magna* STATIC 48-HOUR ACUTE TOXICITY TEST

2. Purpose: To assess the toxicity of the test substance to the marine invertebrate *Daphnia magna* through a 48-hour test.

3. Regulatory Compliance: This study will be conducted according to OPPTS 850.1010, which is intended to meet testing requirements of both FIFRA (7 U.S.C. 136, *et seq.*) and TSCA (15 U.S.C. 2601).

This study will be conducted in compliance with Good Laboratory Practice Standards:
1. EPA FIFRA: 40 CFR 160
2. OECD: C(81)30 (Final)
3. Japanese MAFF

All methods can be found in STILLMEADOW, Inc. Standard Operating Procedures (SOPs).

4. Quality Assurance: The Protocol is reviewed by the Quality Assurance Unit (QAU). The study information will be entered into the Master Schedule. The study will be inspected at least once during its progress. Further inspections may be scheduled as needed to ensure the integrity of the study. Any deviations from SOPs, the Protocol, or Good Laboratory Practice Standards will be immediately reported to the Study Director and Management. The report will be audited and a statement prepared and signed which shall specify the dates inspections were made and findings reported to Management and to the Study Director.

5. Test Substance: Miller 6064. Test substance identification should include the name, batch number, and purity. Information regarding safety, stability, storage conditions, and disposal should also be provided by the Sponsor. The Sponsor assumes responsibility for purity and stability determinations.

6. Proposed Schedule: Testing will begin within approximately three weeks of receipt of the test substance and authorization to conduct the study.

Proposed Start Date: 13 Jun 01
Proposed End Date: 11 Jul 01

7. Study Director: Abigail Campbell, B.S.

A. GENERAL (cont.)

8. Experimental Summary: Test considerations will be determined by a preliminary range finding test which will give an approximate value for the 24 and 48 hr EC_{50} for the test substance. The test substance concentrations chosen will be administered to the test system, *Daphnia magna*, in reconstituted water. All definitive test concentrations will contain 2 replicates with 10 daphnids each. Two control containers will contain 10 test organisms in reconstituted water and no test substance. Dissolved oxygen, temperature, and pH will be measured and recorded in each treatment and the control at test initiation and termination. Observations for immobilization in each test chamber will be made daily. The test will be terminated after 48±1 hours of exposure.

The test will be considered valid if the control immobilization does not exceed 10 percent at 48 hours.

9. Protocol Amendments: Any alteration in the Protocol will be justified, approved by the Study Director, and recorded in writing.

10. Sponsor Audits: The Sponsor may send an authorized representative to inspect the test system and/or data on the STILLMEADOW, Inc. premises during normal working hours.

Appendix C: Protocol and Amendment

B. EXPERIMENTAL DESIGN

1. Organism

a.	Species:	*Daphnia magna*
b.	Justification of Species:	One of the daphnid species specified in the OPPTS regulations.
c.	Age:	Less than 24 hours old at test initiation.
d.	Number:	The range-finding test will use 5 daphnids for each concentration and the control. The definitive test will use 20 daphnids for each concentration and the control (2 replicates each containing 10 daphnids).
e.	Source:	*Daphnia magna* will be obtained from the STILLMEADOW, Inc. culture laboratory. Cultures which should not be used for testing include: cultures containing ephippia, if adults do not produce young before day 12, if more than 20 percent of the culture stock die during the two days preceding the test, or if adults in the culture do not produce an average of at least three young adult per day over a 7-day period prior to test.
f.	Acclimation:	Brood daphnids will be maintained in 100 percent dilution water at the test temperature for at least 48 hours prior to the start of the test.
g.	Identification:	Organisms will be labeled by study number, lot number, date, and number of organisms.

2. Animal Husbandry

a.	Test Medium:	Reconstituted water.
b.	Test Room:	Testing will be conducted in a light/temperature-controlled cabinet.
c.	Temperature:	Test temperature will be 20 ± 2°C.
d.	Photoperiod:	16-hours light and 8-hours dark.
e.	Test Chambers:	Test containers will be 250 mL beakers. Test containers will be loosely covered to reduce the loss of test solution or dilution water due to evaporation and to minimize the entry of dust or other particles into the solutions.
f.	Dissolved Oxygen Concentrations:	Test containers will not be aerated, but dissolved oxygen level for the dilution water at test initiation will 60-105% saturation.
g.	Food:	A variety of foods (e.g. unicellular green algae) is adequate for daphnid culture. However, the organisms will not be fed during the test.

3. Contaminants: No contaminants are expected during the study that are known to be capable of interfering with the purpose or conduct of the study.

Appendix C: Protocol and Amendment

B. EXPERIMENTAL DESIGN (cont.)

4. Pretest Preparation

a. Test Substance Receipt: Test substances will be supplied by the Sponsor in appropriately sized glass containers sealed and delivered to STILLMEADOW, Inc. Samples will be stored according to the Sponsor's instructions until prepared for testing.

b. Range-finding: A preliminary range-finding test will be conducted with several concentrations of the test substance to obtain an approximate value for the 24 and 48 hour EC_{50} for the test substance. Concentrations for the definitive test will be chosen from this data.

c. Test Substance Preparation: The test substance is insoluble in water and will be administered using an appropriate solvent (DMF, ethanol, methanol, etc.) as weight/volume concentrations. A solvent control will be included in the test design. The test substance dilutions will be prepared on the day of treatment.

d. Route of Administration: The test substance will be administered to the test system at test initiation by introduction to the test containers.

e. Reason for Route of Administration: Specified by the cited guidelines for evaluation of the toxicity potential of a test substance.

f. Preparation of Test System: The organisms will be randomized into cups containing holding water. Each cup will contain a maximum of five daphnids. Each test concentration will consist of two replicates of ten daphnids per replicate.

g. Control Group: Two replicates containing ten daphnids will not have test substance added and will be considered the control. This control will be used to demonstrate the condition of the test population.

5. Test Substance Administration

a. Dosing Concentrations: A preliminary range-finding test will be conducted with at least five concentrations of the test substance to obtain an approximate EC_{50} value for the test substance. The concentrations selected in a geometric series will be between a factor of 1.5 and 2.0.

Five test concentrations chosen from the range-finding data will be prepared on the day of test initiation.

b. Initial Measurements: Dissolved oxygen, temperature, and pH of the control and treated containers will be measured and recorded at test initiation.

Appendix C: Protocol and Amendment

B. EXPERIMENTAL DESIGN (cont.)

6. Observations:

a. Biological Monitoring: Containers will be inspected at 24 and 48 hours for immobility. In addition to immobility, any abnormal behavior or appearance will be recorded.

b. Chemical and Physical Monitoring: At a minimum, the following measurements will be made at test termination: dissolved oxygen, temperature, and pH of the control and treated containers.

7. Test Duration: The test will be terminated at the end of 48 ± 1 hours.

8. Evaluation of Results: The test will be considered valid if the control immobilization does not exceed 10 percent at 48 hours.

The 24 and 48 hour EC_{50} values and their respective 95 percent confidence limits will be determined using a linear regression model. Several models are available for EC_{50} determination: Probit, Trimmed Spearman-Karber, and Binomial. The most appropriate model will be selected for the determination if a dose response is exhibited in the study.

9. Test Substance Accountability: A comprehensive inventory of test substance received and used will be kept. The test substance container(s) will be weighed when received at this facility, and a record of all test substance use will be maintained. Test substance and test substance dosing solutions will be stored in the original containers, or in the equivalent thereof, or in glass containers with Teflon-lined caps.

10. Disposal of Unused Test Substance: Unused test substance will be disposed of at the Sponsor's expense after the termination of the study. STILLMEADOW, Inc. will retain a reserve sample.

11. Safety Precautions: General safety precautions required by laboratory SOPs will be followed. The Sponsor will supply basic toxicity data on the test substance to be used. However, since the toxicity of test substances is often not well characterized, this laboratory will be conservative in setting safety procedures. The Sponsor or Sponsor's Representative shall be notified of any exposures requiring a physician's examination or care.

C. DATA MANAGEMENT

1. Records:

The following records will be maintained during the study and transferred to the STILLMEADOW, Inc. archives upon study termination.

a. Protocol and Protocol Amendments (if any).
b. Final report and amendments (if any).
c. Study correspondence.
d. Test culture data.
e. Test substance receipt, identification as supplied by Sponsor, preparation, administration, and disposition.
f. Range-finding data and results.
g. Initial and terminal measurements for dissolved oxygen, temperature, and pH of the control and treated containers.
h. Daily counts for each container.
i. Other pertinent data.

2. Data Storage:

All raw data and a reserve sample of the test substance will be retained at STILLMEADOW, Inc. for a period of at least five years..

3. Data Reporting:

The final report will include all data as described in the Good Laboratory Practice Standards, including:

a. Statement from the Quality Assurance Unit.
b. Signature of the Study Director.
c. A GLP Compliance Statement signed by the Study Director.
d. Names of scientific personnel involved in the study.
e. Dates of study initiation and termination.
f. Identification, description, preparation, and storage of the test substance.
g. All pertinent culture information, preparation of test medium, test conditions, dosing information, and observation methods.
h. Initial and terminal data for dissolved oxygen, temperature, and pH of the control and treated containers.
i. Daily counts for each container and mean number of immobile organisms per treatment.
j. Determination of the validity of the test based on the control data.
k. The 24 and 48 hour EC_{50} values and their respective 95 percent confidence limits.
l. Evaluation of results.
m. A reference to this Protocol.

4. Report Submission:

A report will be submitted after termination of the laboratory portion of the study.

Appendix D: Certificate of Analysis

MILLER CHEMICAL & FERTILIZER CORPORATION
P.O. BOX 333, 120 RADIO ROAD
HANOVER, PENNSYLVANIA 17331 U.S.A.
TELEPHONE: 717-633-8821
FAX NO.: 717-632-4561

1/18/01

CERTIFICATE OF ANALYSIS

Product Name: MILLER 6064
Quantity Produced: 7.4 kilograms
Batch Number: 150386
Date of Formulation: 12/13/00

Appearance: Yellow to Amber Liquid
Odor: Terpenic
Moisture: Negligible (non-aqueous)
Specific Gravity: 0.92 - 0.94 @ 20c
Viscosity @ 20c: 100 to 500 cps
pH(1% solution): 6.0 to 8.0
Solubility in Water: Insoluble (Emulsifies)
Flash Point: over 200 f
Purity: 100%

Signature:

Name: Andrew G. Smith
Title: Compliance Officer

11/23/2003

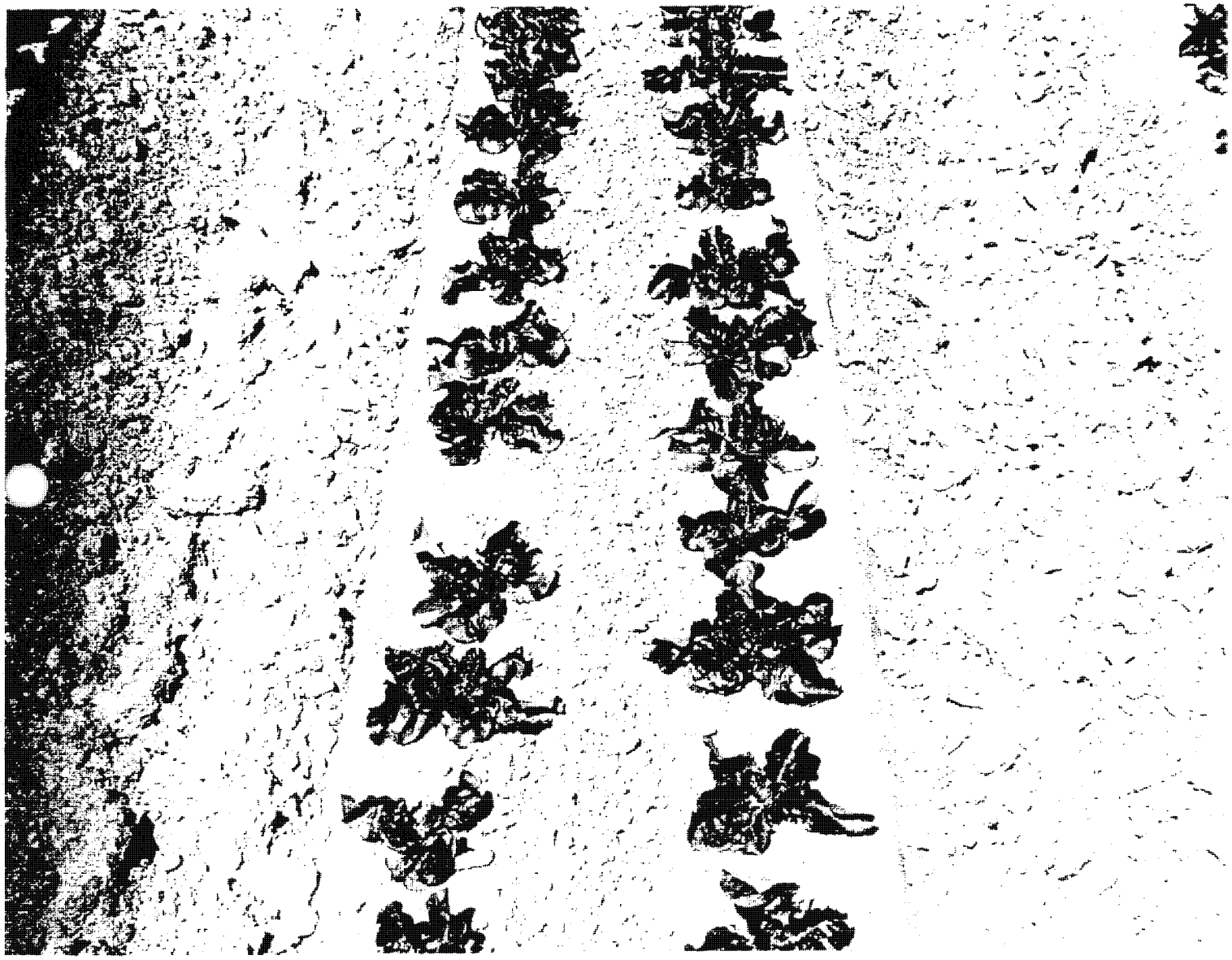
Grower: Salyer Yuma Farm - Dickson Ranch, Bard, CA
Cooperator: Soil Serve - Yuma, AZ
Ground rig, soil applied Kerb on Lettuce

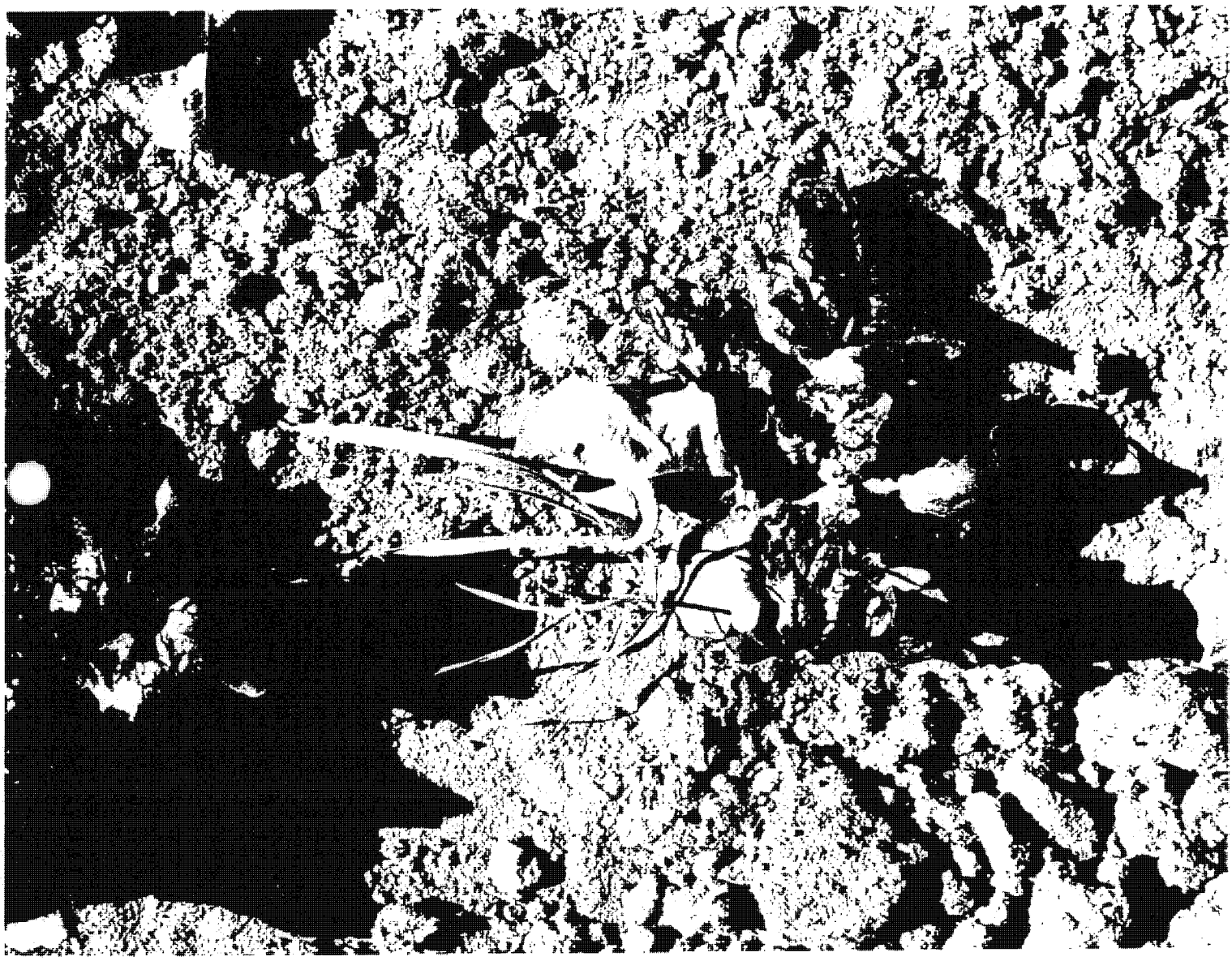
To evaluate if Sustain, soil applied adjuvant will increase efficacy of Kerb pre-emergent herbicide on fall lettuce.

Block #4 - 17 acres
First 19 beds sprayed without any adjuvant (west end of field)
Second 12 beds sprayed with adjuvant, Sustain
Rest of field sprayed without adjuvant (east side of field)
Applied on 10/22/03 at 8:00p.m. Applicator: Soil Serve
First weed counts taken on 11/19/03 by Tommy Wildermuth and Jim Tribby
Weeds present: *London rocket, Nettleleaf goosefoot, Shepherdspurse, Mallow, Foxtail, Sowthistle.*

Results:		**West side**	**Sustain**	**East side**
	#1	5	2	4
	#2	3	2	4
	#3	4	3	4
	#4	4	3	4
	#5	2	4	1
% Higher		**29%**	0	**22%**
Based on 10' samples (.0008034 acres).				
Weeds per acre		**22,402.30**	**17,424**	**21,157.70**

Five random counts were conducted in each of the trial areas. 10' was the standard measure decided upon Lettuce had recently been thinned, and cultivated in the furrows and bed tops. The weeds counted were those emerged since thinning, and were not abated during cultivation. Sizes of weeds ranged from codoled size up to 3-4 true leaves.





ATTACHMENT 25

SUSTAIN® in Carrots 2003

FMG Ag Products

SUSTAIN IN CARROTS 2003

GROWER: GREG LEE, MOSEL LAKE, WA

CO-OPERATOR: BARRY KIRKWOOD
WILBUR ELLIS, MOSES LAKE

CROP: CARROTS

APPLICATION:

LOROX 2#/AC.
SUSTAIN 1PT./AC
HASTEN 1PT./AC

SOIL WAS VERY SANDY AND IRRIGATION WAS VERY HEAVY.
GROWER NOTED BETTER WEED CONTROL ON TOUGHER SPECIES AND LONGER CONTROL AS WELL.

08/16/2004 (2004WDM02) Standardized Summary Page 1 of

FMC Ag Products Group

Spartan/Sustain - Tobacco

Project Code: Master Prt #: Discipline: H
Trial Number: 2004WDM02 Location: Nathalie, VA
Cooperator: Carr By: W.D. Martin

Trt No	Treatment Name	Form Amt	Fm Ds	Rate	Rate Unit	Appl Code	Method/ Timing	NIOTA 05/21/2004 P NE PT 7 A TRAN	NIOTA 05/21/2004 P ST PT 7 A TRAN	NIOTA 05/21/2004 P SR PT 7 A TRAN	NIOTA 05/28/2004 P NE PT 14 A TRAN
Parameter Code											
Date											
Flag											
Pest Stage											
Eval											
Size/Eval Type											
AN											
Day/Before After											
Activity											
Trt-Eval Interval											
PRM Data Type											
# Subsamples, Dec.											
1	Spartan	4	F	0.1875	lb ai/A	A	PPBCPR	0.0	0.0	0.0	0.0
2	Spartan	4	F	0.1875	lb ai/A	B	PPBCIC	0.0	0.0	0.0	0.0
3	Spartan	4	F	0.1875	lb ai/A	A	PPBCPR	0.0	0.0	0.0	0.0
3	Sustain			1	qt pr/A	A	PPBCPR				
4	Spartan	4	F	0.1875	lb ai/A	B	PPBCIC	0.0	0.0	0.0	0.0
4	Sustain			1	qt pr/A	B	PPBCIC				
5	Spartan	4	F	0.25	lb ai/A	A	PPBCPR	0.0	0.0	0.0	0.0
6	Spartan	4	F	0.25	lb ai/A	B	PPBCIC	0.0	0.0	0.0	0.0
7	Spartan	4	F	0.25	lb ai/A	A	PPBCPR	0.0	0.0	0.0	0.0
7	Sustain			1	qt pr/A	A	PPBCPR				
8	Spartan	4	F	0.25	lb ai/A	B	PPBCIC	0.0	0.0	0.0	0.0
8	Sustain			1	qt pr/A	B	PPBCIC				
9	Spartan	4	F	0.3125	lb ai/A	A	PPBCPR	0.0	0.0	0.0	0.0
10	Spartan	4	F	0.3125	lb ai/A	B	PPBCIC	0.0	0.0	0.0	0.0
11	Spartan	4	F	0.3125	lb ai/A	A	PPBCPR	0.0	0.0	0.0	0.0
11	Sustain			1	qt pr/A	A	PPBCPR				
12	Spartan	4	F	0.3125	lb ai/A	B	PPBCIC	0.0	0.0	0.0	0.0
12	Sustain			1	qt pr/A	B	PPBCIC				
13	Untreated							0.0	0.0	0.0	0.0
SD (P=.05)								0.00	0.00	0.00	0.00
tandard Deviation								0.00	0.00	0.00	0.00
CV								0.0	0.0	0.0	0.0

FMC Ag Products Group

Parameter Code							NIOTA	NIOTA	NIOTA	NIOTA
Date							05/21/2004	05/21/2004	05/21/2004	05/28/2004
Flag							P	P	P	P
Pest Stage										
Eval							NE	ST	SR	NE
Size/Eval Type							PT	PT	PT	PT
AN										
Day/Before After							7 A	7 A	7 A	14 A
Activity							TRAN	TRAN	TRAN	TRAN
Trt-Eval Interval										
PRM Data Type										
# Subsamples, Dec.										
Trt No	Treatment Name	Amt	Form Fm Ds	Rate	Rate Unit	Appl Code	Method/ Timing			
Replicate F							0.000	0.000	0.000	0.000
Replicate Prob(F)							1.0000	1.0000	1.0000	1.0000
Treatment F							0.000	0.000	0.000	0.000
Treatment Prob(F)							1.0000	1.0000	1.0000	1.0000

FMC Ag Products Group

Pigweed

Trt No	Treatment Name	Form Amt	Fm Ds	Rate	Rate Unit	Appl Code	Method/ Timing	NIOTA 05/28/2004 P	NIOTA 05/28/2004 P	AMARE 05/28/2004 E	NIOTA 06/04/2004 P
	Parameter Code							NIOTA	NIOTA	AMARE	NIOTA
	Date							05/28/2004	05/28/2004	05/28/2004	06/04/2004
	Flag							P	P	E	P
	Pest Stage										
	Eval							ST	SR	CO	ST
	Size/Eval Type							PT	PT	PT	PT
	AN										
	Day/Before After							14 A	14 A	14 A	21 A
	Activity							TRAN	TRAN	TRAN	TRAN
	Trt-Eval Interval										
	PRM Data Type										
	# Subsamples, Dec.										
1	Spartan	4	F	0.1875	lb ai/A	A	PPBCPR	0.0	0.0	82.5	0.0
2	Spartan	4	F	0.1875	lb ai/A	B	PPBCIC	0.0	0.0	83.8	0.0
3	Spartan	4	F	0.1875	lb ai/A	A	PPBCPR	0.0	0.0	88.8	0.0
3	Sustain			1	qt pr/A	A	PPBCPR				
4	Spartan	4	F	0.1875	lb ai/A	B	PPBCIC	0.0	0.0	90.0	0.0
4	Sustain			1	qt pr/A	B	PPBCIC				
5	Spartan	4	F	0.25	lb ai/A	A	PPBCPR	0.0	0.0	90.0	0.0
6	Spartan	4	F	0.25	lb ai/A	B	PPBCIC	0.0	0.0	90.0	0.0
7	Spartan	4	F	0.25	lb ai/A	A	PPBCPR	0.0	0.0	95.0	0.0
7	Sustain			1	qt pr/A	A	PPBCPR				
8	Spartan	4	F	0.25	lb ai/A	B	PPBCIC	0.0	0.0	97.5	0.0
8	Sustain			1	qt pr/A	B	PPBCIC				
9	Spartan	4	F	0.3125	lb ai/A	A	PPBCPR	0.0	0.0	98.8	0.0
10	Spartan	4	F	0.3125	lb ai/A	B	PPBCIC	0.0	0.0	98.8	0.0
11	Spartan	4	F	0.3125	lb ai/A	A	PPBCPR	0.0	0.0	100.0	0.0
11	Sustain			1	qt pr/A	A	PPBCPR				
12	Spartan	4	F	0.3125	lb ai/A	B	PPBCIC	0.0	0.0	100.0	0.0
12	Sustain			1	qt pr/A	B	PPBCIC				
13	Untreated							0.0	0.0	0.0	0.0
	LSD (P=.05)							0.00	0.00	2.45	0.00
	Standard Deviation							0.00	0.00	1.71	0.00
	CV							0.0	0.0	2.0	0.0
	Replicate F							0.000	0.000	2.182	0.000
	Replicate Prob(F)							1.0000	1.0000	0.1071	1.0000
	Treatment F							0.000	0.000	952.855	0.000
	Treatment Prob(F)							1.0000	1.0000	0.0001	1.0000

FMC Ag Products Group

Trt No	Treatment Name	Form Amt	Fm Ds	Rate	Rate Unit	Appl Code	Method/ Timing	NIOTA 06/04/2004 P SR PT 21 A TRAN	AMARE 06/04/2004 E CO PT 45 A TRAN	NIOTA 06/28/2004 P ST PT 45 A TRAN	NIOTA 06/28/2004 P SR PT 45 A TRAN
	Parameter Code										
	Date										
	Flag										
	Pest Stage										
	Eval										
	Size/Eval Type										
	AN										
	Day/Before After										
	Activity										
	Trt-Eval Interval										
	PRM Data Type										
	# Subsamples, Dec.										
1	Spartan	4	F	0.1875	lb ai/A	A	PPBCPR	0.0	85.0	0.0	0.0
2	Spartan	4	F	0.1875	lb ai/A	B	PPBCIC	0.0	85.0	0.0	0.0
3	Spartan	4	F	0.1875	lb ai/A	A	PPBCPR	0.0	90.0	0.0	0.0
3	Sustain			1	qt pr/A	A	PPBCPR				
4	Spartan	4	F	0.1875	lb ai/A	B	PPBCIC	0.0	90.0	0.0	0.0
4	Sustain			1	qt pr/A	B	PPBCIC				
5	Spartan	4	F	0.25	lb ai/A	A	PPBCPR	0.0	90.0	0.0	0.0
6	Spartan	4	F	0.25	lb ai/A	B	PPBCIC	0.0	93.8	0.0	0.0
7	Spartan	4	F	0.25	lb ai/A	A	PPBCPR	0.0	95.0	0.0	0.0
7	Sustain			1	qt pr/A	A	PPBCPR				
8	Spartan	4	F	0.25	lb ai/A	B	PPBCIC	0.0	98.8	0.0	0.0
8	Sustain			1	qt pr/A	B	PPBCIC				
9	Spartan	4	F	0.3125	lb ai/A	A	PPBCPR	0.0	98.8	0.0	0.0
10	Spartan	4	F	0.3125	lb ai/A	B	PPBCIC	0.0	100.0	0.0	0.0
11	Spartan	4	F	0.3125	lb ai/A	A	PPBCPR	0.0	100.0	0.0	0.0
11	Sustain			1	qt pr/A	A	PPBCPR				
12	Spartan	4	F	0.3125	lb ai/A	B	PPBCIC	0.0	100.0	0.0	0.0
12	Sustain			1	qt pr/A	B	PPBCIC				
13	Untreated							0.0	0.0	0.0	0.0
	LSD (P=.05)							0.00	1.57	0.00	0.00
	Standard Deviation							0.00	1.10	0.00	0.00
	CV							0.0	1.27	0.0	0.0
	Replicate F							0.000	3.600	0.000	0.000
	Replicate Prob(F)							1.0000	0.0226	1.0000	1.0000
	Treatment F							0.000	2355.933	0.000	0.000
	Treatment Prob(F)							1.0000	0.0001	1.0000	1.0000

FMC Ag Products Group

Trt No	Treatment Name	Form Amt	Fm Ds	Rate	Rate Unit	Appl Code	Method/ Timing	AMARE 06/28/2004 E CO PT 45 A TRAN	NIOTA 07/13/2004 P ST PT 60 A TRAN	NIOTA 07/13/2004 P SR PT 60 A TRAN	AMARE 07/13/2004 E CO PT 60 A TRAN
	Parameter Code										
	Date										
	Flag										
	Pest Stage										
	Eval										
	Size/Eval Type										
	AN										
	Day/Before After										
	Activity										
	Trt-Eval Interval										
	PRM Data Type										
	# Subsamples, Dec.										
1	Spartan	4	F	0.1875	lb ai/A	A	PPBCPR	82.5	0.0	0.0	55.0
2	Spartan	4	F	0.1875	lb ai/A	B	PPBCIC	85.0	0.0	0.0	58.3
3	Spartan	4	F	0.1875	lb ai/A	A	PPBCPR	90.0	0.0	0.0	78.3
3	Sustain			1	qt pr/A	A	PPBCPR				
4	Spartan	4	F	0.1875	lb ai/A	B	PPBCIC	90.0	0.0	0.0	77.5
4	Sustain			1	qt pr/A	B	PPBCIC				
5	Spartan	4	F	0.25	lb ai/A	A	PPBCPR	90.0	0.0	0.0	65.0
6	Spartan	4	F	0.25	lb ai/A	B	PPBCIC	92.5	0.0	0.0	77.5
7	Spartan	4	F	0.25	lb ai/A	A	PPBCPR	95.0	0.0	0.0	77.5
7	Sustain			1	qt pr/A	A	PPBCPR				
8	Spartan	4	F	0.25	lb ai/A	B	PPBCIC	98.3	0.0	0.0	75.0
8	Sustain			1	qt pr/A	B	PPBCIC				
9	Spartan	4	F	0.3125	lb ai/A	A	PPBCPR	95.0	0.0	0.0	80.0
10	Spartan	4	F	0.3125	lb ai/A	B	PPBCIC	100.0	0.0	0.0	82.5
11	Spartan	4	F	0.3125	lb ai/A	A	PPBCPR	100.0	0.0	0.0	82.5
11	Sustain			1	qt pr/A	A	PPBCPR				
12	Spartan	4	F	0.3125	lb ai/A	B	PPBCIC	100.0	0.0	0.0	85.0
12	Sustain			1	qt pr/A	B	PPBCIC				
13	Untreated							0.0	0.0	0.0	0.0
	LSD (P=.05)							2.77	0.00	0.00	6.86
	Standard Deviation							1.80	0.00	0.00	4.57
	CV							2.09	0.0	0.0	6.65
	Replicate F							0.302	0.000	0.000	1.175
	Replicate Prob(F)							0.8233	1.0000	1.0000	0.3501
	Treatment F							867.521	0.000	0.000	98.310
	Treatment Prob(F)							0.0001	1.0000	1.0000	0.0001

ATTACHMENT 26

SUSTAIN® with Herbicides on Flue Cured Tobacco.

FMC Ag Products Group 2004 Field Trials.

FMC Ag Products Group

Spartan/Sustain - Tobacco

roject Code: Master Prt #: Discipline: H
Trial Number: 2004WDM02 Location: Nathalie, VA
Cooperator: Carr By: W.D. Martin

Trial Status: INCOMPLETE **Trial Reliability:** H

Objective(s): WEED CONTROL AND CROP PHYTO

Conclusion(s):

TRIAL MANAGEMENT

Trial Number: 2004WDM02 **Trial Type, Category:** HERBICIDE , FIELD
City: NATHALIE **County:** HALIFAX **State:** VA **Country:** USA

Principal Investigator: MARTIN. W.D.
Affiliation: PRIVATE
HALIFAX, VA

Study Director: MARTIN, W.D.
Affiliation: PRIVATE
HALIFAX, VA

STUDY DESCRIPTION

Study Design: RCB **Reps:** 4 **Plot Type:** SMALL PLOT
lot Width/Area, Unit: 30.6, FT **Length, Unit:** 30 , FT
Treated Width/Area, Unit: 7.67, FT **Length, Unit:** 30 , FT
Tillage Type: CONVENTIONAL **Carryover From Trial No.:**

Field Preparation: FIELD WAS DISCED AND BEDDED PRIOR TO APPLICATIONS AND TRANSPLANTING

Fertilizer Applied: 1000 LB/A OF 6-12-18 PRIOR TO PLANTING

Study Description Comments: ALL FIELD PREPARATION AND CULTURAL PRACTICES NORMAL TO TOBACCO PRODUCTION WERE FOLLOWED THROUGH OUT THE SEASON

CROP DESCRIPTION

Multicrop Trial (Y/N): N

Crop 1:TOBACCO, FLUE CURED **Variety:** NC 72 **Planting Date:** 05/14/2004

Planting Method: TRANSPLANT **Depth, Unit:** 4 , IN **Row Spacing, Unit:** 46 , IN

Perennial Age, Unit: , **YEAR**

Field History:

Year Crop	Pesticide	,Type	Year Crop	Pesticide	,Type
	,		,		
	,		,		
	,		,		
	,		,		
	,		,		
	,		,		

08/16/2004 (2004WDM02)

FMC Ag Products Group

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APPLICATION INFORMATION

A

Method/Timing: PPBCPR
Application Date: 04/30/2004
Time of Day: 0130
Air Temp., Unit: 76 , F
% Relative Humidity: 40
Wind Velocity, Unit: 2 , MPH
Wind Direction: SE
Dew Presence (Y/N): N
Soil Temp., Unit: 62 , F
Soil Moisture: Optimum
% Cloud Cover: 20

B

Method/Timing: PPBCIC
Application Date: 04/30/2004
Time of Day: 0130
Air Temp., Unit: 76 , F
% Relative Humidity: 40
Wind Velocity, Unit: 2 , MPH
Wind Direction: SE
Dew Presence (Y/N): N
Soil Temp., Unit: 62 , F
Soil Moisture: Optimum
% Cloud Cover: 20

APPLICATION EQUIPMENT INFORMATION

A

Appl. Equipment: SPRAYER
Spray Pressure, Unit: 35 , PSI
Nozzle Type: FLAT FAN
Nozzle Size: 8003
Nozzle Spacing, Unit: 18 , IN
Nozzles/Row: 2.5
Band Width, Unit: , IN
Swath Width, Unit: 7 , FT
Boom Height, Unit: 24 , IN
Ground Speed, Unit: 3 , MPH
Incorporation Equip.:
Hours to Incorp.:
Incorp. Depth, Unit: 2 , IN
Carrier: WATER
Spray Volume, Unit: 20 , GPA

FMC Ag Products Group

B

Appl. Equipment: SPAYER
Spray Pressure, Unit: 35 , PSI
Nozzle Type: FLAT FAN
Nozzle Size: 8003
Nozzle Spacing, Unit: 18 , IN
Nozzles/Row: 2.5
Band Width, Unit: , IN
Swath Width, Unit: 7 , FT
Boom Height, Unit: 24 , IN
Ground Speed, Unit: 3 , MPH
Incorporation Equip.: PERFECTOR
Hours to Incorp.: 1
Incorp. Depth, Unit: 2 , IN
Carrier: WATER
Spray Volume, Unit: 20 , GPA

Application Comments:

Treatment Comments:

FMC Ag Products Group

Summary Comments:
TOBACCO WAS CULTIVATED ON 6/23/2004 AND LAYBY WAS PERFORMED ON 06/30/2004. BOTH OF THESE OPERATIONS DISRUPTED WEED CONTROL AND SUBSEQUENT GROWTH. PLOT DATA AFTER THESE TWO EVENTS WERE EVALUATED BASED ON SKIP ROWS BESIDE PLOTS. DATA ENTRY WITH A (.) INDICATES NO AREA FOR EVALUATION AS COMPARED TO PLOTS AVAILABLE. GRASS AND WILD RADISH POPULATIONS INCREASED AFTER THESE CULTIVATION EVENTS AND DATA WILL BE REPORTED AT THE 100 DAT AND AFTER HARVEST INTERVALS.

FMC Ag Products Group

SOIL INFORMATION

Soil Analyzed by: A&L LABS

Soil pH : 5.5 **CEC (meq/100g):** 3.1
Buffer pH: 6.9

Phosphorus (P): 139 **Rate Unit:** PPM
Potassium (K) : 82 PPM **% Saturation** **% Composition**
Calcium (Ca) : 311 PPM **% K :** 6.7 **% Sand:** 64
Magnesium (Mg): 67 PPM **% Ca:** 49.5 **% Silt:** 26
% Mg: 17.8 **% Clay:** 10
Organic Matter (%): 1.5 **% H :** 26
ENR (lb/a) :
Texture: SANDY LOAM

RAINFALL INFORMATION

Measurement Type: ACTUAL **Location:** NATHALIE, VA

Seasonal Moisture: MOIST **Rainfall Units:** IN **Distance to Station:** 1 **Unit:** MI

Date	Amt	Remark/Activity
05/01/2004	0.45	
05/02/2004	0.48	
5/03/2004	0.38	
5/16/2004	0.67	
05/19/2004	0.12	
05/25/2004	0.64	
06/04/2004	1.86	
06/22/2004	0.47	
06/23/2004	0.22	
06/25/2004	0.14	
06/26/2004	0.11	
06/27/2004	2.76	
06/28/2004	0.85	
06/30/2004	0.24	
07/04/2004	1.65	
07/10/2004	0.26	
07/11/2004	1.32	
07/14/2004	0.15	
07/18/2004	0.18	
07/21/2004	0.20	
07/25/2004	0.62	
07/26/2004	1.94	
07/30/2004	0.52	

ATTACHMENT 27

Miller 6064

Rice Herbicide Retention Study.

Sills Ag Consulting Group
Summer 2001.

To: Miller Chemical & Fertilizer Co.
Michael D. Fiery

From: Dave Sills, Sills Ag Consulting, Inc., Researcher

Reported Study: Miller 6064 Rice Herbicide Retention

Date of Study: Summer, 2001

Goal of Study

- To evaluate soil retention performance of Miller 6064 when tank mixed with California registered pre-flood rice herbicides.

Applications, Rates, and Conditions of Study

This study was designed to provide data establishing the chronology (days to flooding after treatment) using Miller 6064 to stabilize pre-flood herbicides and thereby improving both efficacy and crop safety.

A 1.5 acre rice station was constructed specifically for this study. This rice station was located at Sopwith Farms located in south Sutter County, 25 miles north of Sacramento. Three basins were constructed within the station in order to provide the timeline of initial flood. That timeline was to flood basin 1 @ 24 hrs after treatment, basin 2 @ 72 hrs after treatment, and basin 3 @ 120 hrs after treatment.
Within basin no. 3, one pass of drill seeded rice was planted pretreatment and preflood. The balance of the rice station was water seeded with pre-soaked seed, as is the common

practice in commercial rice production. The following information will be divided into 2 sections; water-seeded applications and drill-seeded applications.

Water Seeded Applications

All three basins of water seeded rice had the following pre plant, pre-initial flood applications:

1. Ordram 8E @ 5 pints per acre.
2. Abolish 8E @ 4 pints per acre.
3. Ordram 8E @ 5 pints and Abolish 8E @ 3 pints per acre tank mixed.
4. Ordram 8E @ 5 pints plus Miller 6064 @ 1 pint per acre.
5. Abolish 8E @ 4 pints plus Miller 6064 @ 1 pint per acre.
6. Ordram 8E @ 5 pints and Abolish 8E @ 3 pints per acre plus Miller 6064 @ 1 pint per acre tank mixed.

Drill Seeded Applications (third basin)

1. Prowl @ 2.4 pints per acre.
2. Prowl @ 5 pints per acre.
3. Prowl @ 2.4 pints plus Miller 6064 @ 1 pint per acre.
4. Prowl @ 5 pints plus Miller 6064 @ 1 pint per acre.
5. Prowl @ 2.4 pints and Ordram 8E @ 5 pints per acre.
6. Prowl @ 2.4 pints, Ordram 8E @ 5 pints and Miller 6064 @ 1 pint per acre.

Results and Conclusions (Water Seeded Basins)

Miller 6064 does exhibit value at retention of soil applied herbicides. In this study, efficacy of volatile and movement oriented materials was certainly improved and/or enhanced. In regard to volatility, specifically Ordram 8E, performance begins to fall off beyond the 3-day period. The same is true regarding Abolish 8E. The consistently superior tank mix is Ordram 8E @ 5 pints, Abolish8E @ 3 pints with Miller 6064 @ 1 pint as a tank mix. Under this mix, weed control was excellent and persistent, even when flushed several times. The only weed to escape this combination was red stem (purple ammannia). This weed is easily controlled with post emergent broadleaf herbicides.

In the plots where no Miller 6064 was included, weed control was inconsistent.

Results and Conclusions (Drill Seeded Basin)

The central focus of this basin was to evaluate efficacy and safety of Prowl and Ordram8E when applied post plant, but pre flush, directly to the soil surface. Drill seeding depth was 1" to 1.5" from soil surface and herbicide layer.

The Prowl @ 2.4 pints and Miller 6064 @ 1 pint per acre did not cause phytotoxicity. Rice emergence was normal and growth unrestricted. Prowl @ 5 pints with Miller 6064 @ 1 pint per acre did, however, cause significant stand reduction. (Too much total A.I. to hold to the surface?)

Early observations of the Prowl and Miller 6064 combinations indicated similar efficacy to that of the Prowl @ 2.4 pints, Miller 6064 @ 1 pint and Ordram 8E @ 5 pints. Observations at 3 weeks, however, demonstrated that the addition of the 5-pint rate of Ordram 8E to the 6064/Prowl mix gave much longer and sustained performance that made the addition of the Ordram worth the extra expense. The addition of Abolish caused a stand reduction.

Recommendations and Conclusions

This is a successful project. The addition of Miller 6064 to soil applied rice herbicides, under water seeded conditions, is an excellent way to insure stability and performance of a grower's investment and a relatively inexpensive insurance program.

Drill seeded usage of Miller 6064 to keep Prowl tight to the soil surface needs more research and repetition before I would recommend marketing. This potential is not just improvement of efficacy, but centrally, the elimination of phytotoxicity. This safety consistency must be proven and reliable in order to avoid liability issues.

For the year 2002, a supplemental label for Nu-film P should be pursued for soil surface applied rice herbicides in water seeded rice if Miller 6064 is not registered. From a marketing perspective, the program could be promoted this winter at PCA and grower meetings. There are several attractive advantages to the grower that can be exposed:

1. Reduced water hold due to pre flood advantage.
2. Elimination of aerial application cost(s).
3. Grower control over applications.
4. Tank mix flexibility.
5. Possible elimination of steel ground rig applications.

The point still must be emphasized that applications, even with the Miller 6064, should be made as close as is functional, to the initial head of water. Miller 6064 will reduce volatility and leaching of herbicides but not eliminate them entirely.

ATTACHMENT 28

Nu-Film 17, Pinolene B and SUSTAIN®.

Evaluate the Efficacy of Different Surfactants with Pristine for Powdery Mildew on Squash.

Glades Crop Care, Inc. Spring 2004.

GLADES CROP CARE, INC.
Agricultural Consultants

OFFICE (561) 746-3740
FAX (561) 746-3775
www.gladescropcare.com

949 TURNER QUAY
JUPITER, FLORIDA 33458

"Established 1972"

Evaluate the Efficacy of Different Surfactants with Pristine for Powdery Mildew on Squash

Sponsor: Miller Chemical

Spring, 2004

Glades Crop Care Internal No. 04-40

July, 2004

Objective

To determine the efficacy of different surfactants with Pristine for powdery mildew on squash.

General Trial Information

- **Location:** Kitching Creek Research Facility, Hobe Sound, Florida
- **Soil Type:** EauGallie Fine Sand
- **Crop/Variety:** Squash "Medallion"
- **Planting Date:** April 28, 2004

Trial Design	complete randomized block
Plot Size:	25 row feet
Replications:	4
Output Per Treated Acre:	26.4 to 36.2 GPA
Production System:	raised bed with white on black plastic mulch and drip irrigation

General Treatment Information

Treatments	Rates	Application dates
1. Untreated check	-	-
2. Pristine	12.5 oz/A	May 25, June 2, 8 & 15, 2004.
3. Pristine + Nu-Film 17	12.5 oz/A 8 fl oz/A	
4. Pristine + Pinolene B	12.5 oz/A 1 pt/A	
5. Pristine + Sustain	12.5 oz/A 8 fl oz/A	

Evaluations

Disease Evaluations: Weekly observations for disease incidence and severity were made. Twenty-five leaves per plot were examined. The following rating scale was used to evaluate severity. The evaluation is based on leaf area showing disease symptoms.

0 - 1 = 1-5%
1 = 6-15%
2 = 16-30%
3 = 31-50%
4 = 51-75%
5 = 76-100%

All data collected was entered into FieldPro for statistical analysis.

Results and Discussion

All treatments showed better powdery mildew control then the untreated. Until June 21 evaluation, treatments 2, 4 and 5 provided the best control. Pristine combined with Pinolene B provided the best control.

Powdery Mildew Incidence per 25 Leaves Means					
Trt	**May 25**	**Jun 1**	**Jun 7**	**Jun 14**	**Jun 21**
1	0.0	3.3	21.3	24.5	24.8
2	0.0	0.0	3.8	5.3	18.3
3	0.0	1.8	12.0	12.5	16.3
4	0.0	0.0	2.0	2.5	11.3
5	0.0	1.0	3.8	3.5	13.5

Powdery Mildew Severity per 25 Leaves Means					
Trt	**May 25**	**Jun 1**	**Jun 7**	**Jun 14**	**Jun 21**
1	0.00	0.13	1.33	2.55	4.79
2	0.00	0.00	0.15	0.21	1.51
3	0.00	0.07	0.59	0.71	1.31
4	0.00	0.00	0.08	0.10	0.70
5	0.00	0.04	0.15	0.14	1.07

No phytotoxicity was observed in any of the treatments.

Maintenance Applications

Applications for control of whiteflies and worms included Actara, Avaunt, M-Pede, and Assail.

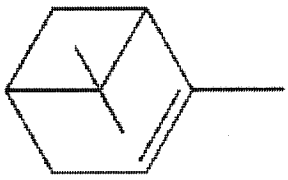
ATTACHMENT 29

α-Pinene and its Insecticidal Properties

« Previous Compound Next Compound »

Compound - alpha-pinene

Discuss this Compound



2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene

Formula:	C10H16
CAS#:	80-56-8
MW:	136.23

[MS spectra]

Species utilize 2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene in its chemical communication system

For help just move the cursor over the abbreviations in green or the red text below

Coleoptera, Chrysomelidae		
Leptinotarsa decemlineata	K	Colorado potato beetle
Coleoptera, Cleridae		
Thanasimus dubius	A	
Thanasimus formicarius	A	European red-bellied clerid
Thanasimus undatulus	A	
Thanasimus undatulus	K	
Coleoptera, Curculionidae		
Anthonomus grandis	A	Cotton boll weevil
Hylobius abietis	A	Large pine weevil
Smicronyx fulvus	A	Sunflower seed weevil
Coleoptera, Nitidulidae		
Epuraea pygmaea	A	
Coleoptera, Scolytidae		
Conophthorus coniperda	K	White pine cone beetle
Dendroctonus frontalis	A	Southern pine beetle
Dendroctonus pseudotsugae	K	Douglas fir beetle
Dendroctonus simplex	A	Eastern larch beetle
Dendroctonus valens	A	Red turpentine beetle
Dryocoetes autographus	K	
Gnathotrichus retusus	A	

Gnathotrichus retusus	K	
Gnathotrichus sulcatus	K	
Hylastes longicollis	A	
Hylastes macer	A	Root-feeding bark beetle
Hylastes nigrinus	A	
Hylastes nigrinus	K	
Hylastes ruber	K	
Hylurgops porosus	A	
Hylurgops subcostulatus	A	
Ips caelatus	A	
Ips latidens	A	
Ips perturbatus	A	Northern spruce engraver
Ips pini	A	Pine engravers
Ips typographus	P	Spruce bark beetle
Phloeotribus scarabaeoides	A	Olive bark beetle
Pseudohylesinus grandis	K	Silver fir beetle
Pseudohylesinus nebulosus	K	
Scolytus ventralis	K	Fir engraver
Tomicus piniperda	K	Pine shoot beetle
Trypodendron lineatum	K	Striped ambrosia beetle
Trypodendron lineatum	P	Striped ambrosia beetle
Coleoptera, Tenebrionidae		
Artystona sp.	Al	
Coleoptera, Trogositidae		
Temnochila chlorodia	A	
Diptera, Dolichopodidae		
Medetera signaticornis	A	
Homoptera, Aphididae		
Megoura viciae	P	Vetch aphid
Isoptera, Rhinotermitidae		
Reticulitermes flavipes	Al	Eastern subterranean termite
Reticulitermes santonensis	Al	
Isoptera, Termitidae		
Nasutitermes costalis	P	
Nasutitermes ephratae	P	
Nasutitermes nigriceps	P	
Nasutitermes princeps	P	
Nasutitermes rippertii	P	
Velocitermes velox	Al	
Velocitermes velox	P	
Coleoptera, Cerambycidae, Lamiinae		
Monochamus alternatus	K	Japanese pine sawyer
Diptera, Tephritidae, Dacinae		
Bactrocera oleae	A	Olive fruit fly
Bactrocera oleae	P	Olive fruit fly
Lepidoptera, Noctuidae, Amphipyrinae		
Spodoptera frugiperda	K	Fall armyworm
Lepidoptera, Papilionidae, Papilioninae		

Papilio demodocus	P	Citrus swallowtail butterfly
Lepidoptera, Pieridae, Pierinae		
Pieris melete	P	White butterfly
Pieris napi japonica	P	
Hymenoptera, Formicidae, Myrmicinae, Myrmicariini		
Myrmicaria natalensis	Al	
Thysanoptera, Phlaeothripidae, Phlaeothripinae, Haplothripini		
Dolichothrips sp.	P	

Free plugin Chime is required to view the molecule in 3D

Page created on 4-November-2004

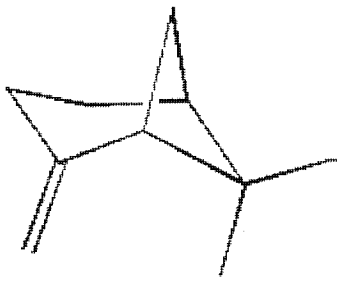
ATTACHMENT 30

β-Pinene and its Insecticidal Properties

« Previous Compound Next Compound »

Compound - beta-pinene

Discuss this Compound



6,6-Dimethyl-2-methylenebicyclo [3.1.1]heptane

Formula:	C10H16
CAS#:	127-91-3
MW:	136.24

[MS spectra]

Species utilize 6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane in its chemical communication system

For help just move the cursor over the abbreviations in green or the red text below

Coleoptera, Cleridae		
Thanasimus formicarius	A	European red-bellied clerid
Thanasimus undatulus	K	
Coleoptera, Curculionidae		
Smicronyx fulvus	A	Sunflower seed weevil
Coleoptera, Nitidulidae		
Epuraea pygmaea	A	
Coleoptera, Scolytidae		
Dendroctonus pseudotsugae	K	Douglas fir beetle
Dendroctonus valens	A	Red turpentine beetle
Dryocoetes autographus	K	
Gnathotrichus retusus	A	
Gnathotrichus retusus	K	
Gnathotrichus sulcatus	K	
Hylastes longicollis	A	
Hylastes macer	A	Root-feeding bark beetle
Hylastes nigrinus	A	
Hylastes nigrinus	K	
Hylastes ruber	K	
Hylurgops palliatus	K	

Hylurgops porosus	A	
Hylurgops subcostulatus	A	
Ips latidens	A	
Ips pini	A	Pine engravers
Ips typographus	P	Spruce bark beetle
Phloeotribus scarabaeoides	A	Olive bark beetle
Pseudohylesinus grandis	K	Silver fir beetle
Pseudohylesinus nebulosus	K	
Scolytus ventralis	K	Fir engraver
Tomicus piniperda	K	Pine shoot beetle
Trypodendron lineatum	K	Striped ambrosia beetle
Coleoptera, Trogositidae		
Temnochila chlorodia	A	
Diptera, Dolichopodidae		
Medetera signaticornis	A	
Heteroptera, Scutelleridae		
Hotea gambiae	Al	
Homoptera, Aphididae		
Megoura viciae	P	Vetch aphid
Isoptera, Rhinotermitidae		
Reticulitermes flavipes	Al	Eastern subterranean termite
Reticulitermes santonensis	Al	
Isoptera, Termitidae		
Nasutitermes costalis	P	
Nasutitermes rippertii	P	
Velocitermes velox	Al	
Coleoptera, Cerambycidae, Lamiinae		
Monochamus alternatus	K	Japanese pine sawyer
Lepidoptera, Pieridae, Pierinae		
Pieris melete	P	White butterfly
Pieris napi japonica	P	
Hymenoptera, Formicidae, Myrmicinae, Myrmicariini		
Myrmicaria natalensis	Al	
Thysanoptera, Phlaeothripidae, Phlaeothripinae, Haplothripini		
Dolichothrips sp.	P	

Free plugin Chime is required to view the molecule in 3D

ATTACHMENT 31

α-Pinene Polymer

PAN Pesticide Database

Identification, Toxicity, Use, Water Pollution Potential, Ecological Toxicity and Regulatory Information

PAN Pesticides Database - Chemicals

Alpha-pinene polymer - Identification, toxicity, use, water pollution potential, ecological toxicity and regulatory information

Note: See Working with the Information on this Page section below for important notes about this data.

Chemical ID	Identifying information, including synonyms, ID numbers, use type, chemical classification, a link to a list of all products containing this chemical and a list of the top crops this pesticide is used on in California.
Poisoning Symptoms	Signs and symptoms of poisoning, first aid, and links to treatment information for this chemical.
Toxicity	Toxicity to humans, including carcinogenicity, reproductive and developmental toxicity, neurotoxicity, and acute toxicity.
Regulatory	Links to world-wide registration status as well as regulatory information for the U.S. and California.
Water	Water quality standards and physical properties affecting water contamination potential.
Ecotoxicity	Toxicity to aquatic organisms.
Related Chems	List of chemicals in the same family, including breakdown products, salts, esters, isomers, and other derivatives.

Chemical Identification and Use for Alpha-pinene polymer

Top

Basic Identification Information About This Chemical

Chemical Name:	Alpha-pinene polymer
CAS Number:	31393-98-3
U.S. EPA PC Code:	
CA DPR Chem Code:	3996
Use Type:	Insecticide
Chem Class:	Polymer , Polymer

View Related Chemicals

Additional Resources About This Chemical Class and Use Type

See the Global Pesticide Resources page for many additional links.

Other Names for this Chemical

About Chemical Synonyms

03996 (CA DPR Chem Code) , 31393-98-3 (CAS Number) , 31393983 (CAS Number) , 3996 (CA DPR Chem Code) , Alpha-

pinene polymer , Alphapinenepolymer

Signs and Symptoms of Alpha-pinene polymer Poisoning

Top

NOTE! There may be other diseases and chemicals that have similar symptoms.

If you have a poisoning emergency in the United States call 1-800-222-1222. If the victim has collapsed or is unconscious, call 911.

Sorry! No symptoms for this chemical or chemical group are available. See related chemicals for possible additional information.

Toxicity Information for Alpha-pinene polymer

Top

Note: Information for many chemicals is incomplete and may not be fully representative of effects on humans. Why?

Summary Toxicity Information

PAN Bad Actor Chemical [1]	Acute Toxicity [2]	Carcinogen	Cholinesterase Inhibitor	Ground Water Contaminant	Developmental or Reproductive Toxin	Endocrine Disruptor
Not Listed	?	?	No	?	?	?

Indicates high toxicity in the given toxicological category.

Indicates no available weight-of-the-evidence summary assessment. For additional information on toxicity from scientific journals or registration documents, see the "Additional Resources for Toxicity " section of the chemical detail page.

1. **PAN Bad Actors** are chemicals that are one or more of the following: highly acutely toxic, cholinesterase inhibitor, known/probable carcinogen, known groundwater pollutant or known reproductive or developmental toxicant. NOTE! Because there are no authoritative lists of Endocrine Disrupting (ED) chemicals, EDs are not yet considered PAN Bad Actor chemicals.

2. The acute toxicity reported on this page is of the pure chemical ingredient only and may not reflect the acute toxicity of individual pesticide products. To view acute toxicity of individual products, click on 'View Products' link in the 'Chemical Identification' section above.

Additional Resources about the Toxicity of this Chemical

Additional Toxicity Info for this Chemical

See the Global Pesticide Resources page for many additional links.

Detailed Toxicity Information	This Chemical	Parent Chemical
Acute Toxicity [2]	Alpha-pinene polymer	Pinene
WHO Acute Hazard	Not Listed	Not Listed
TRI Acute Hazard	Not Listed	Not Listed
Material Safety Data Sheets	Not Available	Not Available
Acute rating from U.S. EPA product label	No Consensus Value	No Consensus Value
U.S. NTP Acute Toxicity Studies	No NTP Studies	Slightly Toxic
View Studies		
Cholinesterase Inhibitor	No	No

2. The acute toxicity reported on this page is of the pure chemical ingredient only and may not reflect the acute toxicity of individual pesticide products. To view acute toxicity of individual products, click on 'View Products' link in the 'Chemical Identification' section above.

Cancer Information

IARC Carcinogens	Not Listed	Not Listed
U.S. NTP Carcinogens	Not Listed	Not Listed
California Prop 65 Known Carcinogens	Not Listed	Not Listed
U.S. EPA Carcinogens	Not Listed	Not Listed
TRI Carcinogen	Not Listed	Not Listed

Endocrine Disruption

Illinois EPA list	Not Listed	Not Listed
Keith list	Not Listed	Not Listed
Colborn list	Not Listed	Not Listed
Benbrook list	Not Listed	Not Listed

Reproductive and Developmental Toxicity

CA Prop 65 Developmental Toxin	Not Listed	Not Listed
U.S. TRI Developmental Toxin	Not Listed	Not Listed
CA Prop 65 Female Reproductive Toxin	Not Listed	Not Listed
CA Prop 65 Male Reproductive Toxin	Not Listed	Not Listed
U.S. TRI Reproductive Toxin	Not Listed	Not Listed

Chemicals of Special Concern

PAN Bad Actors	Not Listed	Not Listed
PAN Dirty Dozen list	Not Listed	Not Listed

Water Pollution Potential and Criteria for Alpha-pinene polymer

Top

Water Pollution Potential	This Chemical	Parent Chemical

	Alpha-pinene polymer	Pinene
PAN Ground Water Contaminant Rating	Insufficient Data	Insufficient Data

Sorry, no water quality standards or criteria have been established for this chemical by the U.S. or Canadian governments; however, there may be criteria established for related chemicals.

Regulatory Information for Alpha-pinene polymer

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International Regulatory Status	This Chemical	Parent Chemical
Worldwide Registration	Worldwide Registration for: Alpha-pinene polymer	Worldwide registration for: Pinene
Click on link at right to view registration information for different countries -->	Number of countries where this chemical is: Banned, Restricted or Cancelled: 0 Not legal for import: 0	Number of countries where this c Banned, Restricted or Cancell Not legal for import: 0

UNEP Persistent Organic Pollutant (POP)	Not Listed	Not Listed
UNEP Prior Informed Consent Chemical (PIC)	Not Listed	Not Listed
WHO Obsolete Pesticide	Not Listed	Not Listed

U.S. and California Regulatory Status

U.S. EPA Registered	No	No
U.S. EPA Hazardous Air Pollutant	Not Listed	Not Listed
U.S. EPA Minimum Risk Pesticide (25b list)	No	No
CA Registered	No	No
CA Groundwater Contaminant	Not Listed	Not Listed
CA Toxic Air Contaminant	Not Listed	Not Listed

Maximum Tolerance and Residue Levels

Codex Alimentarius (UN FAO Maximum Residue Limits)	Go to web site
U.S. Maximum Tolerance Levels	Go to web site
European Union Maximum Residue Levels	Go to web site

Ecotoxicity for Alpha-pinene polymer

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Note! Information for many chemicals is incomplete and may not be fully representative of effects on the environment. Why? Click on underlined terms for definitions and additional information.

Aquatic Ecotoxicity

All Toxic Effects for Organism Group	
Organism Group	Effects Noted
Sorry, no ecotoxicity data available for this chemical. Try related chemicals.	

Summary of Acute Toxicity for Organism Group
Sorry, no acute ecotoxicity data available for this chemical. Try related chemicals.

Terrestrial Ecotoxicity

We have recently secured funding from the U.S. EPA to incorporate terrestrial ecotoxicity data. Watch this space!

Related Chemicals for Alpha-pinene polymer

Top

CAS Number	Relation	Reason	Chemical Name	Chem Detail	Registration	Symptoms	California Use	Chem Use Type	U.S EPA Reg
80-56-8, 127-91-3, 1330-16-1	Parent	P	Pinene	View	View	View	View	Insecticide	No
31393-98-3	Related	16	Alpha-pinene polymer	View	View	View	View	Insecticide	No
127-91-3	Related	6	beta-Pinene	View	View	View	View	Insecticide	No
68240-09-5	Related	6, 16	Beta-pinene polymer	View	View	View	View	Insecticide	No
25719-60-2	Related	6, 16	Beta-pinene polymer -toluene and xylene alkylated with dicyclopentadiene	View	View	View	View		No
	Related	6, 16	Beta-pinene-maleamide copolymer	View	View	View	View		No
53404-49-2	Related	2	Ethylene glycol ether of pinene	View	View	View	View	Insecticide	No
	Related	16	Polymerized pinene	View	View	View	View	Insecticide	No

Working with the Information on this Page

Click on underlined terms for definitions or go to the Pesticide Tutorial overview page.

Any underlined term with a book icon has additional information.

* Data marked with an asterisk indicates that this chemical is not explicitly listed on the corresponding list. Instead, it belongs to a group of chemicals that IS designated on the list. For example, if an agency assigns a classification of reproductive toxicant to "mercury compounds", that classification is applied to all mercury compounds in the PAN Pesticide database, which are then marked with an asterisk.

To print this page, choose **Print**. To export this data, choose **Save As** 'HTML Source' and open it in Excel or equivalent program.

Citation: S. Orme and S. Kegley.*PAN Pesticide Database*. Pesticide Action Network, North America (San Francisco, CA. 2004).
http:www.pesticideinfo.org.

Related Chemicals for Alpha-pinene polymer

Top

CAS Number	Relation	Reason	Chemical Name	Chem Detail	Registration	Symptoms	California Use	Chem Use Type	U.S. EPA Reg	PAN Bad Actor
80-56-8, 127-91-3, 1330-16-1	Parent	P	Pinene	View	View	View	View	Insecticide	No	Not Listed
31393-98-3	Related	16	Alpha-pinene polymer	View	View	View	View	Insecticide	No	Not Listed
127-91-3	Related	6	beta-Pinene	View	View	View	View	Insecticide	No	Not Listed
68240-09-5	Related	6, 16	Beta-pinene polymer	View	View	View	View	Insecticide	No	Not Listed
25719-60-2	Related	6, 16	Beta-pinene polymer -toluene and xylene alkylated with dicyclopentadiene	View	View	View	View		No	Not Listed
	Related	6, 16	Beta-pinene-maleamide copolymer	View	View	View	View		No	Not Listed
53404-49-2	Related	2	Ethylene glycol ether of pinene	View	View	View	View	Insecticide	No	Not Listed
	Related	16	Polymerized pinene	View	View	View	View	Insecticide	No	Not Listed

Working with the Information on this Page

Click on underlined terms for definitions or go to the Pesticide Tutorial overview page.

Any underlined term with a book icon has additional information.

* Data marked with an asterisk indicates that this chemical is not explicitly listed on the corresponding list. Instead, it belongs to a group of

chemicals that IS designated on the list. For example, if an agency assigns a classification of reproductive toxicant to "mercury compounds", that classification is applied to all mercury compounds in the PAN Pesticide database, which are then marked with an asterisk.

To print this page, choose **Print**. To export this data, choose **Save As** 'HTML Source' and open it in Excel or equivalent program.

Related Chemicals for Alpha-pinene polymer

Top

CAS Number	Relation	Reason	Chemical Name	Chem Detail	Registration	Symptoms	California Use	Chem Use Type	U.S EPA Reg
80-56-8, 127-91-3, 1330-16-1	Parent	P	Pinene	View	View	View	View	Insecticide	No
31393-98-3	Related	16	Alpha-pinene polymer	View	View	View	View	Insecticide	No
127-91-3	Related	6	beta-Pinene	View	View	View	View	Insecticide	No
68240-09-5	Related	6, 16	Beta-pinene polymer	View	View	View	View	Insecticide	No
25719-60-2	Related	6, 16	Beta-pinene polymer -toluene and xylene alkylated with dicyclopentadiene	View	View	View	View		No
	Related	6, 16	Beta-pinene-maleamide copolymer	View	View	View	View		No
53404-49-2	Related	2	Ethylene glycol ether of pinene	View	View	View	View	Insecticide	No
	Related	16	Polymerized pinene	View	View	View	View	Insecticide	No

Working with the Information on this Page

Click on underlined terms for definitions or go to the Pesticide Tutorial overview page.

Any underlined term with a book icon has additional information.

* Data marked with an asterisk indicates that this chemical is not explicitly listed on the corresponding list. Instead, it belongs to a group of chemicals that IS designated on the list. For example, if an agency assigns a classification of reproductive toxicant to "mercury compounds", that classification is applied to all mercury compounds in the PAN Pesticide database, which are then marked with an asterisk.

To print this page, choose **Print**. To export this data, choose **Save As** 'HTML Source' and open it in Excel or equivalent program.

ATTACHMENT 32

β-Pinene Polymer

PAN Pesticide Database

Identification, Toxicity, Use, Water Pollution Potential, Ecological Toxicity and Regulatory Information

PAN Pesticides Database - Chemicals

Beta-pinene polymer - Identification, toxicity, use, water pollution potential, ecological toxicity and regulatory information

Note: See Working with the Information on this Page section below for important notes about this data.

Chemical ID	Identifying information, including synonyms, ID numbers, use type, chemical classification, a link to a list of all products containing this chemical and a list of the top crops this pesticide is used on in California.
Poisoning Symptoms	Signs and symptoms of poisoning, first aid, and links to treatment information for this chemical.
Toxicity	Toxicity to humans, including carcinogenicity, reproductive and developmental toxicity, neurotoxicity, and acute toxicity.
Regulatory	Links to world-wide registration status as well as regulatory information for the U.S. and California.
Water	Water quality standards and physical properties affecting water contamination potential.
Ecotoxicity	Toxicity to aquatic organisms.
Related Chems	List of chemicals in the same family, including breakdown products, salts, esters, isomers, and other derivatives.

Chemical Identification and Use for Beta-pinene polymer

Top

Basic Identification Information About This Chemical

Chemical Name:	Beta-pinene polymer
CAS Number:	68240-09-5
U.S. EPA PC Code:	
CA DPR Chem Code:	3998
Use Type:	Insecticide
Chem Class:	Polymer , Polymer

View Related Chemicals

Additional Resources About This Chemical Class and Use Type

See the Global Pesticide Resources page for many additional links.

Historical Use of this Chemical

Top five crops and sites for this pesticide in California

Wine Grapes | Tomatoes for Processing | Almonds | Table and Raisin Grapes | Strawberries
View All Crops and Sites

Other Names for this Chemical
About Chemical Synonyms

03998 (CA DPR Chem Code) , 3998 (CA DPR Chem Code) , 68240-09-5 (CAS Number) , 68240095 (CAS Number) , Beta-pinene polymer , Betapinene Polymer , Betapinenepolymer

Signs and Symptoms of Beta-pinene polymer Poisoning

Top

NOTE! There may be other diseases and chemicals that have similar symptoms.

If you have a poisoning emergency in the United States call 1-800-222-1222. If the victim has collapsed or is unconscious, call 911.

Sorry! No symptoms for this chemical or chemical group are available. See related chemicals for possible additional information.

Toxicity Information for Beta-pinene polymer

Top

Note: Information for many chemicals is incomplete and may not be fully representative of effects on humans. Why?

Summary Toxicity Information

PAN Bad Actor Chemical [1]	Acute Toxicity [2]	Carcinogen	Cholinesterase Inhibitor	Ground Water Contaminant	Developmental or Reproductive Toxin	Endocrine Disruptor
Not Listed	?	?	No	?	?	?

Indicates high toxicity in the given toxicological category.

? Indicates no available weight-of-the-evidence summary assessment. For additional information on toxicity from scientific journals or registration documents, see the "Additional Resources for Toxicity " section of the chemical detail page.

1. **PAN Bad Actors** are chemicals that are one or more of the following: highly acutely toxic, cholinesterase inhibitor, known/probable carcinogen, known groundwater pollutant or known reproductive or developmental toxicant. NOTE! Because there are no authoritative lists of Endocrine Disrupting (ED) chemicals, EDs are not yet considered PAN Bad Actor chemicals.

2. The acute toxicity reported on this page is of the pure chemical ingredient only and may not reflect the acute toxicity of individual pesticide products. To view acute toxicity of individual products, click on 'View Products' link in the 'Chemical Identification' section above.

Additional Resources about the Toxicity of this Chemical

Additional Toxicity Info for this Chemical

See the Global Pesticide Resources page for many additional links.

Detailed Toxicity Information	This Chemical	Parent Chemical
Acute Toxicity [2]	Beta-pinene polymer	Pinene
WHO Acute Hazard	Not Listed	Not Listed
TRI Acute Hazard	Not Listed	Not Listed
Material Safety Data Sheets	Not Available	Not Available
Acute rating from U.S. EPA product label	No Consensus Value	No Consensus Value
U.S. NTP Acute Toxicity Studies	No NTP Studies	Slightly Toxic
View Studies		
Cholinesterase Inhibitor	No	No

2. The acute toxicity reported on this page is of the pure chemical ingredient only and may not reflect the acute toxicity of individual pesticide products. To view acute toxicity of individual products, click on 'View Products' link in the 'Chemical Identification' section above.

Cancer Information

IARC Carcinogens	Not Listed	Not Listed
U.S. NTP Carcinogens	Not Listed	Not Listed
California Prop 65 Known Carcinogens	Not Listed	Not Listed
U.S. EPA Carcinogens	Not Listed	Not Listed
TRI Carcinogen	Not Listed	Not Listed

Endocrine Disruption

Illinois EPA list	Not Listed	Not Listed
Keith list	Not Listed	Not Listed
Colborn list	Not Listed	Not Listed
Benbrook list	Not Listed	Not Listed

Reproductive and Developmental Toxicity

CA Prop 65 Developmental Toxin	Not Listed	Not Listed
U.S. TRI Developmental Toxin	Not Listed	Not Listed
CA Prop 65 Female Reproductive Toxin	Not Listed	Not Listed
CA Prop 65 Male Reproductive Toxin	Not Listed	Not Listed
U.S. TRI Reproductive Toxin	Not Listed	Not Listed

Chemicals of Special Concern

PAN Bad Actors	Not Listed	Not Listed
PAN Dirty Dozen list	Not Listed	Not Listed

Water Pollution Potential and Criteria for Beta-pinene polymer

Top

Water Pollution Potential	This Chemical	Parent Chemical
	Beta-pinene polymer	Pinene
PAN Ground Water Contaminant Rating	Insufficient Data	Insufficient Data

Sorry, no water quality standards or criteria have been established for this chemical by the U.S. or Canadian governments; however, there may be criteria established for related chemicals.

Regulatory Information for Beta-pinene polymer

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International Regulatory Status	This Chemical	Parent Chemical
Worldwide Registration Click on link at right to view registration information for different countries -->	Worldwide Registration for: Beta-pinene polymer Number of countries where this chemical is: Banned, Restricted or Cancelled: 0 Not legal for import: 0	Worldwide registration for: Pinene Number of countries where this c Banned, Restricted or Cancell Not legal for import: 0
UNEP Persistent Organic Pollutant (POP)	Not Listed	Not Listed
UNEP Prior Informed Consent Chemical (PIC)	Not Listed	Not Listed
WHO Obsolete Pesticide	Not Listed	Not Listed

U.S. and California Regulatory Status

U.S. EPA Registered	No	No
U.S. EPA Hazardous Air Pollutant	Not Listed	Not Listed
U.S. EPA Minimum Risk Pesticide (25b list)	No	No
CA Registered	Yes	No
CA Groundwater Contaminant	Not Listed	Not Listed
CA Toxic Air Contaminant	Not Listed	Not Listed

Maximum Tolerance and Residue Levels

Codex Alimentarius (UN FAO Maximum Residue Limits)	Go to web site
U.S. Maximum Tolerance Levels	Go to web site
European Union Maximum Residue Levels	Go to web site

Ecotoxicity for Beta-pinene polymer

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Note! Information for many chemicals is incomplete and may not be fully representative of effects on the environment. Why? Click on underlined terms for definitions and additional information.

Aquatic Ecotoxicity

All Toxic Effects for Organism Group	
Organism Group	Effects Noted
Sorry, no ecotoxicity data available for this chemical. Try related chemicals.	

Summary of Acute Toxicity for Organism Group
Sorry, no acute ecotoxicity data available for this chemical. Try related chemicals.

Terrestrial Ecotoxicity

We have recently secured funding from the U.S. EPA to incorporate terrestrial ecotoxicity data. Watch this space!

Related Chemicals for Beta-pinene polymer

Top

CAS Number	Relation	Reason	Chemical Name	Chem Detail	Registration	Symptoms	California Use	Chem Use Type	U.S EPA Reg
80-56-8, 127-91-3, 1330-16-1	Parent	P	Pinene	View	View	View	View	Insecticide	No
31393-98-3	Related	16	Alpha-pinene polymer	View	View	View	View	Insecticide	No
127-91-3	Related	6	beta-Pinene	View	View	View	View	Insecticide	No
68240-09-5	Related	6, 16	Beta-pinene polymer	View	View	View	View	Insecticide	No
25719-60-2	Related	6, 16	Beta-pinene polymer -toluene and xylene alkylated with dicyclopentadiene	View	View	View	View		No
	Related	6, 16	Beta-pinene-maleamide copolymer	View	View	View	View		No
53404-	Related	2	Ethylene glycol	View	View	View	View	Insecticide	No

49-2			ether of pinene						
	Related	16	Polymerized pinene	View	View	View	View	Insecticide	No

Working with the Information on this Page

Click on underlined terms for definitions or go to the Pesticide Tutorial overview page.

Any underlined term with a book icon has additional information.

* Data marked with an asterisk indicates that this chemical is not explicitly listed on the corresponding list. Instead, it belongs to a group of chemicals that IS designated on the list. For example, if an agency assigns a classification of reproductive toxicant to "mercury compounds", that classification is applied to all mercury compounds in the PAN Pesticide database, which are then marked with an asterisk.

To print this page, choose **Print**. To export this data, choose **Save As** 'HTML Source' and open it in Excel or equivalent program.

Citation: S. Orme and S. Kegley. *PAN Pesticide Database*. Pesticide Action Network, North America (San Francisco, CA. 2004).
http.www.pesticideinfo.org.

PAN Pesticides Database - California Pesticide Use

Beta-pinene polymer - Pesticide use statistics for 2002

Note: See Working with the Information on this Page section below for important notes about this data.

Chemical ID	Identifying information for this chemical, including synonyms, ID numbers, use type, and chemical classification.
Summary Toxicity	Summary toxicity to humans, including carcinogenicity, reproductive and developmental toxicity, neurotoxicity, and acute toxicity.
Regional Use	Use of this pesticide by county for all counties in California, with information on gross pounds used, application rate, acres planted, and number of applications.
Top Crops/Sites	Top crops and sites use of this chemical in 2002, with information on gross pounds used, application rate, acres planted, and number of applications.

Basic Chemical Information for Beta-pinene polymer

Top

For detailed chemical information see the chemical detail page.

Basic Identification Information About This Chemical

Chemical Name	Beta-pinene polymer
CAS Number	68240-09-5
U.S. EPA PC Code	
CA DPR Chem Code	3998
Use Type	Insecticide
Chem Class	Polymer, Polymer

Synonyms

Chemical versus Common Names

03998 (CA DPR Chem Code) , 3998 (CA DPR Chem Code) , 68240-09-5 (CAS Number) , 68240095 (CAS Number) , Beta-pinene polymer , Betapinene Polymer , Betapinenepolymer

Summary Toxicity Information for Beta-pinene polymer

Top

For detailed chemical information see the chemical detail page.

Note: Information for many chemicals is incomplete and may not be fully representative of effects on humans. Why?

Summary Toxicity Information

PAN Bad Actor Chemical [1]	Acute Toxicity [2]	Carcinogen	Cholinesterase Inhibitor	Ground Water Contaminant	Developmental or Reproductive Toxin	Endocrine Disruptor
Not Listed			No			

? ? ? ? ?

Indicates high toxicity in the given toxicological category.

Indicates no available weight-of-the-evidence summary assessment. For additional information on toxicity from scientific journals or registration documents, see the "Additional Resources for Toxicity " section of the chemical detail page.

1. **PAN Bad Actors** are chemicals that are one or more of the following: highly acutely toxic, cholinesterase inhibitor, known/probable carcinogen, known groundwater pollutant or known reproductive or developmental toxicant. NOTE! Because there are no authoritative lists of Endocrine Disrupting (ED) chemicals, EDs are not yet considered PAN Bad Actor chemicals.

2. The acute toxicity reported on this page is of the pure chemical ingredient only and may not reflect the acute toxicity of individual pesticide products. To view acute toxicity of individual products, click on 'View Products' link in the 'Chemical Identification' section above.

Top 50 Crops and Sites for for Beta-pinene polymer use in California in 2002

Top

Crop or Site (Commodity Code)	Gross Pounds	Application Rate pounds per acre treated	Acres Planted where all or part has been sprayed	Acres Treated	Application Count
All Sites (00)	7,645	0.17	36,031	46,282	1,916
Wine Grapes (29143)	1,898	0.08	10,138	22,837	1,108
Tomatoes for Processing (29136)	1,324	0.17	7,696	7,777	61
Almonds (3001)	735.3	0.33	2,153	2,250	35
Table and Raisin Grapes (29141)	675.2	0.34	2,748	1,993	61
Strawberries (1016)	522.0	0.29	788.5	1,804	37
Outdoor Container Nursery (154)	471.1	0.95	504.0	493.4	46
Head Lettuce (13045)	264.8	0.16	1,477	1,634	72
Apricots (5001)	227.3	0.29	599.5	796.5	26
Corn for Forage (22005)	190.9	0.26	838.0	741.0	21
Mustard (29123)	150.0	0.29	705.0	516.7	55
Cherries (5002)	127.3	0.21	208.0	597.0	20
Oranges (2006)	111.7	0.44	363.0	252.0	4
Walnuts (3009)	90.9	0.28	347.0	323.0	11
Celery (29113)	89.9	0.26	1,374	348.6	27
Leaf Lettuce (13031)	88.8	0.16	651.2	544.4	63
Collards (13009)	79.3	0.32	665.0	251.3	21
Kale (13011)	71.8	0.18	665.0	396.2	67
Onions (14011)	59.8	0.17	349.0	349.0	3
Cotton (29121)	57.6	0.17	336.0	336.0	3
Turnips (29137)	45.9	0.29	455.0	159.8	35

Bell Peppers (11003)	45.8	0.10	306.0	450.5	11
Alfalfa for Forage (23001)	44.7	0.29	152.0	151.7	3
Potatoes (14013)	44.6	0.34	260.0	131.0	3
Cabbage (13007)	31.7	0.15	250.0	206.0	25
Asparagus (16002)	27.0	0.08	317.7	317.7	11
Greenhouse Plants (153)	26.3	0.64	100.0	41.0	5
Cauliflower (13008)	26.0	0.17	120.8	149.2	23
Outdoor Flower Nursery (152)	25.5	1.69	15.1	15.1	3
Apples (4001)	21.1	0.41	27.0	51.0	6
Pears (4003)	18.7	0.20	188.0	94.0	5
Tomatoes (11005)	18.6	0.17	38.0	109.0	6
Broccoli (13005)	7.51	0.17	34.0	44.0	6
Nectarines (5003)	6.68	0.26	13.0	26.0	2
Chinese Cabbage (13010)	6.36	0.16	190.5	40.8	8
Radishes (14014)	3.51	0.18	225.0	20.0	4
Right of Way (40)	3.36	-	-	-	5
Bok Choy (13502)	2.41	0.16	139.2	15.2	9
Swiss Chard (13025)	1.92	0.19	370.0	10.0	2
Leeks (14010)	1.53	0.17	225.0	9.00	2
Landscape (30)	0.13	-	-	-	1

Regional Use for Beta-pinene polymer on All Sites in 2002

Top

Region (County Code)	Gross Pounds	Application Rate pounds per acre treated	Acres Planted where all or part has been sprayed	Acres Treated	Application Count
California (00)	7,645	0.17	36,031	46,282	1,916
Fresno (10)	1,287	0.17	7,932	7,706	90
Ventura (56)	984.1	0.27	5,815	3,682	272
Monterey (27)	860.6	0.07	4,197	12,982	778
Stanislaus (50)	756.3	0.26	2,534	2,870	67
Tulare (54)	588.0	0.71	904.0	828.0	21
Orange (30)	497.3	0.93	600.0	534.0	50
Sacramento (34)	493.2	0.17	2,716	2,911	63
Kern (15)	394.0	0.27	1,610	1,441	29
Kings (16)	359.1	0.17	2,243	2,097	9
San Benito (35)	299.8	0.06	1,088	4,870	233
Santa Barbara (42)	273.0	0.16	2,047	1,716	122
San Joaquin (39)	226.0	0.21	1,608	1,090	23
San Luis Obispo (40)	189.9	0.11	932.0	1,687	97
Merced (24)	166.2	0.21	636.0	782.0	25
Yolo (57)	109.5	0.30	386.0	368.0	11

Solano (48)	51.8	0.23	238.0	223.7	8
Amador (03)	48.5	0.17	355.9	283.0	6
Imperial (13)	40.5	0.23	163.0	174.0	8
Santa Cruz (44)	19.8	0.55	22.0	36.0	3
San Bernardino (36)	0.04	0.09	4.00	0.46	1

Working with the Information on this Page

* Complete 2001 data for Kern county was never submitted by the Kern County Agricultural Commissioners office. This missing data includes approximately 32,000 records totaling roughly 10 million pounds. An omission of this scale will significantly impact statewide trends.

NOTE! See methodology and documentation for California Pesticide Use Reporting for important qualifications on these numbers. Click on the commodities name links for special caveats on the data for each commodity. For more detailed use information, including data for other years, search our California Pesticide Use database. Original source for all pesticide use data is the California Pesticide Use Report (PUR) dataset, collected and managed by the California Department of Pesticide Regulation. Significant processing of the original dataset is required to generate the summary data presented here; see documentation for a full discussion of data handling.

NOTE! Comments on the accuracy of Acres Planted reflect PAN's analysis of acreage data from the California Agricultural Statistics Service (CASS) and Department of Pesticide Regulation Pesticide Use Reporting data. The method used to assess acreage accuracy is described in the documentation. Accuracy was evaluated for aggregate, statewide California acreage, but not for smaller regions (e.g., counties). See CASS acreage estimates for additional information.

Click on underlined terms for definitions or go to the Pesticide Tutorial overview page.

Any underlined term with a book icon has additional information.

To print this page, choose **Print**. To export this data, choose **Save As** 'HTML Source' and open it in Excel or equivalent program.

Definitions

- Acres Planted is the planted acreage of the crop in the selected region where the selected chemical was applied. This figure may not be the total acreage of the selected crop. To obtain the total planted acreage for a crop or county, see the crop and county pages available from the Pesticide Use Search Page. See documentation for important distinctions between acres treated and acres planted.
- Acres Treated is the acreage of the crop actually treated with the pesticide. Gross Pounds applied divided by Acres Treated is the application rate of the pesticide. The difference between Acres Planted and Acres Treated is best explained through an example:If a farmer has a 100-acre field and sprays 50 acres, then the Acres Planted will be 100 and the Acres Treated will be 50. If a farmer sprays 50 of their 100 acres three times, then the Acres Planted will remain at 100 acres, but now the value of Acres Treated will be 150. This figure may not be the total acreage of the selected crop. See documentation for further distinctions between acres treated and acres planted.
- Application Count indicates the number of times this chemical was applied.
- Application Rate indicates the total pounds applied to acreage divided by the total treated acres. Only pounds applied to acreage are used to calculate the application rate. This excludes pesticides used to fumigate stored commodities or other non-cropland applications. See documentation for important distinctions between acres treated and acres planted.

- Chemical Class indicates the chemical classification of this chemical.
- Chemical Code is the California Department of Pesticide Regulation code number assigned as an identifier for each chemical.
- Chemical Uses indicates the major uses for this chemical, with the most common use listed first.
- County Code is a code number that various California State agencies use to identify each California county. There are 58 total counties.
- Field Count is the number of fields where this chemical was applied.
- Gross Pounds indicates the total pounds used, including both pounds used on acreage and pounds used in other applications such as commodity fumigation.
- PAN Bad Actor indicates whether this chemical belongs to the group of most toxic pesticides or not. See link for a full definition of this term.

Citation: S. Orme and S. Kegley,*PAN Pesticide Database*, Pesticide Action Network, North America (San Francisco, CA. 2004).

http:www.pesticideinfo.org.

ATTACHMENT 33

EPA Scientific Assessment for α- and β-Pinene Chemicals

Kathryn Boyle
Registration Division, EPA
April 11, 2003

opp-2005-0110-0002

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

APR 20 2005

April 11, 2005

MEMORANDUM

FROM: Kathryn Boyle, Inerts Team
Minor Use, Inerts, and Emergency Response Branch
Registration Division

THROUGH: Pauline Wagner, Inerts Coordinator
Registration Division

TO: Dan Rosenblatt, Chief
Minor Use, Inerts, and Emergency Response Branch
Registration Division

SUBJECT: Science Assessment for alpha- and beta-Pinene Chemicals

The attached science assessment discusses the toxicity of pure alpha- and beta-pinene, and alpha- and/or beta-pinene polymers. This document serves the dual purpose of supporting the tolerance reassessment process for the existing two tolerance exemptions for alpha-pinene (40 CFR 180.920 and 180.930) and the one tolerance exemption for beta-pinene polymers (40 CFR 180.910), and Pesticide Petition 6E4782 to amend the existing exemption for beta-pinene polymers to include alpha- and/or beta-pinene polymers.

The Agency is performing a qualitative assessment. Given the low acute toxicity by the oral, dermal and inhalation routes, the low subchronic toxicity, the lack of reproductive or developmental effects at high dose levels, and the extensive naturally-occurring (primarily inhalation and oral) exposures, a quantitative approach is not needed. Based on this qualitative assessment, EPA finds that exempting alpha- and/or beta-pinene polymers from the requirement of a tolerance will be safe, and that the three existing tolerance exemptions can be reassessed.

Science Assessment for
Alpha- and Beta-Pinene Compounds

I. Executive Summary

This assessment evaluates pure alpha- and beta-pinene, and alpha- and/or beta-pinene polymers. Currently two tolerance exemptions are established for alpha-pinene (40 CFR 180.920 and 180.930), and one tolerance exemption for beta-pinene polymers (40 CFR 180.910). These exemptions must be reassessed under the Food Quality Protection Act. The Agency has been petitioned (6E4782) to amend the existing exemption for beta-pinene polymers to include alpha- and/or beta-pinene polymers.

The data considered in this assessment included information submitted by the petitioner, and information located by OPP on the internet, primarily information prepared by the National Toxicology Program (NTP) and the robust summaries for bicyclic terpene hydrocarbons submitted in 2002 to EPA by the Terpene Consortium of the Flavor and Fragrance High Production Volume Consortia (FFHPVC). The Agency has not conducted a review of the original study, unless specifically stated.

Alpha- and beta-pinene are the major components of turpentine. The two chemicals are closely related, having the same empirical formula of $C_{10}H_{16}$ and the same basic ring structure. The predominant uses of the pure forms of alpha- and beta-pinene are as fragrances.

Alpha- and beta-pinene are of low acute toxicity. Both are irritants to the skin, eye and mucous membranes. The subchronic toxicity of alpha- and beta-pinene compounds appears to be low. In a subchronic oral toxicity study there were no effects at approximately 800 mg/kg/day.

Genotoxicity study summaries indicated no evidence of mutagenicity. No chronic/carcinogenicity studies were identified; however, alpha- and beta-pinene are not structurally related to any known carcinogens.

In three developmental toxicity studies no maternal or developmental effects were noted in mice, hamsters, or rats at the highest dose levels, 560, 600, or 260 mg/kg/day, respectively. Alpha- and beta-pinene are not structurally related to any known developmental/reproductive toxicants.

Most of the turpentine produced in the United States is made up primarily of alpha-pinene (75 to 85%). Turpentine is known to act as a central nervous system (CNS) depressant. Given the relationship of turpentine to alpha-pinene, and the relationship of alpha- to beta-pinene, there could be solvent toxicity concerns for pinene chemicals in the workplace.

The polymers composed of alpha and beta-pinene monomers are of too low a molecular weight to be exempted from the requirement of a tolerance using the criteria specified for

defining a low-risk polymer in 40 CFR 723.250. However, any polymerization process would increase the molecular weight beyond that of the pinene monomers, and therefore decrease absorption. These alpha- and/or beta-pinene polymers should therefore be even less toxic than pure alpha- and beta-pinene.

Exposure to alpha- and beta-pinene can occur from use as a fragrance in consumer products and as a flavoring in foods. However, alpha- and beta-pinene occur naturally in the atmosphere as a result of release from forests, and are present in a variety of foods commonly consumed in the human diet. Alpha- and beta- pinene could be present in sources of drinking water, but are not persistent and would be expected to readily volatilize to the atmosphere. These naturally-occurring exposures are more extensive than such anthropogenic exposures.

The Agency is performing a qualitative assessment. Given the low acute toxicity by the oral, dermal and inhalation routes, the low subchronic toxicity, the lack of reproductive or developmental effects at high dose levels, and the extensive naturally-occurring (primarily inhalation and oral) exposures, a quantitative approach is not needed. Based on this qualitative assessment, EPA finds that exempting alpha- and/or beta-pinene polymers from the requirement of a tolerance will be safe, and that the three existing tolerance exemptions can be reassessed.

Alpha-and beta-pinene are acutely toxic to aquatic organisms, and although documented environmental concentrations are <100 ppb, they may present an acute concern for marine/estuarine invertebrates. Bioconcentration of alpha- and beta-pinene in aquatic organisms may occur. This information was a significant factor in the decision to classify the pinene chemicals as List 4B.

II. Introduction

This review serves two purposes. First, it is conducted to reassess the existing tolerance exemptions as presented in Table 1.

Table 1: Tolerance Exemptions Being Reassessed in this Document

Tolerances Exemption Expression	40 CFR ◊	Uses
beta-Pinene polymers	180.910	Surfactants, related adjuvants of surfactants
alpha-Pinene	180.920 and 180.930	Stabilizer Limitation: Not more than 2% of formulation by weight

◊Residues listed in 40 CFR 180.910[formerly 180.1001(c)] are exempted from the requirement of a tolerance when used in accordance with good agricultural practice as inert (or occasionally active) ingredients in pesticide formulations applied to growing crops or to raw agricultural commodities after harvest.

Residues listed in 40 CFR 180.920 [formerly 180.1001(d)] are exempted from the requirement of a tolerance when used in accordance with good agricultural practice as inert (or occasionally active) ingredients in pesticide formulations applied to growing crops only.

Residues listed in 40 CFR 180.930 [formerly 180.1001(e)] are exempted from the requirement of a tolerance when used in accordance with good agricultural practice as inert (or occasionally active) ingredients in pesticide formulations applied to animals.

Second, this review supports the petition to amend the existing exemption from tolerance for beta-pinene polymers to include alpha- and/or beta-pinene polymers (Petition 6E4782). The chemicals considered include:

Table 2: Chemicals Considered

Common Chemical Name	CAS Nomenclature	CAS Reg. No.	List Classification
alpha-pinene	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl- (9CI) 2-Pinene (8CI)	80-56-8	4B
L-alpha-pinene	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-, (1S,5S) (9CI) 2-Pinene, (1S,5S)-(-)- (8CI)	7785-26-4	---
beta-pinene	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene- (9CI) 2(10)-Pinene (8CI)	127-91-3	---
(S)-beta-pinene	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S,5S)- (9CI) 2(10)-Pinene, (1S,5S)-(-)- (8CI)	18172-67-3	3
Oil of turpentine, alpha-pinene fraction	Terpenes and terpenoids, turpentine oil, alpha-pinene fraction	65996-96-5*	---
Oil of turpentine, beta-pinene fraction	Terpenes and terpenoids, turpentine oil, beta-pinene fraction	65996-97-6**	---

Table 2: Chemicals Considered			
Common Chemical Name	CAS Nomenclature	CAS Reg. No.	List Classification
alpha-pinene polymer	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-, homopolymer	25766-18-1	---
beta-pinene polymer	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, homopolymer (9CI)	25719-60-2	4B
copolymer of alpha- and beta-pinene	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-, polymer with 6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane (9CI)	31393-98-3	---
Polymerized alpha- pinene fraction from turpentine	Terpenes and Terpenoids, turpentine oil, alpha-pinene fraction, polymd.	70750-57-1	---

* Defined by CAS as "The hydrocarbon fraction distilled from oil of turpentine. Contains greater than 80% [alpha]-pinene, the remainder being other terpene hydrocarbons."
** Defined by CAS as "The hydrocarbon fraction distilled from oil of turpentine or produced by the isomerization of [alpha]-pinene. Contains greater than 70% [beta]-pinene"

The Lower Risk Pesticide Chemical Focus Group served as the review body for this assessment of alpha- and beta- pinene compounds.

III. Use Pattern of Alpha and Beta-Pinene

The predominant (non-pesticidal) uses of the pure forms of alpha- and beta-pinene are as fragrances. They are approved by the Food and Drug Administration as food additives (synthetic flavoring substances and adjuvants) for direct addition to food for human consumption (21 CFR 172.515: Synthetic flavoring substances and adjuvants). Alpha-pinene is used as a solvent for protective coatings, polishes, and waxes and in the synthesis of other chemicals such as camphene, camphor, synthetic pine oil and terpine esters. Beta-pinene is used in polyterpene resins and as a feedstock for preparation of menthol, and other flavors and fragrances.

The predominant pesticidal use of the pinene chemicals is as an adjuvant. Adjuvants are used to enhance the performance of a spray application of a pesticide product. Adjuvants are added in the field to the tank mix containing the active pesticidal ingredient. Under 21 CFR 182.99 and 582.99, chemicals used as adjuvants on agricultural food crops must have an

exemption from the requirement of a tolerance under 40 CFR part 180 as established by the Environmental Protection Agency.

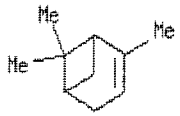
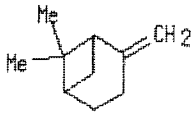
IV. Physical/Chemical Properties of alpha and beta-Pinene

Alpha- and beta-pinene are the major components of turpentine which is manufactured from the resinous sap of pine trees. "Turpentine is a mixture of constituents. The type and amount of specific constituents is dependent on the type of pine tree, the geographical location of the trees, and the season of tree harvest. Turpentine produced in the United States is made up primarily of alpha-pinene (75 to 85%) with varying amounts of beta-pinene (up to 3%)..." New Zealand turpentine is 30 to 50% alpha-pinene and 40 to 60% beta-pinene. (NTPb)

Alpha- and beta-pinene are separated from the turpentine by fractional distillation. Further fractionation in needed to separate the pinenes. They are colorless, transparent, liquids with a turpentine odor. Chemically, they are bicyclic, unsaturated, monoterpene hydrocarbons. The two chemicals are closely related, having the same empirical formula of $C_{10}H_{16}$ and the same basic ring structure, differing only in the placement of a carbon-carbon double bond. Alpha-pinene can be chemically converted to beta-pinene.

Various physical/chemical property data for alpha- and beta-pinene and their structures are presented in Table 3.

Table 3: Chemical/Physical Properties of Alpha- and Beta-Pinene

Property	Alpha-Pinene	Beta-Pinene
Structure		
Molecular Weight	136.24	136.24
Melting point	- 55 °C	----
Boiling point	156.2 °C	----
Vapor Pressure	10 mm Hg at 37.3 °C 4.75 mm Hg at 25 °C	2.93 mm Hg at 25 °C
Log Kow	4.83	4.35 (E)
Koc	1204 (E)	1204 (E)
Water solubility	1.891 mg/L at 25 °C	4.886 mg/L at 25 °C (E)
Henry's Law Constant	2.94×10^{-1} atm-m^3/mole	9.2×10^{-2} atm-m^3/mole (E)
Hydrolysis/Photodegradation	Not expected to occur	Not expected to occur
Biodegradation	Ultimate: weeks to months (E) Primary: days to weeks (E) Complete removal in 250 hours in 3 different soil slurries from Georgia	Ultimate: weeks to months (E) Primary: days to weeks (E)

E= Estimated value as reported in the 2002 EFED Tolerance Review

V. Toxicity Reviews and Evaluation by the Office of Pesticide Programs (OPP)

In August 2001, the Health Effects Division of OPP reviewed the toxicity data submitted in support of Pesticide Petition 6E4782. The following information on dermal irritation, sensitization and subchronic toxicity was extracted from that review and evaluation.

Beta-pinene polymer (CAS Reg. No. 25719-60-2): The amount of absorption is dependent on the molecular weight of the polymer; however, it is considered that beta-pinene polymer is not well-absorbed via any route. No concerns were noted for developmental/reproductive effects, carcinogenicity, or mutagenicity: overall low concern.

VII. Toxicity Reviews and Evaluation by the Terpene Consortium of the Flavor and Fragrance High Production Volume Consortia (FFHPVC)

Alpha- and beta-pinene are sponsored by The Flavor and Fragrance High Production Volume Consortia/The Terpene Consortium under the High Production Volume (HPV) Challenge Program. HPV chemicals are those that are manufactured or imported into the United States in volumes greater than one million pounds per year. Twenty-one companies are current members of the Terpene Consortium. Additional information on the HPV Challenge Program can be found at http://www.epa.gov/chemrtk/volchall.htm.

Alpha-pinene is also sponsored by the Flavor and Fragrance High Production Volume Consortia/The Terpene Consortium in the Voluntary Children's Chemical Evaluation Program. (See http://www.epa.gov/chemrtk/vccep/terpenecons.pdf for the sponsorship letter.) The Agency's website does not indicate the submission of any additional information under VCCEP.

The HPV Test Plan for the Bicyclic Terpene Hydrocarbons was submitted to the Agency in February 2002. (AR201-13610A). HPV submissions are prepared by the submitters. As stated on the Agency's website (see http://www.epa.gov/chemrtk/viewsrch.htm), the submissions are not edited by the Agency. The Agency is unaware of the type of review process used by the submitter in the preparation of the robust summaries.

Summaries of the applicable information on alpha-pinene, beta pinene, l-alpha-pinene, and the pinene fractions of turpentine oil are presented in Tables 4, 5 and 6. Only data rated as reliability code 1 (reliable without restrictions), or reliability code 2 (reliable with restrictions) are included in the tables below. Reliability code 2 is generally assigned if the study was not conducted according to today's standards, but the methodology was comparable, and the results of the study were published in a peer-reviewed journal.

Table 4: Acute Toxicity Studies (FFHPVC-Prepared Robust Summaries for Bicyclic Terpene Hydrocarbons)

Test Substance	Doses	Type of Study (species)	Results	Reliability Code
alpha-Pinene	0, 2020, 3200, 5000, 7800 mg/kg bw	Oral (Rat)	LD_{50} = 3700 mg/kg bw (95% C.L. 2300-5100 mg/kg bw)	1

Table 4: Acute Toxicity Studies
(FFHPVC-Prepared Robust Summaries for Bicyclic Terpene Hydrocarbons)

Test Substance	Doses	Type of Study (species)	Results	Reliability Code
	5000 mg/kg bw	Dermal (Rabbit)	LD_{50} > 5000 mg/kg bw	1
beta- Pinene	5000 mg/kg bw	Oral (Rat)	LD_{50} > 5000 mg/kg bw	1
	5000 mg/kg bw	Dermal (Rabbit)	LD_{50} > 5000 mg/kg bw	1

Table 5: Summary of Toxicological Data for alpha- and beta-Pinene (FFHPVC-Prepared Robust Summaries for Bicyclic Terpene Hydrocarbons)

Test Substance	Doses	Type of Study (species)	Results	Reliability Code
Developmental and Reproductive Toxicity				
Mixture of terpene hydrocarbons (20-25% alpha-pinene and 15 to 18% beta-pinene)	0 (control), 6, 26, 120, 560 mg/kg bw/day	Developmental (Mouse)	"There was no evidence of maternal toxicity or developmental toxicity at dose levels up to and including 560 mg/kg bw/day of test material." Maternal NOAEL is equal to or greater than 560 mg/kg bw/day Maternal LOAEL was not determined, but would be greater than 560 mg/kg bw/day Developmental NOAEL is equal to or greater than 560 mg/kg bw/day Developmental LOAEL was not determined, but would be greater than 560 mg/kg bw/day	2
Mixture of terpene hydrocarbons (20-25% alpha-pinene and 15 to 18% beta-pinene)	0 (control), 6, 28, 130, 600 mg/kg bw/day	Developmental (Hamster)	"The administration of up to and including 600 mg/kg bw/day of test article FDA 71-28 to pregnant golden hamsters on days 6 through 10 of gestation had no effects on nidation, maternal survival or fetal survival. The number and types of abnormalities seen in tissues of the dam or pups of the test groups did not differ for the number and type occurring spontaneously in the positive or negative controls." Maternal NOAEL is equal to or greater than 600 mg/kg bw/day Maternal LOAEL was not determined, but would be greater than 600 mg/kg bw/day Developmental NOAEL is equal to or greater than 600 mg/kg bw/day Developmental LOAEL was not determined, but would be greater than 600 mg/kg bw/day	2

Test Substance	Doses	Type of Study (species)	Results	Reliability Code
Mixture of terpene hydrocarbons (20-25% alpha-pinene and 15 to 18% beta-pinene)	0 (control), 3, 12, 56, 260 mg/kg bw/day	Developmental (Rat)	"The administration of up to and including 260 mg/kg bw/day of test article FDA 71-28 to pregnant Wistar rats on days 6 through 15 of gestation had no effects on nidation, maternal survival or fetal survival. The number and types of abnormalities seen in tissues of the dam or pups of the test groups did not differ for the number and type occurring spontaneously in the positive or negative controls." Maternal NOAEL is equal to or greater than 260 mg/kg bw/day Maternal LOAEL was not determined, but would be greater than 260 mg/kg bw/day	2

It is noted that the reference given for these three developmental toxicity studies indicate that these studies were performed for the Food and Drug Administration.

Table 6: Mutagenicity Studies (FFHPVC-Prepared Robust Summaries for Bicyclic Terpene Hydrocarbons)

Test Substance	Concentration	Species/Strain	Type of Study	Results	Reliability Code
alpha-Pinene	0.5- 300 µL/plate	*Salmonella typhimurium* TA100 and TA98	Ames/ microsome assay Reverse Mutation	No evidence of mutagenicity	2
	4.08, 40.8, 408, and 4080 µg/plate	*Salmonella typhimurium* TA100 and TA98	Ames/ microsome assay Reverse Mutation	No evidence of mutagenicity	2
	25000 µg/plate	*Salmonella typhimurium* TA1535, TA1537, TA1538, TA100 and TA98	Ames/ microsome assay Reverse Mutation	No evidence of mutagenicity	1
	0, 0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, and 25 µL/plate	*Salmonella typhimurium* TA1535, TA1537, TA1538, TA100 and TA98	Ames/ microsome assay Reverse Mutation	No evidence of mutagenicity	1
	0.001, 0.003, 0.01, 0.03, 0.1, 10 µL/mL	Rat hepatocytes	Unscheduled DNA Synthesis Assay	No evidence of genotoxicity	1
beta-Pinene	0, 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0 µL/plate	*Salmonella typhimurium* TA1535, TA1537, TA1538, TA100 and TA98	Ames/ microsome assay Reverse Mutation	No evidence of mutagenicity	1
	Up to 5000 µg/plate	*Salmonella typhimurium* TA1535, TA1537, TA1538, TA100 and TA98	Ames/ microsome assay Reverse Mutation	No evidence of mutagenicity	1
	4.08, 40.8, 408, and 4080 µg/plate	*Salmonella typhimurium* TA100 and TA98	Ames/ microsome assay Reverse Mutation	No evidence of mutagenicity	2
	0, 3.3, 10, 33.3, 100, 333, 1000 µM	Chinese hamster ovary (CHO) cells	Sister Chromatid Exchange	Test substance did not induce sister chromatid exchange in CHO cells	1

VIII. Toxicity Summaries by the National Toxicology Program (NTP)

Health and Safety Summary for alpha-Pinene

The Health and Safety summary for alpha-pinene prepared by NTP indicates that this chemical is a "moderate irritant to skin, eyes and mucous membrane and via oral, inhalation and dermal routes."

Of significant interest is the information that NTP is currently conducting or planning to conduct toxicity studies using alpha-pinene. (NTPc). The 14-day inhalation toxicity study in rats is listed as **on test.** A 13-week inhalation toxicity study in rats and a 2-year inhalation carcinogenicity study in rats are listed as **assigned.** However, given that the background document on which this testing is based is the Turpentine Review (discussed below), the Agency believes that the test substance is actually turpentine.

Turpentine Review of Toxicological Literature

Although this report researched turpentine toxicological literature, given the chemical relationship of turpentine to alpha- and beta-pinene, toxicity data generated using alpha- and beta-pinene were used for assessing the toxicity of turpentine. The results of the author's data search which are not duplicative to previously reported information are discussed below.

Solvent Toxicity:

Turpentine (which is mostly alpha-pinene) is known to act as a central nervous system (CNS) depressant. Typical symptoms of CNS can include headache; fatigue; dizziness; an effect similar to that of ethanol intoxication; difficulty in breathing; irritation of the skin, eyes, nose, and mucous membranes; and can progress to convulsions and death. OSHA's permissible exposure limit (PEL), as an 8-hour time-weighted average, for turpentine is 100 ppm or 560 mg/m^3 as measured in breathing-zone air samples.

Inhalation Acute Toxicity:

Table 7: Acute Inhalation Toxicity Studies (Taken from Table 18 - Toxicological Summary for Turpentine)

Species Tested	Chemical Tested	Test Results
Mouse, rat, mouse, guinea pig	α-pinene from wood turpentine	LC_{100} = 4666 ppm (26,000 mg/m^3) (5 h) (all species)

Mouse, rat, mouse, guinea pig	α-pinene from sulfate turpentine	LC_{100} = 5025 ppm (28,000 mg/m^3) (5 h) (all species)
Mouse, rat, guinea pig	β-pinene from sulfate turpentine	LC_{100} = 3517 ppm (19,596 mg/m^3) (5 h) (all species)
Mouse	(+)- β-pinene	RD_{50} = 1279 ppm (7126 mg/m^3) (30 min)
Mouse	(-)- β-pinene	RD_{50} = 4663 ppm (25,981 mg/m^3) (30 min)
Rat	α-pinene	LCLo = 625 mg/kg (4.59 mmol/kg)
Guinea pig	α-pinene	LCLo = 0.572 mg/m^3) (103 ppb)

LC_{100} is the concentration (dose) that is lethal to 100% of the test animals;
LCLo is the lowest concentration (dose) that resulted in the death of test animals;
RD_{50} is the concentration that causes 50% decrease in respiratory frequency

Short-Term Inhalation: Wistar rats were treated with a commercial turpentine mixture that was 95% alpha-pinene. The dose level was 300 ppm (1670 mg/m^3), 6 hours/day, 5 days/week for up to 8 weeks. "No behavioral abnormalities were observed in treated rats. Exposures resulted in the accumulation of alpha-pinene in fat. One and two week exposures resulted in a reduction of RNA content in the brain, similar to the effects of other solvents."

Chronic/carcinogenic: No alpha-pinene chronic or carcinogenicity studies were identified.

IX. Hazard Characterization

The toxicity of alpha- and beta-pinene is defined by studies from open-literature conducted with alpha-pinene, beta-pinene and various alpha- and beta pinene mixtures and/or polymers. There is also a structure-activity-relationship (SAR) assessment. The database is sufficient for the purposes of this document. Alpha- and beta-pinene are of low acute toxicity via the oral, dermal and inhalation routes. Both alpha-and beta-pinene are irritants to the skin, eye and mucous membranes. Alpha- and beta-pinene are well-absorbed by all routes of exposure.

The subchronic toxicity of alpha- and beta-pinene compounds appears to be low. A subchronic oral toxicity study reviewed by HED during evaluation of Petition 6E4782 indicated minor changes in liver and thryoid weights at the two higher dose levels, which were not considered treatment related. There were no effects at approximately 800 mg/kg/day.

Genotoxicity study summaries indicated no evidence of mutagenicity in several *Salmonella typhimurium* reverse mutation assays, one unscheduled DNA assay, and one sister

Dermal Irritation

"Undiluted [alpha] pinene applied to the backs of hairless mice and swine was not irritating. However, once applied to intact or abraded rabbit skin for 24 hr under occlusion it was a **moderate irritant**. When tested in 10% petroleum it produced no irritation after a 48 hr close patch test on two different panels of human subjects. Beta pinene applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was a **moderate irritant**. When tested in 12% petroleum it produced no irritation after a 48 hr close patch test on human subjects."

Dermal Sensitization

"In a dermal human sensitization study, [alpha] and [beta]-pinene produced no dermal sensitization when tested at concentration of 10% and 12% in petroleum, respectively."

Subchronic toxicity

In a 3-month oral toxicity study, rats were fed an alpha pinene resin or pinene polymer made predominantly from alpha-pinene. (The ratio of alpha- and beta-pinene was 10:1.) The dose levels were 0, 1, 3 or 5% in the diet. Effects seen at 5% (3967 mg/kg/day) included an increase in relative liver weight in both sexes, and absolute liver weight in females only. Increased relative thyroid weights in males were noted at the 3 and 5% dose levels. In the absence of histopathological alterations, these changes were not considered treatment related. No effects were noted at 1%, which corresponds to roughly 800 mg/kg/day.

VI. Structure-Activity-Relationship (SAR) Assessments Performed by OPPT

In the early 1990s, the Office of Pollution Prevention and Toxic Substances (OPPT) performed as part of an OPP inert ingredient list reclassification project SARs for several hundred inert ingredients including alpha-pinene and beta-pinene polymers. Toxicity for these two chemicals was assessed by a process called structure-activity relationship. In this process, the chemical's structural similarity to other chemicals (for which data are available) is used to determine toxicity. For human health, this process can be used to assess absorption and metabolism, mutagenicity, carcinogenicity, developmental and reproductive effects, neurotoxicity, systemic effects, immunotoxicity, and sensitization and irritation. This is a qualitative assessment using terms such as good, not likely, poor, moderate, or high. The conclusions of the team performing the SAR are as follows.

alpha-Pinene (CAS Reg. No. 80-56-8): Alpha-pinene is well-absorbed via the skin, lungs, and gastro-intestinal tract. No concerns were noted for developmental/reproductive effects, carcinogenicity, or mutagenicity.

chromatid exchange assay. No chronic/carcinogenicity studies were identified; however, alpha- and beta-pinene are not structurally related to any known carcinogens.

A mixture of alpha- and beta-pinene (and other terpene hydrocarbons) were tested in three developmental toxicity studies. Summaries of the results of these studies report that no maternal or developmental effects were noted in mice, hamsters, or rats at the highest dose levels, 560, 600, or 260 mg/kg/day, respectively. Alpha- and beta-pinene are not structurally related to any known developmental/reproductive toxicants.

The available information does not indicate that any of these chemicals are of higher toxicity. For alpha- and beta-pinene, the irritation effects are of concern. Additionally, given the fact that the turpentine produced in the United States is made up primarily of alpha-pinene (75 to 85%), and that turpentine is known to act as a CNS depressant, by extrapolation, there could be solvent neurotoxicity concerns for pinene chemicals from dermal and inhalation exposures. Exposures generally need to be "high" and/or "prolonged" for these solvent toxicity effects to occur. Also, for acute exposures such effects, generally, are reversible. Concerns are for occupational exposures since the potential for day in/day out exposure can occur in the workplace.

The polymers composed of alpha and beta-pinene monomers are of a low molecular weight, and thus cannot be exempted from the requirement of a tolerance using the criteria specified for defining a low-risk polymer in 40 CFR 723.250. An MSDS for a pinene polymer (CAS Reg. No. 31393-98-3) describes the chemical as a viscous liquid. Processes that could increase the molecular weight beyond that of alpha- or beta-pinene include formation of a dimer (two "pinenes" in a single molecule), formation of a trimer (three "pinenes" in a single molecule), or polymerization. Greater molecular weight means decreased absorption. Alpha- and/or beta-pinene dimers, trimers, or polymers should therefore be even less toxic than pure alpha- and beta-pinene.

X. Exposure Assessment

Exposure to alpha- and beta-pinene can occur from use as a fragrance in consumer products and as a flavoring in foods. However, as discussed below, the naturally-occurring exposures to alpha- and beta-pinene are more extensive. For this section, generally only the exposures of alpha-pinene are discussed. According to Toxnet's beta-pinene summary (TOXNETb), beta-pinene naturally occurs with the alpha-pinene, but at lower concentrations.

Atmospheric

Total US emission of alpha-pinene from deciduous and coniferous forests amounted to 6.6 mega tons annually. An estimated emission rate of alpha-pinene from natural sources to the atmosphere is 1.84×10^{-10} g/sq cm/sec. (TOXNETa)

In a compilation of published and non-published data on the atmospheric concentration of volatile organic compounds determined between 1970 to 1987, the daily mean concentration of alpha-pinene in suburban and urban areas is 0.147 ppb and 0.120 ppb, respectively. the daily mean concentration of alpha-pinene in remote and rural areas is 0.035 ppb, 0.030 ppb, respectively. (USEPA as cited in TOXNETa)

Dietary

According to Toxnet's alpha-pinene summary, alphà-pinene is a component of trees, fruits, grasses, bushes, fungi, herbs, and flowers. Alpha-pinene has been detected in filberts, chicken, mangos, fresh grapefruit juice (0.054 ppm), guava, carrots, pistachio, safflower, sorghum, tomato, walnut, ginger, celery, unpasteurized orange juice (0.10-1.09 ppm), shrimp, and crab. Alpha-pinene is also a constituent of over 400 essential oils.

Environmental Fate Characterization/ Drinking Water Consideration

Alpha-pinene is not expected to persist in the environment. Alpha-pinene has been detected in the river water and sea water. Concentrations were reported in the low parts per billion (<100 ppb). Alpha-pinene will readily volatilize from soil and flowing water to the atmosphere within hours, and within days from lakes. Once in the atmosphere, alpha-pinene degrades with an estimated half-life of 4 hours. Reaction with ozone occurs with a half-life of approximately 40 minutes. Night-time reactions with nitrate radicals occur with a half-life of 6 minutes. Biological degradation is expected to occur rapidly in aerobic soils, and can proceed to complete removal. Degradation in seawater samples occurred with a half-life of approximately 6-8 hours.

Beta-pinene is also not expected to persist in the environment, and will readily volatilize from soil and flowing water to the atmosphere within hours and within days from lakes. Once in the atmosphere, beta-pinene will degrade with photochemically-produced hydroxyl radicals with an estimated half-life of 4.9 hours. Reaction with ozone occurs with a half-life of approximately 22 hours. Biological degradation is expected to occur rapidly in aerobic soils.

Neither alpha- nor beta-pinene are persistent in the environment. Given the ready volatilization and rapid degradation of alpha- and beta-pinene, it is unlikely to be present in any significant amounts in sources of drinking water.

XI. Aggregate Exposures

In examining aggregate exposure, section 408 of the FFDCA directs EPA to consider available information concerning exposures from the pesticide residue in food and all other non-occupational exposures, including drinking water from ground water or surface water and exposure through pesticide use in gardens, lawns, or buildings (residential and other indoor uses).

Given their volatility, pinenes are present in the atmosphere. They are present in the foods that are consumed on a daily basis. They could be present in sources of drinking water, but are not persistent and would be expected to readily volatilize to the atmosphere. The uses regulated by EPA are much smaller than the naturally-occurring exposures.

XII. Cumulative Exposure

Section 408(b)(2)(D)(v) of the FFDCA requires that, when considering whether to establish, modify, or revoke a tolerance, the Agency consider "available information" concerning the cumulative effects of a particular pesticide's residues and "other substances that have a common mechanism of toxicity."

EPA has not made a common mechanism of toxicity finding as to alpha- or beta-pinene and any other substances. They do not appear to produce a toxic metabolite produced by other substances. For the purposes of this tolerance action, therefore, EPA has not assumed that alpha- and beta-pinene have a common mechanism of toxicity with other substances. For information regarding EPA's efforts to determine which chemicals have a common mechanism of toxicity and to evaluate the cumulative effects of such chemicals, see the policy statements released by EPA's Office of Pesticide Programs concerning common mechanism determinations and procedures for cumulating effects from substances found to have a common mechanism on EPA's website at http://www.epa.gov/pesticides/cumulative/.

XIII. Safety Factor for Infants and Children

FFDCA section 408 provides that EPA shall apply an additional tenfold margin of safety for infants and children in the case of threshold effects to account for prenatal and postnatal toxicity and the completeness of the data unless EPA concludes that a different margin of safety will be safe for infants and children. Three developmental toxicity studies (rat, mouse and hamster) conducted using a mixture of alpha- and beta-pinenes at high dose levels did not identify either maternal or developmental NOAELs. There are no indications of increased susceptibility. These pinene chemicals are not structurally related to any known developmental/reproductive toxicants. Therefore, EPA has not used a safety factor analysis to assess the risk. For the same reasons a tenfold safety factor is unnecessary.

XIV. Determination of Safety for US Population, and Infants and Children

The database considered for this action included mostly toxicity data derived using alpha- and beta-pinene. Alpha- and beta-pinene exhibit low acute toxicity by the oral, dermal and inhalation routes, and low subchronic toxicity. Polymers composed of alpha and beta-pinene monomers, even those of low molecular weight, should be even less toxic than alpha- and beta-pinene considering that their absorption is decreased. Based on the available information on toxicity and exposure, EPA concludes that there is a reasonable certainty of no harm from aggregate exposure to residues of alpha-pinene, beta-pinene, and alpha- and/or beta-pinene polymers. EPA finds that amending the existing exemption from the requirement of a tolerance

for beta-pinene polymers to include alpha- and/or beta-pinene polymers will be safe for the general population including infants and children.

XV. Conclusions Pertinent to the Food Quality Protection Act

In Consideration Pesticide Petition 6E4782:

EPA finds that exempting alpha- and/or beta-pinene polymers from the requirement of a tolerance will be safe. The existing exemption for beta-pinene polymers in 40 CFR 180.910 can be amended to include alpha- and/or beta pinene polymers. The following CAS Reg. Nos. have thus far been identified: 25719-60-2, 25766-18-1, 31393-98-3, and 70750-57-1.

In Consideration of Tolerance Reassessment:

The existing exemptions for alpha-pinene and beta-pinene polymers can be reassessed. There are several forms of alpha-pinene (CAS Reg. Nos. 80-56-8, 7785-26-4, and 65996-96-5), all of which are included in the reassessment. The limitation of 2% by weight in the formulation is to remain; however, the exemption should be corrected to indicate that the use of alpha-pinene is as a solvent or fragrance component, not as a stabilizer.

Toxicologically speaking, there is little difference between alpha- and beta-pinene. Given the available information a tolerance exemption could be established for beta-pinene (CAS Reg. Nos. 127-91-3, 18172-67-3, and 65996-97-6). The limitation and use pattern is the same as that for alpha-pinene.

XVI. Ecotoxicity Assessment Developed by OPP/EFED

Alpha-pinene is very highly toxic to aquatic organisms on an acute basis, and may present an acute concern for marine/estuarine invertebrates based on documented environmental concentrations near the levels of predicted toxicity. Acute toxicity estimates are 0.72 mg/L for freshwater fish, 0.51 mg/L for marine/estuarine fish, 0.93 mg/L for *Daphnia magna*, 0.042 mg/L for mysid shrimp, and 0.66 mg/L for green algae. Chronic toxicity estimates for freshwater fish is 0.138 mg/L. Terrestrial animal toxicity based on available rat data would indicate that alpha-pinene is practically non-toxic on an acute basis.

Beta-pinene is very highly toxic to aquatic organisms on an acute basis, and may present an acute concern for marine/estuarine invertebrates based on probable environmental concentrations at or near the levels of predicted toxicity. Acute toxicity estimates are 0.62 mg/L for freshwater fish, 0.45 mg/L for marine/estuarine fish, 0.79 mg/L for *Daphnia magna*, 0.034 mg/L for mysid shrimp, and 0.56 mg/L for green algae. Chronic toxicity estimates for freshwater fish is 0.117 mg/L. Terrestrial animal toxicity based on available rat data would indicate that β-pinene is practically non-toxic on an acute basis.

Bioconcentration of alpha-pinene in aquatic organisms may occur based on an estimated BCF of 2800. For beta-pinene, bioconcentration in aquatic organisms may occur based on an estimated BCF of 444.

XVII. List Reclassification:

Given the ecotoxicity assessment which indicated concerns for aquatic organisms and bioconcentration, and the specification in the tolerance exemption for a limitation of 2% by weight in the formulation, all CAS Reg. No.s discussed in this document are confirmed or reclassified as List 4B.

XVIII. References

EPA/OPP; Environmental Fate and Effects Division (EFED) Tolerance Review of Compounds of Group 13, Lignins, Cellulose and Pinenes as Inert Ingredients in Terrestrial and/or Aquatic Agricultural and Non-Agricultural Uses; April 10, 2002; Memorandum from Sid Abel (EFED) to Kathryn Boyle (RD).

EPA/OPP; Health Effects Division (HED): An Evaluation of Hercules Inc. Petition for the Expansion of the Existing Exemption from Tolerance for β-Pinene Polymers to Include Polymers Made with α-Pinenes and Mixtures of α and β Pinenes (Petition #: 6E04782); August 16, 2001; Memorandum from Waheeda Mani Tehseen (HED) to Robert Forest (RD).

FFHPVC (Flavor and Fragrance High Production Volume Consortia); The Terpene Consortium; Robust Summaries for Bicyclic Terpene Hydrocarbons; AR201-13610B; accessed February 19, 2002; http://www.epa.gov/chemrtk/bictrphy/c13610rs.pdf

NTPa (National Toxicology Program); Health and Safety Information for alpha-Pinene (CAS No. 80-56-8);(accessed March 14, 2002). Note: Information has since been removed to http://ntp-db.niehs.nih.gov/htdocs/H&S_archive.zip

NTPb; Turpentine, Toxicological Literature Review; accessed February 28, 2005; http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/turpentine.pdf

NTPc; Testing Status: Alpha Pinene, accessed February 28, 2005; http://ntp.niehs.nih.gov/INDEX.CFM?OBJECTID=07105185-B741-02BD-FE4334DA286D3041

TOXNETa. Hazardous Substance Data Bank (HSDB). On-line Scientific Search Engine, National Library of Medicine, National Institute of Health; (http://www.toxnet.nlm.nih.gov.) Search terms: 80-56-8

TOXNETb. Hazardous Substance Data Bank (HSDB). On-line Scientific Search Engine, National Library of Medicine, National Institute of Health; (http://www.toxnet.nlm.nih.gov.) Search terms: 127-91-3

Acknowledgement: The Agency was assisted in the preparation of this document by Versar, Inc. Under GSA Contract Number EP-05-W-000253.

ATTACHMENT 34

Acute Oral Toxicity Study in Rats on Miller 6064

STILLMEADOW
INCORPORATED

VOLUME __ OF __ OF SUBMISSION

Miller 6064

FINAL REPORT

ACUTE ORAL TOXICITY STUDY IN RATS

OPPTS NO. 870.1100

AUTHOR:

Janice O. Kuhn, Ph.D., D.A.B.T.

STUDY INITIATION DATE: 26 December 2000
STUDY COMPLETION DATE: 26 March 2001

CONDUCTED BY:
STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, TX 77478

LABORATORY STUDY NUMBER:

6206-00

VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 19

SUBMITTED TO:
Miller Chemical and Fertilization Corp.
P.O. Box 333
Hanover, PA 17331

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA § 10 (d) (1) (A), (B) or (C).

Company: Miller Chemical and Fertilization Corp.

Company Agent: _____ Date: _____

_____ _____

Title Signature

These data are the property of Miller Chemical and Fertilization Corp., and as such, are considered to be confidential for all purposes other than compliance with FIFRA § 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other statute or in any other country.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was designed and performed in STILLMEADOW, Inc.'s AAALAC accredited laboratory in compliance with:

- United States Environmental Protection Agency FIFRA; GLP Standards 40 CFR 160 with exception of Sec. 160.31 (d), and 160.105 (b)(e): stability information was not provided
- United States Environmental Protection Agency TSCA 40 CFR 792 with exception of Sec. 792.31 (d), and 792.105 (b)(e): stability information was not provided
- Organization for Economic Cooperation & Dev. Principles of GLP, Annex 2, C(97)186 with exception of Sec. 6.2 (4): stability information was not provided
- Japan Ministry of Agriculture, Forestry & Fisheries, Notification No. 59 Nohsan 3850, Director-General of Agricultural Prod. Bureau, 10 Aug 84 with exception of Art. 5 (2)(9), and 21 (3): stability information was not provided

Janice O. Kuhn, Ph.D., D.A.B.T.
Study Director, STILLMEADOW, Inc.

26 Mar 01
Date

Signature of Agent of Sponsor

Date

Agent Name
Sponsor: Miller Chemical and Fertilization Corp.

Signature of Agent of Submitter

Date

Agent Name
Submitter: Mandava Associates

TABLE OF CONTENTS

QUALITY ASSURANCE STATEMENT

Study Number: 6206-00
Test Substance: Miller 6064
Study Title: Acute Oral Toxicity Study in Rats

The study report and data have been audited in accordance with STILLMEADOW, Inc. Standard Operating Procedures (SOPs). The findings from the inspection and audit were reported to the Study Director and management as follows:

Phase of Study Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
Dosing	10 Jan 01	11 Jan 01	11 Jan 01
Report/Data Audit	20 Feb 01	20 Feb 01	20 Feb 01

Darrell Zeno

Darrell Zeno, M.S.
Quality Assurance Unit, STILLMEADOW, Inc.

26 Mar 01

Date

SUMMARY

The test substance, Miller 6064, was evaluated for its acute oral toxicity potential in albino rats when administered as a single gavage dose at a level of 5050 mg/kg to males and females. No mortality occurred during the study. Clinical signs included diarrhea, nasal discharge, polyuria and salivation, which were no longer evident by Day 6. There was no effect on body weight gain. The gross necropsy conducted at termination of the study revealed no observable abnormalities, except a herniated liver in one animal. The acute oral LD_{50}, as indicated by the data, was determined to be greater than 5050 mg/kg.

INTRODUCTION

The objective of this study was to assess the acute oral toxicity potential of the test substance when administered by gavage to rats in accordance with US EPA OPPTS 870.1100, which is intended to meet testing requirements of FIFRA 7 USC 136, et seq, and TSCA 15 USC 2601. This study was conducted for Miller Chemical and Fertilization Corp., according to the approved protocol and STILLMEADOW, Inc. SOPs. There were no deviations from the protocol which affected the quality or outcome of the study. All procedures used in this study are in compliance with Animal Welfare Act Regulations. The protocol, raw data and this report are archived at STILLMEADOW, Inc. The animals were treated as follows:

Dose		Male Treatment		Female Treatment		In-life Termination Date	
mg/kg	mL/kg	Date	Time	Date	Time	Males	Females
5050	5.50	10 Jan 01	0920	10 Jan 01	0925	24 Jan 01	24 Jan 01

TEST SUBSTANCE

Identification:	Miller 6064
Date & Quantity Received:	19 Dec 00; 2 x 1 gal
Physical Description:	Amber liquid
Storage:	Room temperature
Density:	0.9179 g/mL
Purity & Composition:	Refer to Certificate of Analysis (Appendix A)
Stability:	Not provided by sponsor

Records pertaining to stability, characterization and verification of test substance identity are the responsibility of the sponsor. A copy of the Certificate of Analysis is included in the report.

TEST SYSTEM

Experimental Animals

Species & Strain:	Albino rat; Sprague-Dawley
Justification of Species:	The rat is a representative rodent species preferred by various regulatory agencies for use in an acute oral study.
Source:	Texas Animal Specialties, Humble, TX
Date Received:	4 Jan 01
Quarantine Period:	5 days
Quantity & Sex:	5 males and 5 females (nulliparous and non-pregnant) were selected for testing
Group Identification:	Cage cards
Animal Identification:	Ear punch
Fasted Wt on Dosing Day:	Males: 236-244 g; Females: 159-181 g
Date of Birth:	14 Nov 00

Animal Husbandry

Cage Type:	Suspended, wire bottom, stainless steel
Housing:	1 per cage
Environmental Controls Set to Maintain:	· Temperature Range 22°C±3° · Humidity Range 30-70% · 12-hour light/dark cycle · 10-12 air changes/hour
Food:	PMI Feeds Inc.™ Formulab #5008; available *ad libitum* except for approximately 16 hours before dosing
Water:	Municipal water supply analyzed by TNRCC Water Utilities Division; available *ad libitum* from automatic water system.

Animal husbandry and housing at STILLMEADOW, Inc. comply with Animal Welfare Act Regulations. No contaminants were expected to have been present in the feed or water which would have interfered with or affected the results of the study.

PROCEDURES

Test Substance Administration

The test substance was administered as received and was not diluted. An individual dose was calculated for each animal based on its fasted body weight and administered by gavage at a volume of 5.50 mL/kg. Each dose was administered using an appropriately sized syringe and stainless steel ball-tipped intubation needle. The animals were returned to their cages immediately after dosing.

In-life Observations

Observations for mortality and clinical/behavioral signs of toxicity were made at least three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14.

Postmortem Observations

On Day 14 after dosing, each animal was euthanized by an overdose of CO_2. All study animals were subjected to gross necropsy and all abnormalities were recorded.

RESULTS AND DISCUSSION

Mortality/Estimated Lethality Values

There was no mortality during the study. The estimated acute oral LD_{50}, as indicated by the data, was determined to be greater than 5050 mg/kg.

Clinical Signs

Clinical signs are presented in Table 2. Clinical signs included diarrhea in both sexes, and nasal discharge, polyuria and salivation in females. Animals were asymptomatic by Day 6.

Body Weights

Individual body weights are presented in Table 1. Body weight gain was unaffected by the administration of the test substance.

Necropsy Findings

Individual necropsy findings are presented in Table 1. The gross necropsy conducted at termination of the study revealed no observable abnormalities, except for a herniated liver in one male.

CONCLUSION

The test substance, Miller 6064, was evaluated for its acute oral toxicity potential when administered to albino rats. The acute oral LD_{50}, as indicated by the data, is greater than 5050 mg/kg in males and females.

Study Director: Janice O. Kuhn, Ph.D., D.A.B.T. — Date: 26 Mar 01
Senior Toxicologist, STILLMEADOW, Inc.

STUDY PERSONNEL

Technical Staff: Carol Morris, B.A.; Hector Fuentes; Paul Siemens, B.A.; Michelle Gantt, B.S.

Data Services: Connie Pavatte, Report Preparation

TABLE 1
ACUTE ORAL TOXICITY STUDY IN RATS
Body Weights, Time of Death, and Gross Necropsy
Test Substance: Miller 6064
Dose Level: 5050 mg/kg (5.50 mL/kg)

Animal Number	Body Weights (g)			Time of Death*	Gross Necropsy Findings
	Day 0	Day 7	Final		
111-M	237	284	341	Day 14	NOA
112-M	236	274	309	Day 14	NOA
113-M	244	295	307	Day 14	NOA
114-M	240	300	342	Day 14	NOA
115-M	238	283	322	Day 14	Liver herniated through diaphragm.
116-F	169	201	226	Day 14	NOA
117-F	166	193	215	Day 14	NOA
118-F	172	197	229	Day 14	NOA
119-F	181	223	249	Day 14	NOA
120-F	159	186	215	Day 14	NOA

* - Day of dosing considered Day 0; Day 14 is terminal sacrifice.
M - Male; F - Female; NOA - No Observable Abnormalities

TABLE 2
ACUTE ORAL TOXICITY STUDY IN RATS
Pharmacologic and/or Toxicologic Signs
Test Substance: Miller 6064
Dose Level: 5050 mg/kg (5.50 mL/kg)
Sex: Males and Females

	Time After Treatment																
	HOURS			DAYS													
Reaction and Severity	1	2	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Males																	
Diarrhea (s-m)	1	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Females																	
Polyuria (v-m)	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0
Salivation (m)	2	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Clear nasal discharge (m)	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diarrhea (v-m)	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0

v - very slight; s - slight; m - moderate; e - extreme
Note: Digits indicate number of animals exhibiting reaction.

APPENDIX A

MILLER® CHEMICAL & FERTILIZER CORPORATION

P.O. BOX 333, 120 RADIO ROAD
HANOVER, PENNSYLVANIA 17331 U.S.A.
TELEPHONE: 717-632-8921
FAX NO.: 717-632-4581

1/18/01

CERTIFICATE OF ANALYSIS

Product Name: MILLER 6064
Quantity Produced: 7.4 kilograms
Batch Number: 150386
Date of Formulation: 12/13/00

Appearance: Yellow to Amber Liquid
Odor: Terpenic
Moisture: Negligible (non-aqueous)
Specific Gravity: 0.92 - 0.94 @ 20c
Viscosity @ 20c: 100 to 500 cps
pH(1% solution): 6.0 to 8.0
Solubility in Water: Insoluble (Emulsifies)
Flash Point: over 200 f
Purity: 100%

Signature:

Name: Andrew G. Smith
Title: Compliance Officer

APPENDIX B

STILLMEADOW
INCORPORATED

PROTOCOL FOR STUDY 6206-00

Study Title: ACUTE ORAL TOXICITY STUDY IN RATS (OPPTS 870.1100)

Test Substance: MILLER 6064

Test Facility: STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, Texas 77478

Approved: _____ 26 Dec 00
Janice O. Kuhn, Ph.D., D.A.B.T. Date
Study Director
STILLMEADOW, Inc.

Approved: _____ 6 Dec 00
Mark S. Holbert Date
Vice President
STILLMEADOW, Inc.

Reviewed: _____ 6 Dec 2000
Vicki S. Crutchfield, RQAP Date
Director, Quality Assurance Unit
STILLMEADOW, Inc.

Sponsor: Miller Chemical and Fertilization Corp.
P.O. Box 333
Hanover, PA 17331

Sponsor Representative: Mandava Associates
1730 M Street, N.W., Suite 906
Washington, DC 20036

Approved: _____ December 26, 2000
N. Bhushan Mandava Date
Agent to Miller Chemical and Fertilization Corp.

APPENDIX B (cont.)

PROTOCOL FOR STUDY 6206-00

A. GENERAL

1. Study Title: ACUTE ORAL TOXICITY STUDY IN RATS

2. Purpose: To assess the acute oral toxicity potential of the test substance when administered by the oral route (gavage) to rats.

3. Regulatory Compliance: This study will be conducted according to OPPTS 870.1100, which is intended to meet testing requirements of both FIFRA (7 U.S.C. 136, *et seq.*) and TSCA (15 U.S.C. 2601).

This study will be conducted in compliance with Good Laboratory Practice Standards:
1. EPA FIFRA: 40 CFR 160
2. EPA TSCA: 40 CFR 792
3. OECD: C(97)186
4. Japanese MAFF

All procedures in this protocol are in compliance with Animal Welfare Act Regulations. All methods can be found in STILLMEADOW, Inc. Standard Operating Procedures (SOPs).

4. Quality Assurance: The Protocol is reviewed by the Quality Assurance Unit (QAU). The study information will be entered into the Master Schedule. The study will be inspected at least once during its progress. Further inspections may be scheduled as needed to ensure the integrity of the study. Any deviations from SOPs, the Protocol, or Good Laboratory Practice Standards will be immediately reported to the Study Director and Management. The report will be audited and a statement prepared and signed which shall specify the dates inspections were made and findings reported to Management and to the Study Director.

5. Test Substance: MILLER 6064. Test substance identification should include the name, batch number, and purity. Information regarding safety, stability, storage conditions, and disposal should also be provided by the Sponsor. The Sponsor assumes responsibility for purity and stability determinations. Analysis and archiving of reserve samples will be the responsibility of the Sponsor.

6. Proposed Schedule: Testing will begin within approximately three weeks of receipt of the test substance and authorization to conduct the study.

Proposed Start Date: 20 Dec 00
Proposed End Date: 03 Jan 01

The study will be extended if several dose levels are required.

APPENDIX B (cont.)

A. GENERAL (cont.)

7.	Study Director:	Janice O. Kuhn, Ph.D., D.A.B.T.
8.	Experimental Summary:	The test substance will be administered to rats orally by gavage. The animals will be observed three times on the day of dosing for mortality and signs of pharmacologic and/or toxicologic effects and once daily thereafter for at least 14 days. If a sufficient number of dose levels are tested, an LD_{50} with slope function and 95% confidence limits will be calculated, and/or a Toxicity Category will be assigned.
9.	Protocol Amendments:	Any alteration in the Protocol will be justified, approved by the Study Director, and recorded in writing.
10.	Sponsor Audits:	The Sponsor may send an authorized representative to inspect the test system and/or data on the STILLMEADOW, Inc. premises during normal working hours.

B. EXPERIMENTAL DESIGN

1. Animals

a. Species:	Albino rat
b. Strain/Source:	Sprague-Dawley (Texas Animal Specialties, Humble Texas or other suitable source)
c. Justification of Species:	The rat is conventionally used to provide an index of toxicity on which human hazard can be judged, and is the species preferred by the regulatory agencies.
d. Quantity and Sex:	Five males and five females (nulliparous and non-pregnant) for the initial dose level and 5/sex for any additional dose levels, if required (see B.3.g.).
e. Age/Weight:	Young adult (8 - 12 weeks) Males: approximately 225 - 330 grams Females: approximately 175 - 250 grams. Weight variation should not exceed ±20% of the mean for each sex.
f. Identification:	Ear punch
g. Acclimation and Health Status:	Animals will be acclimated for at least five days prior to testing. Normal weight gain, appearance, and behavior will be factors used to select healthy animals for testing. Only naive animals will be selected.
h. Randomization:	Unless a control group is required (see B.3.f.) no formal randomization procedure will be required. If a control group is necessary, a weight-stratified randomization procedure will be employed.

2. Animal Husbandry

a. Cages:	Stainless steel, suspended, wire bottom
b. Number per Cage:	Animals will be housed individually during the study.
c. Food:	PMI Feeds, Inc.™ Formulab #5008, available *ad libitum* prior to fasting and after dosing, or equivalent. Analyzed by manufacturer for nutritional content.
d. Water:	Tap water; available *ad libitum* (automatic system). Municipal water supply analyzed by Texas Natural Resource Conservation Commission (TNRCC) Water Utilities Division.
e. Contaminants:	There are no known contaminants in the feed or water available to laboratory animals that would be expected to interfere with this study.
f. Environment:	Target temperature: approximately 22°C ± 3°C. Target relative humidity: approximately 30 - 70%. 12-hour light/dark cycle (regulated automatically). Room ventilation of approximately 10 - 12 air changes per hour.

APPENDIX B (cont.)

B. EXPERIMENTAL DESIGN (cont.)

3. Test Substance Administration

a. Preparation of Animals: Animals will be fasted for at least 16 hours prior to dosing. Food will be made available immediately after dosing.

b. Reason for Route of Administration: Historically, the oral route has been a route of choice for evaluation of the toxicity potential of a test substance and is a potential route of human exposure.

c. Assignment of Animals to Groups: Animals will be randomly selected for dose groups so that individual body weights will not exceed ±20% of the mean weight for each sex.

d. Preparation of Test Substance: The test substance will be administered as received, if possible, or diluted in an appropriate vehicle to the most concentrated workable dilution. If the substance is an aerosol, it will be discharged into a container and administered as a liquid. All animals in a dose group will receive the same concentration of the test substance. Maximum dose volume will not exceed 1 mL/100 g, or for aqueous solutions, 2 mL/100 g. The dosing solutions will be prepared on the day of dosing and the prepared dosing solutions will be stored at room temperature until administration, within three hours after mixing.

e. Dosing: The animals will be dosed by gavage with an appropriately-sized stainless steel ball-tipped dosing needle and syringe. Individual doses will be calculated based upon the animal's body weight on the day of test substance administration.

f. Control Groups: If the test substance is administered as received, a control group is not necessary. If the test substance is administered in a vehicle for which the toxicity is not known, then a vehicle control group (five males and five females) will be required.

g. Dose Level: Unless available toxicologic information indicates otherwise, a single dose level of approximately 5050 mg/kg (or 2020 mg/kg if requested by Sponsor) will be administered to five animals per sex. If no mortality occurs at this level, no further testing is required.

If mortality meets or exceeds 40% in either or both sexes at the level tested, then at least two additional dose levels may be tested for those sexes. There will be at least five animals (five males and/or five females) per dose level. The number and spacing of dose levels will be chosen so that an LD_{50} and/or a Toxicity Category can be determined. If both sexes are tested at a given dose level, then the group will consist of an equal number of males and females. If animals of one sex are markedly more sensitive, testing in animals of the other sex may be dispensed with.

B. EXPERIMENTAL DESIGN (cont.)

4. Observations

a. Clinical Signs: Observations for mortality and signs of pharmacologic and/or toxicologic effects will be made three times on the day of dosing, and once daily thereafter for 14 days. The duration of the study should be determined by the toxic reactions and may be extended beyond 14 days when considered necessary. The nature, onset, severity, and duration of all gross or visible pharmacologic or toxicologic signs will be recorded.

Observations will include but not necessarily limited to evaluation of skin, fur, eyes and mucous membranes, respiratory and circulatory effects, autonomic effects such as salivation, lacrimation, excessive urination, diarrhea, central nervous system effects, including tremors, and convulsions, changes in the level of activity, gait and posture, reactivity to handling or sensory stimuli, altered strength, and stereotypies or bizarre behavior (e.g., self mutilation, walking backwards).

b. Body Weights: Body weights will be recorded on the day prior to dosing (Day -1), the day of dosing (Day 0), and weekly thereafter (Days 7 and 14), or at the time of discovery after death.

c. Sacrifice of Animals: All surviving animals will be euthanized by an overdose of carbon dioxide.

d. Necropsy: A gross necropsy will be conducted on each animal at termination of the study or at the time of discovery after death, and the results recorded. The gross necropsy shall include terminal body weight and gross observations of external surfaces; all orifices; and thoracic abdominal, and pelvic cavities.

5. Evaluation of Results: Unless only a single dose level is tested, an LD_{50} with slope function and 95% confidence limits will be calculated for males, females, and males and females combined (if necessitated by mortality in one or both sexes) by the method of Litchfield and Wilcoxon (Litchfield, J.T., Jr., and Wilcoxon, F.: A Simplified Method of Evaluating Dose-Effect Experiments, J. Pharm. & Exp. Ther., 96, 99-115, 1949) or other appropriate method. If requested by the Sponsor, a Toxicity Category may be determined instead of an LD_{50} or in addition to an LD_{50}.

6. Test Substance Accountability: A comprehensive inventory of test substance received and used will be kept. The test substance container(s) will be weighed when received at this facility, and a record of all test substance use will be maintained. Test substance and test substance dosing solutions will be stored in the original containers, or in the equivalent thereof, or in glass containers with Teflon-lined caps.

APPENDIX B (cont.)

B. EXPERIMENTAL DESIGN (cont.)

7. Disposal of Unused Test Substance:

Unused test substance will be returned or disposed of at the Sponsor's expense after the termination of the study. The Sponsor should specify return or disposal at the time of study authorization. If returned, the recipient of the test substance will be notified in advance of shipping. The sample will be accompanied by a chain of custody letter describing the sample(s) contained in the shipment.

8. Safety Precautions:

General safety precautions required by laboratory SOPs will be followed. The Sponsor will supply basic toxicity data on the test substance to be used. However, since the toxicity of test substances is often not well characterized, this laboratory will be conservative in setting safety procedures. The Sponsor or Sponsor's Representative shall be notified of any exposures requiring a physician's examination or care.

C. DATA MANAGEMENT

1. Records:

The following records will be maintained during the study and transferred to the STILLMEADOW, Inc. archives upon study termination.

a. Protocol and Protocol Amendments (if any).
b. Final report and amendments (if any).
c. Study correspondence.
d. Animal receipt/acclimation data.
e. Test substance receipt, identification as supplied by Sponsor, preparation, administration, and disposition.
f. Test animal information: number, sex, source, strain.
g. Body weight data.
h. Daily observation data for signs of pharmacologic and/or toxicologic effects.
i. Mortality data, gross necropsy findings, and histopathology findings, if requested.
j. Calculations (if any) of the LD_{50} and slope determinations with 95% confidence limits.
k. Other pertinent data.

2. Data Storage:

All raw data will be retained at STILLMEADOW, Inc without charge to the Sponsor for a period of five years.

APPENDIX B (cont.)

C. DATA MANAGEMENT (cont.)

3. Data Reporting:

The final report will include all data as described in the Good Laboratory Practice Standards, including:

a. Statement from the Quality Assurance Unit.
b. Signature of the Study Director.
c. A GLP Compliance Statement signed by the Study Director.
d. Names of scientific personnel involved in the study.
e. Dates of study initiation and termination.
f. Identification, description, preparation, and storage of the test substance.
g. All pertinent animal data, animal husbandry, dosing information, and observation methods.
h. Description of the test procedures.
i. If calculated, the LD_{50} and slope function data with 95% confidence limits for males, females, and males and females combined (if necessitated by mortality in one or both sexes), and/or the Toxicity Category.
j. Individual body weights.
k. Observations on the nature, onset, severity, and duration of all gross or visible pharmacologic and/or toxicologic signs. Nonroutine findings will be addressed in a discussion section in which the relationship to treatment and historical data will be evaluated.
l. Individual mortality data, gross necropsy findings, and histopathology findings, if requested.
m. A reference to this Protocol.

4. Report Submission:

A report will be submitted after termination of the in-life portion of the study (subject to completion of histopathology, if requested).

ATTACHMENT 35

Acute Dermal Toxicity Study in Rabbits on Miller 6064

STILLMEADOW
INCORPORATED

VOLUME _ OF _ OF SUBMISSION

Miller 6064

FINAL REPORT

ACUTE DERMAL TOXICITY STUDY IN RABBITS

OPPTS NO. 870.1200

AUTHOR:

Janice O. Kuhn, Ph.D., D.A.B.T.

STUDY INITIATION DATE: 26 December 2000
STUDY COMPLETION DATE: 26 March 2001

CONDUCTED BY:
STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, TX 77478

LABORATORY STUDY NUMBER:

6207-00

VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 22

SUBMITTED TO:
Miller Chemical and Fertilization Corp.
P.O. Box 333
Hanover, PA 17331

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA § 10 (d) (1) (A), (B) or (C).

Company: Miller Chemical and Fertilization Corp.

Company Agent: _____ Date: _____

_____ _____

Title Signature

These data are the property of Miller Chemical and Fertilization Corp., and as such, are considered to be confidential for all purposes other than compliance with FIFRA § 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other statute or in any other country.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was designed and performed in STILLMEADOW, Inc.'s AAALAC accredited laboratory in compliance with:

- United States Environmental Protection Agency FIFRA: GLP Standards, 40 CFR 160 with exception of Sec. 160.31 (d), and 160.105 (b)(e): stability information was not provided
- United States Environmental Protection Agency TSCA 40 CFR 792 with exception of Sec. 792.31 (d), and 792.105 (b)(e): stability information was not provided
- Organization for Economic Cooperation & Dev. Principles of GLP, Annex 2, C(97)186 with exception of Sec. 6.2 (4): stability information was not provided
- Japan Ministry of Agriculture, Forestry & Fisheries, Notification No. 59 Nohsan 3850, Director-General of Agricultural Prod. Bureau, 10 Aug 84 with exception of Art. 5 (2)(9), and 21 (3): stability information was not provided

Janice O. Kuhn — 26 Mar 01

Janice O. Kuhn, Ph.D., D.A.B.T.
Study Director, STILLMEADOW, Inc.

Date

Signature of Agent of Sponsor

Date

Agent Name
Sponsor: Miller Chemical and Fertilization Corp.

Signature of Agent of Submitter

Date

Agent Name
Submitter: Mandava Associates

TABLE OF CONTENTS

QUALITY ASSURANCE STATEMENT

Study Number: 6207-00
Test Substance: Miller 6064
Study Title: Acute Dermal Toxicity Study in Rabbits

The study report and data have been audited in accordance with STILLMEADOW, Inc. Standard Operating Procedures (SOPs). The findings from the inspection and audit were reported to the Study Director and management as follows:

Phase of Study Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
Dosing	4 Jan 01	9 Jan 01	9 Jan 01
Report/Data Audit	14 Feb 01	14 Feb 01	14 Feb 01

Darrell Zeno

Darrell Zeno, M.S.
Quality Assurance Unit, STILLMEADOW, Inc.

26 Mar 01
Date

SUMMARY

The test substance, Miller 6064, was evaluated for its dermal toxicity potential and relative skin irritancy when a single undiluted dose of 5050 mg/kg was applied to the intact skin of albino rabbits. No mortality occurred during the study. Clinical signs included decreased defecation, soft feces and not eating, which were no longer observed on Day 4 of the study. Signs of dermal irritation included erythema, edema, atonia, desquamation and shallow fissuring. There was no effect on body weight gain, with the exception of three animals that lost or failed to gain weight during the first week. The gross necropsy conducted at termination of the study revealed no observable abnormalities, except for discolored lungs in six animals. The estimated LD_{50}, as indicated by the data, was determined to be greater than 5050 mg/kg.

INTRODUCTION

The objective of this study was to assess the systemic toxicity potential and relative skin irritancy of the test substance when administered to rabbits in accordance with US EPA OPPTS 870.1200, which is intended to meet testing requirements of FIFRA 7 USC 136, et seq, and TSCA 15 USC 2601. This study was conducted for Miller Chemical and Fertilization Corp., according to the approved protocol and STILLMEADOW, Inc. SOPs. There were no deviations from the protocol that affected the quality or outcome of the study. All procedures used in this study are in compliance with Animal Welfare Act Regulations. The protocol, raw data and this report are archived at STILLMEADOW, Inc. The animals were treated as follows:

Dose		Male Treatment		Female Treatment		In-life Termination Date	
mg/kg	mL/kg	Date	Time	Date	Time	Males	Females
5050	5.50	4 Jan 01	0940	4 Jan 01	0946	18 Jan 01	18 Jan 01

TEST SUBSTANCE

Identification:	Miller 6064
Date & Quantity Received:	19 Dec 00; 2 x 1 gal
Physical Description:	Amber liquid
Storage:	Room temperature
Measured Density:	0.9179 g/mL
Purity & Composition:	Refer to Certificate of Analysis (Appendix A)
Stability:	Not provided by sponsor

Records pertaining to stability, characterization and verification of test substance identity are the responsibility of the sponsor. A copy of the Certificate of Analysis is included in the report.

TEST SYSTEM

Experimental Animals

Species & Strain:	Albino rabbit; New Zealand White
Justification of Species:	The rabbit is a representative species preferred by various regulatory agencies for use in acute dermal testing.
Source:	Ray Nichols Rabbitry; Lumberton, TX
Date Received:	28 Dec 00
Quarantine Period:	5 days
Quantity & Sex:	5 males and 5 females (nulliparous and non-pregnant) were selected for testing
Group Identification:	Cage cards
Animal Identification:	Ear tag
Weight on Dosing Day:	Males: 2.075-2.950 kg; Females: 2.275-2.475 kg
Date of Birth:	8 Oct 00

Animal Husbandry

Cage Type:	Suspended, wire bottom, stainless steel
Housing:	1 per cage
Environmental Controls Set to Maintain:	· Temperature Range 20°C± 3° · Humidity Range 30-70% · 12-hour light/dark cycle · 10-12 air changes/hour
Food:	PMI Feeds, Inc.™ Lab Rabbit Diet #5321, in measured amounts
Water:	Municipal water supply analyzed by TNRCC Water Utilities Division; tap water, available *ad libitum* (automatic system)

Animal husbandry and housing at STILLMEADOW, Inc. comply with Animal Welfare Act Regulations. No contaminants were expected to have been present in the feed or water which would have interfered with or affected the results of the study.

PROCEDURES

Test Substance Administration

Healthy albino rabbits were released from quarantine. Each animal was prepared on the day prior to treatment by clipping the dorsal surface of the trunk free of hair to expose not less than 10% of the total body surface area. Care was taken to avoid abrading the skin. Only those animals with exposure areas free of pre-existing skin irritation or defects were used for this study. All animals were treated with 5050 mg/kg (5.50 mL/kg) of undiluted test substance. An individual dose was calculated for each animal based on its Day 0 body weight just before exposure. The test substance was applied evenly to each exposure area in a thin, uniform layer. The area of application was covered with an appropriately sized surgical gauze patch (4 ply, 8 x 4 in) and secured with non-irritating adhesive tape. The trunk of each animal was then wrapped with orthopedic stockinette which was secured in place with non-irritating adhesive tape to prevent possible ingestion of the test substance.

PROCEDURES (cont.)

Removal of Test Substance

After 24 hours, the wrappings were removed. The test sites were gently washed with room temperature tap water and a clean cloth to remove as much residual test substance as possible.

In-life Observations

Observations for mortality and clinical/behavioral signs of toxicity were made at least three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14.

Dermal Irritation Observations

Observations for evidence of dermal irritation were made at approximately 60 minutes after removal of wrappings, and on Days 4, 7, 11 and 14.

Postmortem Observations

On Day 14 after dosing, animals were euthanized by an intracardiac injection of Fatal Plus® (Vortech Pharmaceuticals, Dearborn, MI 48126). All study animals were subjected to gross necropsy and all abnormalities were recorded. After necropsy, the animal carcasses were discarded.

RESULTS AND DISCUSSION

Mortality/Estimated LD_{50} Values

There was no mortality during the study. The estimated acute dermal LD_{50}, as indicated by the data, was determined to be greater than 5050 mg/kg body weight.

Clinical Signs

Clinical signs are presented in Table 2. Prominent in-life observations included decreased defecation, soft feces and not eating. Animals were asymptomatic by Day 4.

Dermal Irritation

Signs of dermal irritation are presented in Table 3. Irritation included very slight to well-defined erythema, very slight edema, atonia, desquamation and shallow fissuring.

Body Weights

Individual body weights are presented in Table 1. Body weight gain was unaffected by the administration of the test substance, with the exception of two males that failed to gain weight and one male that lost weight during the first week of the study.

RESULTS AND DISCUSSION (cont.)

Necropsy Findings

Individual necropsy findings are presented in Table 1. The gross necropsy conducted at termination of the study revealed no observable abnormalities, except for discolored lungs in four males and two females.

CONCLUSION

The test substance, Miller 6064, was evaluated for its acute dermal toxicity potential and relative skin irritancy when administered to albino rabbits. The acute dermal LD_{50}, as indicated by the data, is greater than 5050 mg/kg in males and females.

Study Director: Janice O. Kuhn 26 Mar 01

Janice O. Kuhn, Ph.D., D.A.B.T.
Senior Toxicologist, STILLMEADOW, Inc.

Date

STUDY PERSONNEL

Technical Staff: Carol Morris, B.A.
Hector Fuentes
Paul Siemens, B.A.
Michelle Gantt, B.S.

Data Services: Connie Pavatte, Report Preparation

TABLE 1
ACUTE DERMAL TOXICITY STUDY IN RABBITS
Body Weights, Time of Death, and Gross Necropsy
Test Substance: Miller 6064
Dose Level: 5050 mg/kg (5.50 mL/kg)

Animal Number	Body Weights (kg)			Time of Death*	Gross Necropsy Findings
	Day 0	Day 7	Final		
2308-M	2.350	2.325	2.450	Day 14	NOA
2310-M	2.600	2.725	2.825	Day 14	Lungs pale.
2316-M	2.950	2.950	3.200	Day 14	Lungs pale.
2318-M	2.075	2.075	2.275	Day 14	Lungs mottled.
2322-M	2.800	3.000	3.175	Day 14	Lungs pale.
2317-F	2.400	2.450	2.675	Day 14	NOA
2321-F	2.400	2.625	2.775	Day 14	NOA
2323-F	2.475	2.750	2.850	Day 14	Lungs mottled.
2325-F	2.325	2.500	2.800	Day 14	Lungs pale.
2327-F	2.275	2.425	2.600	Day 14	NOA

* - Day of dosing considered Day 0; Day 14 is terminal sacrifice.
M - Male; F - Female; NOA - No Observable Abnormalities

TABLE 2
ACUTE DERMAL TOXICITY STUDY IN RABBITS
Pharmacologic and/or Toxicologic Signs
Test Substance: Miller 6064
Dose Level: 5050 mg/kg (5.50 mL/kg)
Sex: Males and Females

	Time After Treatment															
	HOURS			DAYS												
Reaction and Severity	1.0	2.0	4.0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Males																	
Soft feces	0	3	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0
Decreased defecation	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Not eating	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Females																	
Soft feces	0	1	1	4	4	0	0	0	0	0	0	0	0	0	0	0	0
Not eating	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0

Note: Digits indicate number of animals exhibiting reaction.

TABLE 3
ACUTE DERMAL TOXICITY STUDY IN RABBITS
Signs of Dermal Irritation
Test Substance: Miller 6064
Dose Level: 5050 mg/kg (5.50 mL/kg)

Animal Number	Day 1			Day 4			Day 7			Day 11			Day 14		
	Er	Ed	Other	Er	Ed	Other	Er	Ed	Other	Er	Ed	Other	Er	Ed	Other
2308-M	1	0	-	1	0	D	0	0	-	0	0	-	0	0	-
2310-M	1	1	-	1	0	D,W	0	0	-	0	0	-	0	0	-
2316-M	1	0	-	1	0	D,W	0	0	-	0	0	-	0	0	-
2318-M	1	1	-	1	0	D,W	0	0	-	0	0	-	0	0	-
2322-M	2	0	-	1	0	D,W	0	0	-	0	0	-	0	0	-
2317-F	2	0	-	1	0	D,W	0	0	-	0	0	-	0	0	-
2321-F	2	0	-	1	0	A,D,W	0	0	-	0	0	-	0	0	-
2323-F	2	1	-	1	0	A,D,W	0	0	-	0	0	-	0	0	-
2325-F	1	1	-	1	0	A,D,W	0	0	-	0	0	-	0	0	-
2327-F	2	1	-	1	0	A,D,W	0	0	-	0	0	-	0	0	-

Er = Erythema; 0 = None, 1 = Very slight, 2 = Well-defined, 3 =Moderate, 4 = Severe
Ed = Edema; 0 = None, 1 = Very slight, 2 = Slight, 3 = Moderate, 4 = Severe
A = Atonia; D = Desquamation; W = Shallow lateral fissuring
Note: A dash (-) is used if no other irritation is observed.

APPENDIX A

MILLER® CHEMICAL & FERTILIZER CORPORATION

P O. BOX 333, 120 RADIO ROAD
HANOVER, PENNSYLVANIA 17331 U.S.A.
TELEPHONE: 717-632-8921
FAX NO.: 717-632-4581

1/18/01

CERTIFICATE OF ANALYSIS

Product Name: MILLER 6064
Quantity Produced: 7.4 kilograms
Batch Number: 150386
Date of Formulation: 12/13/00

Appearance: Yellow to Amber Liquid
Odor: Terpenic
Moisture: Negligible (non-aqueous)
Specific Gravity: 0.92 - 0.94 @ 20c
Viscosity @ 20c: 100 to 500 cps
pH(1% solution): 6.0 to 8.0
Solubility in Water: Insoluble (Emulsifies)
Flash Point: over 200 f
Purity: 100%

Signature:

Name: Andrew G. Smith
Title: Compliance Officer

APPENDIX B

STILLMEADOW
INCORPORATED

PROTOCOL FOR STUDY 6207-00

Study Title: ACUTE DERMAL TOXICITY STUDY IN RABBITS
(OPPTS 870.1200)

Test Substance: MILLER 6064

Test Facility: STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, Texas 77478

Approved: _____ 26 Dec 00
Janice O. Kuhn, Ph.D., D.A.B.T. Date
Study Director
STILLMEADOW, Inc.

Approved: _____ 6 Dec 00
Mark S. Holbert Date
Vice President
STILLMEADOW, Inc.

Reviewed: _____ 6 Dec. 2000
Vicki S. Crutchfield, RQAP Date
Director, Quality Assurance Unit
STILLMEADOW, Inc.

Sponsor: Miller Chemical and Fertilization Corp.
P.O. Box 333
Hanover, PA 17331

Sponsor Representative: Mandava Associates
1730 M Street, N.W., Suite 906
Washington, DC 20036

Approved: _____ December 26, 2000
N. Bhushan Mandava Date
Agent to Miller Chemical and Fertilization Corp.

PROTOCOL FOR STUDY 6207-00

A. GENERAL

1. Study Title: ACUTE DERMAL TOXICITY STUDY IN RABBITS

2. Purpose: To assess the systemic toxicity and relative skin irritancy of a test substance when applied to the skin of rabbits.

3. Regulatory Compliance: This study will be conducted according to OPPTS 870.1200, which is intended to meet testing requirements of both FIFRA (7 U.S.C., 136 *et seq.*) and TSCA (15 U.S.C. 2601).

This study will be conducted in compliance with Good Laboratory Practice Standards:

1. EPA FIFRA: 40 CFR 160
2. EPA TSCA 40 CFR 792
3. OECD: C(97)186
4. Japanese MAFF

All procedures in this protocol are in compliance with Animal Welfare Act Regulations. All methods can be found in STILLMEADOW, Inc. Standard Operating Procedures (SOPs).

4. Quality Assurance: The Protocol is reviewed by the Quality Assurance Unit (QAU). The study information will be entered into the Master Schedule. The study will be inspected at least once during its progress. Further inspections may be scheduled as needed to ensure the integrity of the study. Any deviations from SOPs, the Protocol, or Good Laboratory Practice Standards will be immediately reported to the Study Director and Management. The report will be audited and a statement prepared and signed which shall specify the dates inspections were made and findings reported to Management and to the Study Director.

5. Test Substance: MILLER 6064. Test substance identification will include the name, batch number, and purity. Information regarding safety, stability, storage conditions, and disposal will also be provided by the Sponsor. The Sponsor assumes responsibility for purity and stability determinations. Analysis and archiving of reserve samples will be the responsibility of the Sponsor.

6. Proposed Schedule: Testing will begin within approximately three weeks of receipt of the test substance and authorization to conduct the study.

Proposed Start Date: 21 Dec 00
Proposed End Date: 04 Jan 01

The study will be extended if several dose levels are required.

A. GENERAL (cont.)

7. Study Director:	Janice O. Kuhn, Ph.D., D.A.B.T.
8. Experimental Summary:	The test substance will be applied to the intact skin of albino rabbits and maintained in contact with the skin for 24 hours. The animals will be observed three times on the day of dosing and once daily thereafter for mortality and for signs of pharmacologic and/or toxicologic effects, for at least 14 days. If a sufficient number of dose levels are tested, an LD_{50} with slope function and 95% confidence limits will be calculated and/or a Toxicity Category will be assigned.
9. Protocol Amendments:	Any alteration in the Protocol will be justified, approved by the Study Director, and recorded in writing.
10. Sponsor Audits:	The Sponsor may send an authorized representative to inspect the test system and/or data on the STILLMEADOW, Inc. premises during normal working hours.

APPENDIX B (cont.)

B. EXPERIMENTAL DESIGN

1. Animals

a. Species:	Albino rabbit
b. Strain/Source:	New Zealand White (Ray Nichols Rabbitry, Lumberton, Texas or other suitable source)
c. Justification of Species:	The rabbit is conventionally used in dermal toxicity tests to provide information on which human hazard can be judged, and is one of the species preferred by the regulatory agencies.
d. Quantity and Sex:	At least five males and five females (nulliparous and non-pregnant) for the initial dose level; additional animals may be required (refer to B.3.g.).
e. Age/Weight:	Young adult (12 weeks - 6 months); approximately 2 - 4 kg. Weight variation should not exceed ±20% of the mean for each sex.
f. Identification:	Ear tag
g. Acclimation and Health Status:	Animals will be acclimated for at least five days prior to testing. Normal weight gain, appearance, and behavior will be factors used to select healthy animals for testing. Only naive animals will be selected.
h. Randomization:	Unless a control group is required (see B.3.f.) no formal randomization procedure will be required. If a control group is necessary, a weight-stratified randomization procedure will be employed.

2. Animal Husbandry

a. Cages:	Stainless steel, suspended, wire bottom
b. Number per Cage:	Animals will be individually housed.
c. Food:	A measured amount of PMI Feeds, Inc.™ Laboratory Rabbit Diet #5321. The feed is routinely analyzed by the manufacturer for nutritional components and environmental contaminants.
d. Water:	Tap water; available *ad libitum* (automatic system). Municipal water supply analyzed by Texas Natural Resource Conservation Commission (TNRCC) Water Utilities Division.
e. Contaminants:	There are no known contaminants in the feed or water available to laboratory animals that would be expected to interfere with this study.
f. Environment:	Target temperature: approximately 20°C ± 3°C. Target relative humidity: approximately 30 - 70%. 12-hour light/dark cycle (regulated automatically), and room ventilation of approximately 10 - 12 air changes per hour.

B. EXPERIMENTAL DESIGN (cont.)

3. Test Substance Administration

a. Preparation of Animals: Animals will be prepared by clipping the back of the trunk of each animal free of hair to expose not less than 10% of the total body surface area. Care will be taken to avoid abrading the skin. Clipping of the animals will be done at least 12 hours before dosing. Animals with exposure areas free from pre-existing skin irritation or defects will be selected for testing. The animals will be clipped as needed throughout the study.

b. Reason for Route of Administration: Dermal contact is a potential route of human exposure.

c. Assignment of Animals to Groups: Animals will be randomly selected for dose groups so that individual body weights will not exceed ±20% of the mean weight for each sex.

d. Preparation of Test Substance: The test substance will be administered as received. If the substance is an aerosol, it will be discharged into a container and administered as a liquid.

e. Application of Test Substance: All animals will receive a single administration of the test substance on Day 0 based on the body weight on the day of treatment. The test substance will be applied evenly to the exposure area to make as thin and uniform a layer as possible under a 4-ply surgical gauze square. If the test substance is a solid, it will be pulverized, if necessary, and moistened with a sufficient quantity of deionized water or saline to make a thick paste before application. If an aqueous vehicle is not appropriate, corn oil should be considered first. Other acceptable vehicles include gum arabic, ethanol plus water, glycerol, propylene glycol, carboxymethyl cellulose, PEG, vegetable oil, and mineral oil. Justification for use of a vehicle other than water or saline must be supplied in the report. If the test substance is a liquid, it will be applied as received. The area of application will be covered with an appropriately sized 4 ply gauze patch (4 in x 8 in. or larger if necessary) and secured with non-irritating adhesive tape. The trunk of each animal will be wrapped with stockinette and secured in place with non-irritating adhesive tape.

f. Removal of Test Substance: The wrappings will be removed after a 24-hour exposure period. Depending on the presence of test substance residue, the skin may be wiped with a dry cloth or washed with room temperature tap water and dried with a dry cloth to remove as much residual test substance as possible. Control animals, if any, will be treated in a similar manner.

B. EXPERIMENTAL DESIGN (cont.)

3. Test Substance Administration (cont.)

g. Dose Level: Unless available toxicologic information indicates otherwise, a test will be conducted with five animals per sex at a dose level of approximately 5050 mg/kg unless 2020 mg/kg is requested by the Sponsor. If no mortality occurs among these animals, no further testing is necessary.

If mortality meets or exceeds 40% in either or both sexes at the level tested, then at least two additional dose levels may be tested for those sexes. There will be at least five animals (five males or five females) per dose level. The number and spacing of dose levels will be chosen so that an LD_{50} and/or a Toxicity Category can be determined. If both sexes are tested at a given dose level, the group will consist of an equal number of males and females. If animals of one sex are markedly more sensitive, testing of animals of the other sex may be dispensed with.

h. Control Groups: If the test substance is administered as received, a control group is not necessary. If the test substance is administered in a vehicle for which the toxicity is not known, a vehicle control group (five males and five females) will be required.

4. Observations

a. Clinical Signs: Observations for mortality and signs of pharmacologic and/or toxicologic effects will be made three times on the day of dosing, and once daily thereafter for 14 days. The duration should be determined by the toxic reactions and may be extended beyond 14 days when considered necessary. The nature, onset, severity, and duration of all gross or visible pharmacologic or toxicologic signs will be recorded.

Observations will include, but not be limited to evaluation of skin, fur, eyes and mucous membranes, respiratory and circulatory effects, autonomic effects such as salivation, lacrimation, excessive urination, diarrhea, central nervous system effects, including tremors and convulsions, changes in the level of activity, gait and posture, reactivity to handling or sensory stimuli, altered strength, and stereotypies or bizarre behavior (e.g., self mutilation, walking backwards).

b. Body Weights: Body weights will be recorded on the day of dosing (Day 0), and weekly thereafter (Days 7 and 14), or at the time of discovery after death.

c. Dermal Irritation: Approximately 60 minutes after removal of wrappings, the exposure area of each animal will be examined for evidence of dermal irritation (Appendix A). Additional observations for dermal irritation will be made on Study Days 4, 7, 11, and 14.

B. EXPERIMENTAL DESIGN (cont.)

4. Observations (cont.)

d. Sacrifice of Animals: All surviving animals will be sacrificed with a cardiac injection of Fatal Plus® (Vortech Pharmaceuticals, Dearborn, Michigan 48126).

e. Necropsy: A gross necropsy will be conducted on each animal at termination of the study or at the time of discovery after death, and the results recorded. The gross necropsy shall include terminal body weight, and gross observations of external surfaces; all orifices; and thoracic, abdominal, and pelvic cavities.

5. Evaluation of Results: Unless only a single dose level is tested, an LD_{50} with slope function and 95% confidence limits will be calculated for males, females, and males and females combined (if necessitated by mortality in one or both sexes) by the method of Litchfield and Wilcoxon (Litchfield, J.T., Jr., and Wilcoxon, F.: A Simplified Method of Evaluating Dose-Effect Experiments, J. Pharm. & Exp. Ther., 96, 99-115, 1949) or other appropriate method. If requested by the Sponsor, a Toxicity Category may be determined instead of an LD_{50} or in addition to an LD_{50}.

6. Test Substance Accountability: A comprehensive inventory of test substance received and used will be kept. The test substance container(s) will be weighed when received at this facility, and a record of all test substance use will be maintained. Test substance and test substance dosing solutions will be stored in the original containers, or in the equivalent thereof, or in glass containers with Teflon-lined caps.

7. Disposal of Unused Test Substance: Unused test substance will be returned or disposed of at the Sponsor's expense after the termination of the study. The Sponsor should specify return or disposal at the time of study authorization. If returned, the recipient of the test substance will be notified in advance of shipping. The sample will be accompanied by a chain of custody letter describing the sample(s) contained in the shipment.

8. Safety Precautions: General safety precautions required by laboratory SOPs will be followed. The Sponsor will supply basic toxicity data on the test substance to be used. However, since the toxicity of test substances is often not well characterized, this laboratory will be conservative in setting safety procedures. The Sponsor or Sponsor's Representative shall be notified of any exposures requiring a physician's examination or care.

C. DATA MANAGEMENT

1. Records:

The following records will be maintained during the study and transferred to the STILLMEADOW, Inc. archives upon study termination.

a. Protocol and Protocol Amendments (if any).
b. Final report and amendments (if any).
c. Study correspondence.
d. Animal receipt/acclimation data.
e. Test substance receipt, identification as supplied by Sponsor, preparation, administration and disposition.
f. Test animal information: species, strain, sex, source and number.
g. Body weight data.
h. Daily observation data for signs of pharmacologic and/or toxicologic effects.
i. Mortality data, gross necropsy findings, and histopathology findings, if requested.
j. Calculations (if any) of the LD_{50} and slope determinations with 95% confidence limits.
k. Observations for dermal irritation.
l. Other pertinent data.

2. Data Storage:

All raw data will be retained at STILLMEADOW, Inc.

3. Data Reporting:

The final report will include all data as described in the Good Laboratory Practice Standards, including:

a. Statement from the Quality Assurance Unit.
b. Signature of the Study Director.
c. A GLP Compliance Statement signed by the Study Director.
d. Names of scientific personnel involved in the study.
e. Dates of study initiation and termination.
f. Identification, description, and storage of the test substance.
g. All pertinent animal data, animal husbandry, dosing information, and observation methods.
h. Description of the test procedures.
i. If calculated, the LD_{50} and slope function data with 95% confidence limits for males, females, and males and females combined (if necessitated by mortality in one or both sexes), and/or the Toxicity Category.
j. Individual body weights.
k. Observations on the nature, onset, severity, and duration of all gross or visible pharmacologic and/or toxicologic signs. Nonroutine findings will be addressed in a discussion section in which the relationship to treatment and historical data will be evaluated.
l. Individual mortality data, gross necropsy findings, and histopathology findings, if applicable.
m. Dermal irritation observations.
n. A reference to this Protocol.

4. Report Submission:

A report will be submitted after termination of the in-life portion of the study (subject to completion of histopathology, if requested).

APPENDIX B (cont.)

Appendix A
ACUTE DERMAL TOXICITY STUDY IN RABBITS
Evaluation of Skin Reactions

Primary Dermal Irritation Scoring Scale
(Draize Technique)

Erythema and Eschar Formation	Score
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Maximum Possible	4

Edema Formation	Score
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4
Maximum Possible	4

Other observations may be made when needed, for example: Staining of the test site skin, necrosis, blanching, desquamation, sloughing, eschar, coriaceousness (leathery texture), atonia, etc.

ATTACHMENT 36

Acute Inhalation Toxicity Study in Rats on Miller 6064

STILLMEADOW
INCORPORATED

VOLUME _ OF _ OF SUBMISSION

Miller 6064

FINAL REPORT

ACUTE INHALATION TOXICITY STUDY IN RATS

OPPTS NO. 870.1300

AUTHOR:

Lori Carter, B.A.

STUDY INITIATION DATE: 26 December 2000
STUDY COMPLETION DATE: 22 March 2001

CONDUCTED BY:
STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, TX 77478

LABORATORY STUDY NUMBER:

6208-00

VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 29

SUBMITTED TO:
Miller Chemical and Fertilization Corp.
P.O. Box 333
Hanover, PA 17331

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA § 10 (d) (1) (A), (B) or (C).

Company: Miller Chemical and Fertilization Corp.

Company Agent: _____ Date: _____

_____ _____

Title Signature

These data are the property of Miller Chemical and Fertilization Corp., and as such, are considered to be confidential for all purposes other than compliance with FIFRA § 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other statute or in any other country.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was designed and performed in STILLMEADOW, Inc.'s AAALAC accredited laboratory in compliance with:

- United States Environmental Protection Agency FIFRA; GLP Standards 40 CFR 160 with exception of Sec. 160.31 (d), and 160.105 (b)(e): stability information was not provided
- United States Environmental Protection Agency TSCA 40 CFR 792 with exception of Sec. 792.31 (d), and 792.105 (b)(e): stability information was not provided
- Organization for Economic Cooperation & Dev. Principles of GLP, Annex 2, C(97)186 with exception of Sec. 6.2 (4): stability information was not provided
- Japan Ministry of Agriculture, Forestry & Fisheries, Notification No. 59 Nohsan 3850, Director-General of Agricultural Prod. Bureau, 10 Aug 84 with exception of Art. 5 (2)(9), and 21 (3): stability information was not provided

Lori Carter — 22 Mar 01

Lori Carter, B.A.
Study Director, STILLMEADOW, Inc. — Date

Signature of Agent of Sponsor — Date

Agent Name
Sponsor: Miller Chemical and Fertilization Corp.

Signature of Agent of Submitter — Date

Agent Name
Submitter: Mandava Associates

TABLE OF CONTENTS

QUALITY ASSURANCE STATEMENT

Study Number: 6208-00
Test Substance: Miller 6064
Study Title: Acute Inhalation Toxicity Study in Rats

The study has been inspected and the report and data have been audited in accordance with STILLMEADOW, Inc. Standard Operating Procedures (SOPs). The findings from the inspection and audit were reported to the Study Director and Management as follows:

Phase of Study Inspected	Date Inspected	Reported to Study Director	Reported to Management
Body weights	16 Jan 01	17 Jan 01	17 Jan 01
Report/Data Audit	23 Feb 01	23 Feb 01	23 Feb 01

B. Lynn Murphy, B.S.
Quality Assurance Unit, STILLMEADOW, Inc.

23 March 01
Date

SUMMARY

The test substance, Miller 6064, was evaluated for its acute inhalation toxicity potential in albino rats. Five males and five females were exposed for four hours to an aerosol generated from the undiluted liquid test substance at a level of 5.26 mg/L. There was no mortality during the study. Clinical signs included activity decrease, respiratory gurgle and chirp, which were no longer evident by Day 6. Body weights were unaffected by exposure, except in two animals that lost weight during the first week. The gross necropsy revealed no observable abnormalities. As indicated by the data, the acute inhalation LC_{50} is greater than 5.26 mg/L.

INTRODUCTION

The objective of this study was to determine the acute inhalation toxicity potential of the test substance in accordance with US EPA OPPTS 870.1300, which is intended to meet testing requirements of FIFRA 7 USC 136, et seq, and TSCA 15 USC 2601. This study was conducted for Miller Chemical and Fertilization Corp., according to the approved protocol and STILLMEADOW, Inc. SOPs. There were no deviations from the protocol which affected the quality or outcome of the study. All procedures used in this study are in compliance with Animal Welfare Act Regulations. The protocol, raw data and this report are archived at STILLMEADOW, Inc. The animals were exposed as follows:

Beginning of 4 Hour Exposure					Termination of In-Life Observations	
Dose (mg/L)	Males		Females		Date	
	Date	Time	Date	Time	Males	Females
5.26	9 Jan 01	0745	9 Jan 01	0745	23 Jan 01	23 Jan 01

TEST SUBSTANCE

Identification:	Miller 6064
Date & Quantity Received:	19 Dec 00; 2 x 1 gal
Physical Description:	Amber liquid
Storage:	Room temperature
Purity & Composition:	Refer to Certificate of Analysis (Appendix B)
Stability:	Not provided by sponsor

Records pertaining to stability, characterization, and verification of test substance identity are the responsibility of the sponsor. A copy of the Certificate of Analysis is included in the report.

TEST SYSTEM

Experimental Animals

Species & Strain:	Rat; Sprague-Dawley
Justification of Species:	The rat is a representative rodent species preferred by various regulatory agencies for use in acute inhalation toxicity studies.
Source:	Texas Animal Specialties, Humble, TX
Quarantine Period:	5 days
Date Received:	4 Jan 01
Quantity & Sex:	5 males and 5 females (nulliparous and non-pregnant)
Animal Identification:	Ear punch and cage cards
Weight When Tested:	Males: 268-307 g; Females: 189-194 g
Date of Birth:	14 Nov 00

Animal Husbandry

Cage Type:	Suspended, wire bottom, stainless steel
Housing:	One per cage
Environmental Controls Set to Maintain:	·Temperature Range 22°C±3° ·Humidity Range 30-70% ·12-hour light/dark cycle ·10-12 air changes/hour
Food:	PMI™ Lab Diet Formula #5008, available *ad libitum* except during the exposure period
Water:	Municipal water supply analyzed by TNRCC Water Utilities Division; from automatic water system, available *ad libitum* except during the exposure period

Animal husbandry and housing at STILLMEADOW, Inc. comply with Animal Welfare Act Regulations. No contaminants were expected to have been present in the feed or water which would have interfered with or affected the results of the study.

PROCEDURES

Prestudy Testing

Trial assays were conducted to determine which method(s) of aerosolizing the test substance into the exposure chamber would produce an acceptable concentration and mass median aerodynamic diameter (MMAD).

Exposure Chamber

A 500 L nose-only stainless steel, dynamic flow inhalation chamber was utilized in this experiment (Diagram 1). The body of the chamber has 25 ports in 5 rows. Polycarbonate tubes are inserted into 10 designated individual ports. The test substance is introduced through the opening in the top of the chamber. The bottom section has a corresponding air outlet and a drain valve for cleaning the chamber. The individual polycarbonate tubes are tapered at one end to fit the shape of the animal's head and the back portion is sealed with a rubber cap. The tubes containing the animals fit tightly into the ports, and are sealed with "O" rings.

PROCEDURES (cont.)

Generation of Test Atmosphere

The aerosol was generated by a pressure operated Spraying System Company air atomizer (1/4 JSS) which aspirated the test substance from a container, then sprayed the resulting aerosol directly into the exposure chamber. Air flow into the chamber was maintained through the use of a calibrated orifice plate at a rate of 13.6 air changes per hour. Air flow was recorded at 30 minute intervals during the exposure period, and was sufficient to ensure an oxygen content of at least 19% of the exposure atmosphere. Temperature and humidity were recorded at 30 minute intervals during the exposure period from a Taylor wet bulb/dry bulb hygrometer located in the exposure chamber.

Test Substance Administration

Healthy albino rats were released from quarantine, and five males and five females were selected for testing. The animals were exposed to an aerosol generated from the undiluted liquid test substance for a period of four hours. When 99% concentration (t-99) was attained, the animals which were individually housed in polycarbonate exposure tubes were inserted into a 500 L stainless steel nose-only inhalation chamber for the specified exposure period. At the termination of the exposure period, the animals were returned to their stock laboratory cages.

Determination of Concentration

The concentration of test substance in the exposure atmosphere (taken from the breathing zone of the animals) was determined analytically once per hour and nominally at the end of the exposure. The analytical determination was made using a Beckman DU-65 UV Spectrophotometer (Appendix A). The nominal concentration was determined by dividing the loss in weight of the test substance after the exposure by the total volume of air which passed through the chamber.

Particle Size Distribution

Particle size, taken from the breathing zone of the animals, was determined twice during the exposure, using a cascade impactor, at a rate of 8.5 L/minute for a duration of 30 seconds. The MMAD and particle size distributions are calculated from these data by a computer program utilizing probit analysis.

In-life Observations

Observations for mortality and signs of pharmacologic and/or toxicologic effects were made frequently on the day of exposure and at least once daily thereafter for 14 days (Day 0 is day of exposure). Individual body weights were recorded just prior to the inhalation exposure and on Days 7 and 14.

PROCEDURES (cont.)

Postmortem Observations

On Day 14 after exposure, each animal was euthanized by an intraperitoneal injection of Fatal Plus® (Vortech Pharmaceuticals, Dearborn, MI 48126). All study animals were subjected to gross necropsy, and all abnormalities were recorded.

Statistical Analysis

In order to calculate a mean exposure, the Mean Value Theorem of Calculus was used to properly weight the concentration, since the concentrations could not be measured continuously (see Table 4). This method weights concentrations based on the time span of each concentration. A concentration can be calculated for each minute, which better represents the exposure concentration received by each animal.

RESULTS AND DISCUSSION

Mortality/Estimated LC_{50} Values

There was no mortality during the study. As indicated by the data, the acute inhalation LC_{50} for Miller 6064 is greater than 5.26 mg/L.

Body Weights

Individual body weights are presented in Table 1. Body weight gain was unaffected by the administration of the test substance, except in one male and one female that lost 1-6 g during the first week.

Clinical Signs

Clinical signs are presented in Table 2. Prominent in-life observations included activity decrease, respiratory gurgle and chirp. Animals were asymptomatic by Day 6.

Necropsy Findings

Individual necropsy findings are presented in Table 1. The gross necropsy conducted on each animal at termination of the study revealed no observable abnormalities.

Inhalation Chamber Conditions

Chamber operating parameters are presented in Table 3. Concentration determinations and calculations are presented in Table 4. Particle size distributions are presented in Table 5. Pretest data are presented in Table 6. The exposure concentration of 5.26 mg/L had an average MMAD of 3.6 µm.

CONCLUSION

Miller 6064 was evaluated for its acute inhalation toxicity potential in albino rats. As indicated by the data, the acute inhalation LC_{50} is greater than 5.26 mg/L in males and females.

Lori Carter — 22 Mar 01

Lori Carter, B.A.
Study Director, STILLMEADOW, Inc.

Date

STUDY PERSONNEL

Technical Staff: Liangbao Rao, B.S.
Hector Fuentes
Paul H. Siemens, B.A.

Data Services: Connie Pavatte, Report Preparation

TABLE 1
ACUTE INHALATION TOXICITY STUDY IN RATS
Body Weights, Time of Death, and Gross Necropsy
Test Substance: Miller 6064
Exposure Concentration: 5.26 mg/L

Animal Number	Body Weights (g)			Time of Death*	Gross Necropsy Findings
	Day 0	Day 7	Final		
91-M	276	281	289	Day 14	NOA
92-M	268	262	296	Day 14	NOA
93-M	279	292	319	Day 14	NOA
94-M	274	278	313	Day 14	NOA
95-M	307	318	351	Day 14	NOA
96-F	194	193	220	Day 14	NOA
97-F	193	232	242	Day 14	NOA
98-F	189	199	215	Day 14	NOA
99-F	190	201	251	Day 14	NOA
100-F	192	195	213	Day 14	NOA

* - Day of dosing considered Day 0; Day 14 is terminal sacrifice.
M - Male; F - Female; NOA - No Observable Abnormalities

TABLE 2 (cont.)
ACUTE INHALATION TOXICITY STUDY IN RATS
Pharmacologic and/or Toxicologic Signs
Test Substance: Miller 6064
Exposure Concentration: 5.26 mg/L
Sex: Males and Females

	Time After Exposure Begins																	
	HOURS					DAYS												
Reaction and Severity	0.5	1.0	2.5	4.5	6.0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Males																			
Respiratory gurgle (v-s)	0	0	0	4	5	5	5	5	5	5	0	0	0	0	0	0	0	0	0
Activity decrease (v-s)	0	0	0	0	4	4	5	5	1	0	0	0	0	0	0	0	0	0	0
Females																			
Respiratory gurgle (v)	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
Respiratory chirp (v-s)	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

v - very slight; s - slight; m - moderate; e - extreme
Note: Digits indicate number of animals exhibiting reaction.

TABLE 3
ACUTE INHALATION TOXICITY STUDY IN RATS
Chamber Operating Parameters
Test Substance: Miller 6064

Exposure Concentration: 5.26 mg/L

Time (Hour)	Temp. (°F)	RH (%)	Air Flow (Lpm)
0.0	71	42	113
0.5	71	39	113
1.0	71	39	113
1.5	72	40	113
2.0	72	40	113
2.5	72	40	113
3.0	72	40	113
3.5	72	40	113
4.0	72	40	113
Mean:	72	40	113

t-99 Determination

Initial Chamber Air Flow	113 Lpm
Exposure Chamber Size	500 L
Baffling Chamber	Not used
t-99 Value	21 min

Air Atomizer Setting

Sprayer Air Flow	127 Lpm
Sample Intake	~1.3 mL/min

TABLE 4
ACUTE INHALATION TOXICITY STUDY IN RATS
Analytical Concentration Calculations
Test Substance: Miller 6064

Standard Curve

Standard	Conc. (mg/mL)	Abs. @ 270 nm
A	1.5360	2.2490
B	1.1520	1.7170
C	1.0240	1.5455
D	0.7550	1.1680
E	0.5662	0.9035
F	0.5033	0.8090

Corr (r) = 0.999970 y intercept = 0.114211 slope = 1.391966

Calculation for 5.26 Concentration

Sample No	Abs. @ 270 nm	Conc. (mg/ml)	Multi. Factor*	Chamber Conc. (mg/L)
1	1.5600	1.03867	x 4.99	5.183
2	1.5975	1.06561	x 4.99	5.317
3	1.6010	1.06812	x 4.99	5.330
4	1.5705	1.04621	x 4.99	5.221

* - Multiplication factor (mL/L) calculated from figures in Appendix A using following formula:

$$\frac{\text{Solvent volume}}{(\text{Duration})(\text{Sample rate})} = \text{Multifactor}$$

TABLE 4 (cont.)
ACUTE INHALATION TOXICITY STUDY IN RATS
Analytical Concentration Determination
Test Substance: Miller 6064

	Dose Level: 5.26 mg/L	
Event*	Time Period	Concentration
Start-up	0724	
t-99 (begin exposure)	0745	
Extrapolation calculated	0745-0800	5.183 mg/L
Sample 1 taken	0800-0805	5.183 mg/L
Interpolation calculated	0805-0900	5.250 mg/L
Sample 2 taken	0900-0905	5.317 mg/L
Interpolation calculated	0905-1000	5.324 mg/L
Sample 3 taken	1000-1005	5.330 mg/L
Interpolation calculated	1005-1100	5.275 mg/L
Sample 4 taken	1100-1105	5.221 mg/L
Extrapolation calculated	1105-1145	5.221 mg/L
End exposure	1145	
MEAN EXPOSURE CONC.		5.26 mg/L
Nominal Concentration		29.1 mg/L

* - Sample # taken is the concentration measured during the sampling period.
Extrapolation is the measured concentration of the adjacent event period carried over to the present event period.
Interpolation is the concentration calculated as the average of the measured concentration before and after the present event period.
Mean exposure concentration is the sum of the actual time weighted concentrations divided by the sum of elapsed times and represents the mean value of the exposure concentration.

TABLE 5
ACUTE INHALATION TOXICITY STUDY IN RATS
Particle Size Distribution*
Test Substance: Miller 6064
Concentration: 5.26 mg/L
½ Hour Distribution

Stage	Size Range (µm)	EPD** (µm)	Amount Collected (mg)	% in Size Range	Cumulative % Less Than Size Range
1	>16.7	16.7	0.1	1.09	98.91
2	10.0 - 16.7	10.0	0.9	9.78	89.13
3	4.0 - 10.0	4.0	2.4	26.09	63.04
4	2.4 - 4.0	2.4	2.4	26.09	36.96
5	1.5 - 2.4	1.5	2.6	28.26	8.70
6	0.9 - 1.5	0.9	0.8	8.70	0.00
7	0.5 - 0.9	0.5	0.0	0.00	0.00
8	0.3 - 0.5	0.3	0.0	0.00	0.00
Backup Filter	0.0 - 0.3	0.0	0.0	0.00	0.00

Calculated CHI^2 = 43.0 with 6 Degrees of Freedom.
Values of T and CHI^2 for P=.05 are: T = 2.45 CHI^2 = 12.6

Particle Size (Microns)	% of Particles Collected
≤ 0.3	5
≤ 0.8	16
≤ 3.2	50
≤ 13.5	84
≤ 34.0	95

Mass Median Aerodynamic Diameter = 3.2 µm
Geometric Standard Deviation = 4.2

* - Finney, D.J.: PROBIT ANALYSIS, 3rd ed., Chapters 3 and 4, 1971, Cambridge University Press.
** - Equivalent particle diameter @ 8.5 Lpm

TABLE 5 (cont.)
ACUTE INHALATION TOXICITY STUDY IN RATS
Particle Size Distribution*
Test Substance: Miller 6064
Concentration: 5.26 mg/L
3 ¾ Hour Distribution

Stage	Size Range (µm)	EPD** (µm)	Amount Collected (mg)	% in Size Range	Cumulative % Less Than Size Range
1	>16.7	16.7	0.9	4.69	95.31
2	10.0 - 16.7	10.0	3.0	15.63	79.69
3	4.0 - 10.0	4.0	5.5	28.65	51.04
4	2.4 - 4.0	2.4	4.4	22.92	28.13
5	1.5 - 2.4	1.5	4.1	21.35	6.77
6	0.9 - 1.5	0.9	1.3	6.77	0.00
7	0.5 - 0.9	0.5	0.0	0.00	0.00
8	0.3 - 0.5	0.3	0.0	0.00	0.00
Backup Filter	0.0 - 0.3	0.0	0.0	0.00	0.00

Calculated CHI^2 = 40.2 with 6 Degrees of Freedom.
Values of T and CHI^2 for P=.05 are: T = 2.45 CHI^2 = 12.6

Particle Size (Microns)	% of Particles Collected
≤ 0.3	5
≤ 0.8	16
≤ 4.0	50
≤ 19.0	84
≤ 51.7	95

Mass Median Aerodynamic Diameter = 4.0 µm
Geometric Standard Deviation = 4.7

* - Finney, D.J.: PROBIT ANALYSIS, 3rd ed., Chapters 3 and 4, 1971, Cambridge University Press.
** - Equivalent particle diameter @ 8.5 Lpm

TABLE 6
ACUTE INHALATION TOXICITY STUDY IN RATS
Pretest Data
Test Substance: Miller 6064

Trial assays were conducted to ascertain results, summarized below, under different chamber conditions.

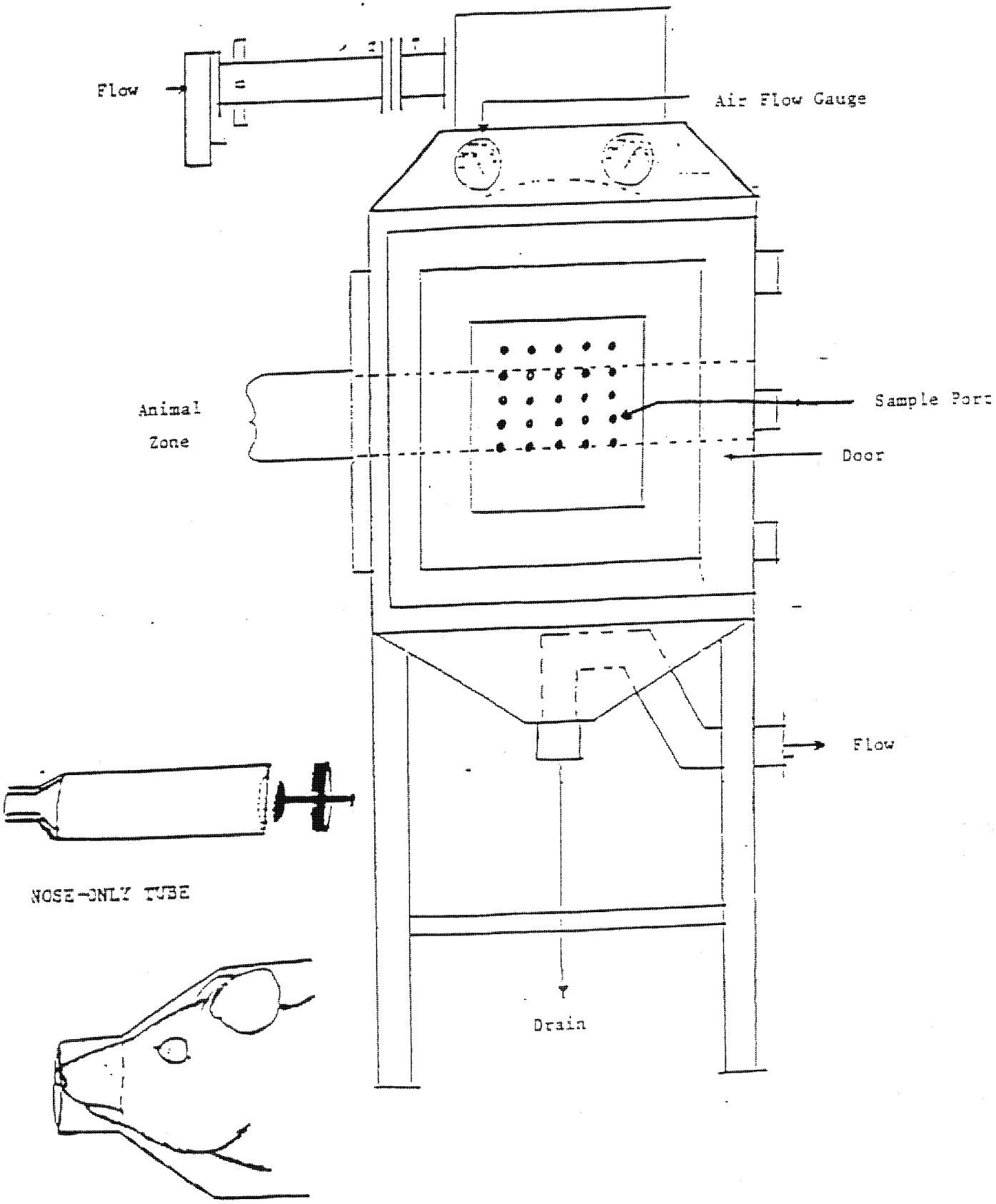
MMAD (um)	Concentration (mg/L)
ND	7.20
4.2	5.02
3.6	3.92

ND - Not determined

Exposure concentration - 5.26 mg/L with an average MMAD of 3.6 µm

Diagram 1

NOSE-ONLY INHALATION CHAMBER



APPENDIX A
ACUTE INHALATION TOXICITY STUDY IN RATS
Operating Parameters for UV Analysis
Test Substance: Miller 6064

Instrument	Beckman DU-65 UV Spectrophotometer
Wavelength	270 nm
Extraction Solvent	1,4 Dioxane
Sampling Method	Double impingement into 25 mL of solvent
Sampling Rate	1.002 Lpm
Sampling Duration	5 minutes
Volume of Chamber Air Sampled	5.01 L

APPENDIX B

MILLER® CHEMICAL & FERTILIZER CORPORATION

P O. BOX 333, 120 RADIO ROAD
HANOVER, PENNSYLVANIA 17331 U.S.A.
TELEPHONE: 717-632-8921
FAX NO.: 717-632-4561

1/18/01

CERTIFICATE OF ANALYSIS

Product Name: MILLER 6064
Quantity Produced: 7.4 kilograms
Batch Number: 150386
Date of Formulation: 12/13/00

Appearance: Yellow to Amber Liquid
Odor: Terpenic
Moisture: Negligble (non-aqueous)
Specific Gravity: 0.92 - 0.94 @ 20c
Viscosity @ 20c: 100 to 500 cps
pH(1% solution): 6.0 to 8.0
Solubility in Water: Insoluble (Emulsifies)
Flash Point: over 200 f
Purity: 100%

Signature:

Name: Andrew G. Smith
Title: Compliance Officer

APPENDIX C

STILLMEADOW
INCORPORATED

PROTOCOL FOR STUDY 6208-00

Study Title: ACUTE INHALATION TOXICITY STUDY IN RATS
(OPPTS 870.1300)

Test Substance: MILLER 6064

Test Facility: STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, Texas 77478

Approved: Lori Carter — 26 Dec 00 (Date)
Lori Carter, B.A.
Study Director
STILLMEADOW, Inc.

Approved: [signature] — 6 Dec 00 (Date)
Mark S. Holbert
Vice President
STILLMEADOW, Inc.

Reviewed: [signature] — 6 Dec 2000 (Date)
Vicki S. Crutchfield, RQAP
Director, Quality Assurance Unit
STILLMEADOW, Inc.

Sponsor: Miller Chemical and Fertilization Corp.
P.O. Box 333
Hanover, PA 17331

Sponsor Representative: Mandava Associates
1730 M Street, N.W., Suite 906
Washington, DC 20036

Approved: N. Bhushan Mandava — December 26, 2000 (Date)
N. Bhushan Mandava
Agent to Miller Chemical and Fertilization Corp.

PROTOCOL FOR STUDY 6208-00

A. GENERAL

1. Study Title: ACUTE INHALATION TOXICITY STUDY IN RATS

2. Purpose: To determine the acute inhalation toxicity potential of the test substance in rats.

3. Regulatory Compliance: This study will be conducted according to OPPTS 870.1300, which is intended to meet testing requirements of both FIFRA (7 U.S.C. 136, *et seq.*) and TSCA (15 U.S.C. 2601).

This study will be conducted in compliance with Good Laboratory Practice Standards:
1. EPA FIFRA: 40 CFR 160
2. EPA TSCA: 40 CFR 792
3. OECD: C(97)186
4. Japanese MAFF

All procedures in this protocol are in compliance with Animal Welfare Act Regulations. All methods can be found in STILLMEADOW, Inc. Standard Operating Procedures (SOPs).

4. Quality Assurance: The Protocol is reviewed by the Quality Assurance Unit (QAU). The study information will be entered into the Master Schedule. The study will be inspected at least once during its progress. Further inspections may be scheduled as needed to ensure the integrity of the study. Any deviations from SOPs, the Protocol, or Good Laboratory Practice Standards will be immediately reported to the Study Director and Management. The report will be audited and a statement prepared and signed which shall specify the dates inspections were made and findings reported to Management and to the Study Director.

5. Test Substance: MILLER 6064. Test substance identification should include the name, batch number and purity. Information regarding safety, stability, storage conditions and disposal should also be provided by the Sponsor. The Sponsor assumes responsibility for purity and stability determinations. Analysis and archiving of reserve samples will be the responsibility of the Sponsor.

6. Proposed Schedule: Definitive study or necessary preliminary analyses will begin within 30 days of receipt of test substance and authorization to conduct study. In the event of a delay, Sponsor will be notified within this 30-day period.

Proposed Start Date: 13 Dec 00
Proposed End Date: 27 Dec 00

The in-life portion of each exposure level generally will be 14 days. The study will be extended if several dose levels are required.

APPENDIX C (cont.)

A. GENERAL (cont.)

7. Study Director:	Lori Carter, B.A.
8. Experimental Summary:	The test substance will be administered for 4 hours in either a 15 L nose-only chamber or a 200 or 500 L stainless steel, dynamic flow test chamber either nose-only or whole body. Nominal, gravimetric, and/or analytical determinations of chamber concentration as well as particle size determinations will be made. The animals will be observed frequently on the day of exposure for mortality and signs of pharmacologic and/or toxicologic effects and once daily thereafter for 14 days. Histopathology will be available upon Sponsor request. If a sufficient number of dose levels are tested, an LC_{50} with slope function and 95% confidence limits will be calculated.
9. Protocol Amendments:	Any alteration in the Protocol will be justified, approved by the Study Director, and recorded in writing.
10. Sponsor Audits:	The Sponsor may send an authorized representative to inspect the test system and/or data on the STILLMEADOW, Inc. premises during normal working hours.

B. EXPERIMENTAL DESIGN

1. Animals

a. Species:	Albino rat
b. Strain/Source:	Sprague-Dawley (Texas Animal Specialties, Humble Texas or other suitable source)
c. Justification of Species:	The rat is conventionally used to provide an index of toxicity on which human hazard can be judged, and is preferred by the regulatory agencies.
d. Quantity and Sex:	Five males and five females (nulliparous and non-pregnant) for the initial dose level and 5/sex for any additional dose levels, if required (see B.3.f.).
e. Age/Weight:	Young adult (8 - 12 weeks of age) Males: approximately 225 - 330 grams Females: approximately 175 - 250 grams. Weight variation should not exceed ±20% of the mean for each sex.
f. Identification:	Ear punch
g. Acclimation and Health Status:	Animals will be acclimated for at least five days prior to testing. Normal weight gain, appearance, and behavior will be factors used to select healthy animals for testing. Only naive animals will be selected.

2. Animal Husbandry

a. Cages:	Stainless steel, suspended, wire bottom
b. Number per Cage:	Animals will be housed individually during exposure and observation periods.
c. Food:	PMI Feeds, Inc.™ Formulab #5008; available *ad libitum* prior to and after exposure. Analyzed by manufacturer.
d. Water:	Tap water (available *ad libitum* prior to and after the exposure period). Automatic system. Municipal water supply analyzed by Texas Natural Resource Conservation Commission (TNRCC) Water Utilities Division.
e. Contaminants:	There are no known contaminants in the feed or water available to laboratory animals that would be expected to interfere with this study.
f. Environment:	Target temperature: approximately 22°C ± 3°C Target relative humidity: approximately 30 - 70% 12-hour light/dark cycle (regulated automatically) Room ventilation of approximately 10 - 12 air changes per hour.

B. EXPERIMENTAL DESIGN (cont.)

3. Test Substance Administration

a. Reason for Route of Administration:	Inhalation is a potential route of human exposure.
b. Test Chamber and Housing:	A test substance atmosphere will be established inside either a 15 L nose-only chamber (animals individually housed in polycarbonate tubes) or a 200 or 500 L New York University design, stainless steel, dynamic flow test chamber either nose-only or whole body (animals individually housed in stainless steel wire mesh cages).
c. Preliminary Analysis:	A variety of techniques will be used in an attempt to attain a concentration of 2 mg/L (EPA) or 5 mg/L (OECD) of the test substance in the chamber while also obtaining a particle size distribution with a mass median aerodynamic diameter of 1 - 4 microns. If the particle size generated is too large and a concentration of 2 mg/L (EPA) or 5 mg/L (OECD) is not attained, the Sponsor will be notified. For gaseous test substances, particle size will not be assayed.
d. Dosing:	The test substance will be administered as either an aerosol or a gas in the test chamber. Filtered air will be used for dilution.
e. Duration of Exposure:	Four hours after equilibration of the chamber conditions.
f. Number of Animals and Selection of Dose Levels:	Ten rats (five males and five females) will be exposed for four hours to the optimum concentration determined by pre-exposure testing and/or Sponsor request. If the LC_{50} is shown to be greater than 2 mg/L (EPA) or 5 mg/L (OECD), no further testing will be required. If mortality meets or exceeds 40% in either or both sexes, then at least two additional concentrations of the test substance will be tested for those sexes. There will be at least five animals (five males and/or five females) per exposure level, and the number and spacing of exposure levels will be chosen so that an LC_{50} can be determined. If both sexes are tested at a given exposure level, the group will contain equal numbers of males and females.
g. Control Groups:	At the request of the Sponsor, a sham control, vehicle control, or negative control group can be run concurrently.

B. EXPERIMENTAL DESIGN (cont.)

3. Test Substance Administration (cont.)

h. Operating Parameters: Operating parameters to be measured include air flow, t-99, temperature, humidity, exposure concentration, and particle size.

Air flow: Monitored through the use of a calibrated orifice plate and will be sufficient to insure adequate oxygen content (at least 19%) of the exposure atmosphere. Air flow will equal at least 12 to 15 air changes per hour.

t-99: Calculated for each exposure and depends on the air flow. Start time of exposure will be adjusted accordingly.

Temperature and humidity: Measurements will be taken at 30 minute intervals during the exposure period. (Targeted conditions are approximately 22°±2°C and 40 - 60% RH).

Analytical concentration determination: Determined at least hourly for each exposure concentration, using the procedures supplied by the Sponsor or developed by STILLMEADOW, Inc.

Gravimetric concentration determination: When applicable, at least once per hour.

Nominal concentration: Determined once for each exposure.

Particle size: Determined at least twice with a cascade impactor. Results reported will include the aerodynamic mass median size and geometric standard deviation (aerosols only).

4. Observations

a. Clinical Signs: Observations for mortality and signs of pharmacologic and/or toxicologic effects will be made frequently on the day of exposure and once daily thereafter for 14 days. The duration should be determined by the toxic reactions and may be extended beyond 14 days when considered necessary. The nature, onset, severity, and duration of all gross or visible pharmacologic or toxicologic signs will be recorded.

Observations will include: skin, fur, eyes and mucous membranes, somatomotor activity, and behavior pattern. Particular attention will be given to tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma.

b. Body Weights: Body weights will be recorded on the day of exposure (Day 0), and on Days 7 and 14, or at the time of discovery after death.

B. EXPERIMENTAL DESIGN (cont.)

4. Observations (cont.)

c. Sacrifice of Animals: All surviving animals will be sacrificed with an IP injection of Fatal Plus® (Vortech Pharmaceuticals, Dearborn, Michigan 48126).

d. Necropsy: A gross necropsy will be conducted on each animal at termination of the study or at the time of discovery after death, and the results recorded. The gross necropsy shall include the following:

1. Terminal body weight.
2. Gross observations of external surfaces; all orifices; and thoracic, abdominal, and pelvic cavities.
3. Upon request of the Sponsor, sections of abnormal tissues may be saved in 10% neutral buffered formalin for possible histopathologic examination. Tissues will be discarded if histopathology is not performed.

5. Evaluation of Results: Unless only a single exposure concentration is tested, an LC_{50} with slope function and 95% confidence limits will be calculated for males, females, and males and females combined (if necessitated by mortality in one or both sexes) by the method of Litchfield, J.T., Jr., and Wilcoxon, F.: A Simplified Method of Evaluating Dose-Effect Experiments, Journal of Pharmacology & Experimental Therapeutics, 96, 99-115, 1949, or other appropriate method.

6. Test Substance Accountability: A comprehensive inventory of test substance received and used will be kept. The test substance container(s) will be weighed when received at this facility, and a record of all test substance use will be maintained. Test substance and test substance dosing solutions will be stored in the original containers, or in the equivalent thereof, or in glass containers with Teflon-lined caps.

7. Disposal of Unused Test Substance: Unused test substance will be returned or disposed of at the Sponsor's expense after the termination of the study. The Sponsor should specify return or disposal at the time of study authorization. If returned, the recipient of the test substance will be notified in advance of shipping. The sample will be accompanied by a chain of custody letter describing the sample(s) contained in the shipment.

8. Safety Precautions: General safety precautions required by laboratory SOPs will be followed. The Sponsor will supply basic toxicity data on the test substance to be used. However, since the toxicity of test substances is often not well characterized, this laboratory will be conservative in setting safety procedures. The Sponsor or Sponsor's Representative shall be notified of any exposures requiring a physician's examination or care.

APPENDIX C (cont.)

C. DATA MANAGEMENT

1. Records:

The following records will be maintained during the study and transferred to the STILLMEADOW, Inc. archives upon study termination.

a. Protocol and Protocol Amendments (if any).
b. Final report and amendments (if any).
c. Study correspondence.
d. Animal receipt/acclimation data.
e. Test substance receipt, identification as supplied by Sponsor, administration, and disposition.
f. Test animal information: number, species, age, sex, source, strain.
g. Body weight data.
h. Daily observation data for signs of pharmacologic and/or toxicologic effects.
i. Mortality data and gross necropsy findings, and histopathology data, if requested.
j. Calculations (if any) of the LC_{50} and slope determinations with 95% confidence limits.
k. Chamber operating parameters.
l. Other pertinent data.

2. Data Storage:

All raw data will be retained at STILLMEADOW, Inc.

3. Data Reporting:

The final report will include all data as described in the Good Laboratory Practice Standards, including:

a. Statement from the Quality Assurance Unit.
b. Signature of the Study Director.
c. A GLP Compliance Statement signed by the Study Director.
d. Names of scientific personnel involved in the study.
e. Dates of study initiation and termination.
f. Identification, description, preparation, and storage of the test substance.
g. All pertinent animal data, animal husbandry, exposure information, operating parameters, and observation methods.
h. Description of the test procedures.
i. If calculated, the LC_{50} and slope function data with 95% confidence limits for males, females, and males and females combined (if necessitated by mortality in one or both sexes).
j. Individual body weights.
k. Observations on the nature, onset, severity, and duration of all gross or visible pharmacologic and/or toxicologic signs. Nonroutine findings will be addressed in a discussion section in which the relationship to treatment and historical data will be evaluated.
l. Individual mortality data, gross necropsy findings, and histopathology findings, if applicable.
m. Chamber operating parameters.
n. A reference to this Protocol.

4. Report Submission:

A report will be submitted after termination of the in-life portion of the study (subject to completion of histopathology, if requested).

ATTACHMENT 37

Acute Dermal Irritation Study in Rabbits on Miller 6064

STILLMEADOW
INCORPORATED

VOLUME __ OF __ OF SUBMISSION

Miller 6064

FINAL REPORT

ACUTE DERMAL IRRITATION STUDY IN RABBITS

OPPTS NO. 870.2500

AUTHOR:

Janice O. Kuhn, Ph.D., D.A.B.T.

STUDY INITIATION DATE: 26 December 2000
STUDY COMPLETION DATE: 26 March 2001

CONDUCTED BY:
STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, TX 77478

LABORATORY STUDY NUMBER:

6210-00

VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 23

SUBMITTED TO:
Miller Chemical & Fertilization Corp.
P.O. Box 333
Hanover, PA 17331

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA § 10 (d) (1) (A), (B) or (C).

Company: Miller Chemical & Fertilization Corp.

Company Agent: _____ Date: _____

_____ _____

Title Signature

These data are the property of Miller Chemical & Fertilization Corp., and as such, are considered to be confidential for all purposes other than compliance with FIFRA § 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other statute or in any other country.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was designed and performed in STILLMEADOW, Inc.'s AAALAC accredited laboratory in compliance with:

- United States Environmental Protection Agency FIFRA: GLP Standards, 40 CFR 160 with exception of Sec. 160.31 (d), and 160.105 (b)(e): stability information was not provided
- United States Environmental Protection Agency TSCA 40 CFR 792 with exception of Sec. 792.31 (d), and 792.105 (b)(e): stability information was not provided
- Organization for Economic Cooperation & Dev. Principles of GLP, Annex 2, C(97)186 with exception of Sec. 6.2 (4): stability information was not provided
- Japan Ministry of Agriculture, Forestry & Fisheries, Notification No. 59 Nohsan 3850, Director-General of Agricultural Prod. Bureau, 10 Aug 84 with exception of Art. 5 (2)(9), and 21 (3): stability information was not provided

Janice O Kuhn — 26 Mar 01

Janice O. Kuhn, Ph.D., D.A.B.T.
Study Director, STILLMEADOW, Inc.

Date

Signature of Agent of Sponsor

Date

Agent Name
Sponsor: Miller Chemical & Fertilization Corp.

Signature of Agent of Submitter

Date

Agent Name
Submitter: Mandava Associates

TABLE OF CONTENTS

QUALITY ASSURANCE STATEMENT

Study Number: 6210-00
Test Substance: Miller 6064
Study Title: Acute Dermal Irritation Study in Rabbits

The study report and data have been audited in accordance with STILLMEADOW, Inc. Standard Operating Procedures (SOPs). The findings from the inspection and audit were reported to the Study Director and management as follows:

Phase of Study Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
Dosing	2 Jan 01	2 Jan 01	2 Jan 01
Report/Data Audit	7 Feb 01	7 Feb 01	7 Feb 01

Darrell Zeno

Darrell Zeno, M.S.
Quality Assurance Unit, STILLMEADOW, Inc.

26 Mar 01
Date

SUMMARY

A primary dermal irritation study was conducted on three albino rabbits using test substance Miller 6064. There was one intact test site per animal. Each test site was treated with 0.5 mL of the undiluted test substance and covered with a semi-permeable dressing. The test substance was maintained in contact with the skin for 4 hours. Observations for dermal irritation and defects were made at 1, 24, 48 and 72 hours after removal of the dressings.

Irritation scores derived from the respective erythema and edema scores through the 72 hour observations for each animal are presented below.

	Erythema Hours after Unwrap				**Edema** Hours after Unwrap				Irritation Scores
	1	24	48	72	1	24	48	72	
2280-M	1	1	0	0	0	0	0	0	0.50
2279-F	0	0	0	0	0	0	0	0	0.00
2281-F	1	1	0	0	0	0	0	0	0.50
Primary Irritation Index (PII)=									0.3

Based on the PII of 0.3, the test substance is rated slightly irritating. Based on the scores at the 72 hour observation only, the test substance is assigned to Toxicity Category IV.

INTRODUCTION

The objective of this study was to assess the relative primary skin irritation level of the test substance on rabbits in accordance with US EPA OPPTS 870.2500, which is intended to meet testing requirements of FIFRA 7 USC 136, et seq, and TSCA 15 USC 2601. This study was conducted for Miller Chemical & Fertilization Corp., according to the approved protocol and STILLMEADOW, Inc. SOPs. There were no deviations from the protocol which affected the quality or outcome of the study. All procedures used in this study are in compliance with Animal Welfare Act Regulations. The protocol, raw data and this report are archived at STILLMEADOW, Inc. The animals were treated with the test substance between 1120 and 1124 on 2 Jan 01. The in-life portion of the study was terminated on 5 Jan 01.

TEST SUBSTANCE

Identification:	Miller 6064
Date & Quantity Received:	19 Dec 00; 2 x 1 gal
Physical Description:	Amber liquid
Storage:	Room temperature
Purity & Composition:	Refer to Certificate of Analysis (Appendix A)
Stability:	Not provided by sponsor

Records pertaining to stability, characterization and verification of test substance identity are the responsibility of the sponsor. A copy of the Certificate of Analysis is included in the report.

TEST SYSTEM

Experimental Animals

Species & Strain:	Albino rabbit; New Zealand White
Justification of Species:	The rabbit is preferred by the various regulatory agencies for use in primary dermal irritation testing.
Source:	Ray Nichols Rabbitry; Lumberton, TX
Date Received:	29 Nov 00
Quarantine Period:	5 days
Quantity & Sex:	1 male and 2 females
Group Identification:	Cage cards
Animal Identification:	Ear tag
Initial Body Weight:	Male: 2.600 kg; Females: 2.775-3.025 kg
Date of Birth:	10 Sep 00

Animal Husbandry

Cage Type:	Suspended, wire bottom, stainless steel
Housing:	1 per cage
Environmental Controls Set to Maintain:	· Temperature Range 20°C±3° · Humidity Range 30-70% · 12-hour light/dark cycle · 10-12 air changes/hour
Food:	PMI Feeds, Inc.™ Lab Rabbit Diet #5321, in measured amounts
Water:	Municipal water supply analyzed by TNRCC Water Utilities Division; available *ad libitum* from automatic water system

Animal husbandry and housing at STILLMEADOW, Inc. comply with Animal Welfare Act Regulations. No contaminants were expected to have been present in the feed or water which would have interfered with or affected the results of the study.

PROCEDURES

Test Substance Administration

Prior to starting the study, the pH of the test substance was determined to be 7.13. Each animal was prepared on the day prior to treatment by clipping the dorsal area of the trunk free of hair to expose an area at least 8 x 8 cm. Only those animals with exposure areas free of pre-existing skin irritation or defects were selected for testing. A single intact exposure site was selected as the test site while the contralateral intact site served as a control site.

On Day 0, 0.5 mL of the undiluted test substance was applied to each test site and covered with a surgical gauze patch measuring 2.5 x 2.5 cm and four single layers thick. Each patch was secured in place with a strip of non-irritating adhesive tape. The entire trunk of each animal was loosely wrapped with a semi-permeable dressing (orthopedic stockinette) which was secured on both edges with strips of tape to retard evaporation of volatile substances and to prevent possible ingestion of the test substance.

Removal of Test Substance

After four hours, the patches and wrappings were removed. The test sites were gently washed with room temperature tap water and a clean cloth to remove as much residual test substance as possible.

Observations

The test sites were observed for erythema and edema formation, and any other dermal defects or irritation at 1, 24, 48 and 72 hours after unwrap.

Irritation Scoring Method

The scoring scale used to rate dermal irritation is presented in the Legend to Table 1. For each animal, all of the erythema and edema scores through 72 hours were added, and the sum was divided by 4 to obtain an individual irritation score. The primary irritation index was determined by calculating the mean of the irritation scores for all the animals and was used to obtain a rating for the test substance. A Toxicity Category (based only on the observations at 72 hours) was assigned according to the scale presented in the Legend to Table 1.

RESULTS AND DISCUSSION

Evaluation

Signs of dermal irritation or defects are presented in Table 1. Very slight erythema was present in two animals at each observation through 24 hours. Edema was not observed at any time throughout the study. No other signs of irritation were observed during the study.

CONCLUSION

The primary irritation index of 0.3 out of a possible 8.0 was obtained from the 1, 24, 48 and 72 hour observations and was used to give Miller 6064 a descriptive rating of slightly irritating. Based on the 72 hour observations only, Miller 6064 is assigned to Toxicity Category IV.

Study Director: Janice O. Kuhn 26 Mar 01

Janice O. Kuhn, Ph.D., D.A.B.T.
Senior Toxicologist, STILLMEADOW, Inc.

Date

STUDY PERSONNEL

Technical Staff: Carol Morris, B.A.
Hector Fuentes
Michelle Gantt, B.S.

Data Services: Connie Pavatte, Report Preparation

LEGEND TO TABLE 1
ACUTE DERMAL IRRITATION STUDY IN RABBITS
Primary Dermal Irritation Scoring Scale (Draize Technique*)

Evaluation of Skin Reactions

Erythema Formation	Score
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Maximum Possible	4

Edema Formation	Score
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4
Maximum Possible	4

* - Draize, John H., Woodard, Geoffrey, and Calvery, H.O., Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes. J. Pharm & Ther. 82, 377 (1944).

LEGEND TO TABLE 1 (cont.)
ACUTE DERMAL IRRITATION STUDY IN RABBITS
Classification of Test Substance

Descriptive Rating	Primary Irritation Index
Non-irritating	0.0
Slightly Irritating	0.1 - 1.9
Moderately Irritating	2.0 - 5.0
Severely Irritating	5.1 - 8.0

The primary irritation index is calculated using only the observations scheduled through 72 hours.

Dermal Irritation Toxicity Categories (per Proposed Rule, FR Vol. 49, No. 188)

Toxicity Category	Criteria
I	Corrosive
II	Severe irritation at 72 hours
III	Moderate irritation at 72 hours
IV	Non-irritating, mild, or slight irritation at 72 hours

TABLE 1
ACUTE DERMAL IRRITATION STUDY IN RABBITS
Signs of Dermal Irritation
Test Substance: Miller 6064

Animal Number	Erythema							Edema										Primary Irritation Scores
	Hours				Days			Hours				Days						
	1	24	48	72	7	10	14	1	24	48	72	7	10	14				
2280-M	1	1	0	0				0	0	0	0				2	/4	=	0.50
2279-F	0	0	0	0				0	0	0	0				0	/4	=	0.00
2281-F	1	1	0	0				0	0	0	0				2	/4	=	0.50
Primary Irritation Index* =					1.00		/3=	0.3										
Descriptive Rating =								Slightly Irritating										
Toxicity Category** =								IV										

* - Only the first four observation times are used for calculations.

** - Based only on the mean 72 hour score for erythema and edema.

Study Duration - 72 hours

M – Male; F – Female

TABLE 1 (cont.)
ACUTE DERMAL IRRITATION STUDY IN RABBITS
Signs of Dermal Irritation
Test Substance: Miller 6064

Animal Number	Other Observations						
	Hours				Days		
	1	24	48	72	7	10	14
2280-M	-	-	-	-			
2279-F	-	-	-	-			
2281-F	-	-	-	-			

Note: A dash (-) is used if there are no other signs of dermal irritation.
Study Duration - 72 hours
M - Male; F - Female

APPENDIX A

MILLER® CHEMICAL & FERTILIZER CORPORATION

P.O. BOX 333, 120 RADIO ROAD
HANOVER, PENNSYLVANIA 17331 U.S.A.
TELEPHONE: 717-632-8921
FAX NO.: 717-632-4581

1/18/01

CERTIFICATE OF ANALYSIS

Product Name: MILLER 6064
Quantity Produced: 7.4 kilograms
Batch Number: 150386
Date of Formulation: 12/13/00

Appearance: Yellow to Amber Liquid
Odor: Terpenic
Moisture: Negligble (non-aqueous)
Specific Gravity: 0.92 - 0.94 @ 20c
Viscosity @ 20c: 100 to 500 cps
pH(1% solution): 6.0 to 8.0
Solubility in Water: Insoluble (Emulsifies)
Flash Point: over 200 f
Purity: 100%

Signature:

Name: Andrew G. Smith
Title: Compliance Officer

APPENDIX B

STILLMEADOW
INCORPORATED

PROTOCOL FOR STUDY 6210-00

Study Title: ACUTE DERMAL IRRITATION STUDY IN RABBITS (OPPTS 870.2500)

Test Substance: MILLER 6064

Test Facility: STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, Texas 77478

Approved: _____ 26 Dec 00
Janice O. Kuhn, Ph.D., D.A.B.T. Date
Study Director
STILLMEADOW, Inc.

Approved: _____ 6 Dec 00
Mark S. Holbert Date
Vice President
STILLMEADOW, Inc.

Reviewed: _____ 6 Dec 2000
Vicki S. Crutchfield, RQAP Date
Director, Quality Assurance Unit
STILLMEADOW, Inc.

Sponsor: Miller Chemical and Fertilization Corp.
P.O. Box 333
Hanover, PA 17331

Sponsor Representative: Mandava Associates
1730 M Street, N.W., Suite 906
Washington, DC 20036

Approved: _____ December 26, 2000
N. Bhushan Mandava Date
Agent to Miller Chemical and Fertilization Corp.

PROTOCOL FOR STUDY 6210-00

A. GENERAL

1. Study Title: ACUTE DERMAL IRRITATION STUDY IN RABBITS

2. Purpose: To assess the relative level of primary skin irritation produced when rabbits are exposed to the test substance under semioccluded conditions.

3. Regulatory Compliance: This study will be conducted according to OPPTS 870.2500, which is intended to meet testing requirements of both FIFRA (7 U.S.C. 136, *et seq.*) and TSCA (15 U.S.C. 2601).

This study will be conducted in compliance with Good Laboratory Practice Standards:

1. EPA FIFRA: 40 CFR 160
2. EPA TSCA: 40 CFR 792
3. OECD: C(97)186
4. Japanese MAFF

All procedures in this protocol are in compliance with Animal Welfare Act Regulations. All methods can be found in STILLMEADOW, Inc. Standard Operating Procedures (SOPs).

4. Quality Assurance: The Protocol is reviewed by the Quality Assurance Unit (QAU). The study information will be entered into the Master Schedule. The study will be inspected at least once during its progress. Further inspections may be scheduled as needed to ensure the integrity of the study. Any deviations from SOPs, the Protocol, or Good Laboratory Practice Standards will be immediately reported to the Study Director and Management. The report will be audited and a statement prepared and signed which shall specify the dates inspections were made and findings reported to Management and to the Study Director.

5. Test Substance: MILLER 6064. Test substance identification should include the name, batch number and purity. Information regarding safety, stability, storage conditions and disposal should also be provided by the Sponsor. The Sponsor assumes responsibility for purity and stability determinations. Analysis and archiving of reserve samples will be the responsibility of the Sponsor.

6. Proposed Schedule: Testing will begin within approximately three weeks of receipt of the test substance and authorization to conduct the study.

Proposed Start Date: 19 Dec 00
Proposed End Date: 02 Jan 01

If dermal effects are resolved prior to 14 days after treatment, the study may end as early as 72 hours after treatment.

APPENDIX B (cont.)

A. GENERAL (cont.)

7.	Study Director:	Janice O. Kuhn, Ph.D., D.A.B.T.
8.	Experimental Summary:	The test substance will be applied to a single intact skin test site on each of three rabbits. The test substance will be maintained in contact with the skin for 4 hours. The test sites will then be washed as thoroughly as possible with room temperature tap water and/or an appropriate solvent without irritating the skin. The test sites will be scored 30-60 minutes later for signs of skin irritation. The sites will be scored again at 24, 48 and 72 hours after the end of the exposure period (post patch removal) and every 2 - 4 days thereafter until reversible irritation subsides (maximum of 14 days). The Primary Irritation Index will be determined from the scores through 72 hours. Unless otherwise requested by the Sponsor, a Toxicity Category will be assigned based on the scores at 72 hours.
9.	Protocol Amendments:	Any alteration in the Protocol will be justified, approved by the Study Director, and recorded in writing.
10.	Sponsor Audits:	The Sponsor may send an authorized representative to inspect the test system and/or data on the STILLMEADOW, Inc. premises during normal working hours.

B. EXPERIMENTAL DESIGN

1. Animals

a. Species:	Albino rabbit
b. Strain/Source:	New Zealand White (Ray Nichols Rabbitry, Lumberton, Texas or other suitable source)
c. Justification of Species:	The rabbit is conventionally used in primary dermal irritation studies to provide information on which human hazard can be judged, and is preferred by the regulatory agencies.
d. Quantity and Sex:	Three animals; males and/or females may be used
e. Age/Weight:	Young adult (12 weeks - 6 months); approximately 2 - 4 kg
f. Identification:	Ear tag
g. Acclimation and Health Status:	Animals will be acclimated for at least five days prior to testing. Normal weight gain, appearance, and behavior will be factors used to select healthy animals for testing. Only naive animals will be used.

2. Animal Husbandry

a. Cages:	Stainless steel, suspended, wire bottom.
b. Number per Cage:	Animals will be individually housed.
c. Food:	A measured amount of PMI Feeds, Inc.™ Laboratory Rabbit Diet #5321. The food is routinely analyzed by the manufacturer for nutritional components and environmental contaminants.
d. Water:	Tap water; available *ad libitum* (automatic system). Municipal water supply analyzed by Texas Natural Resource Conservation Commission (TNRCC) Water Utilities Division.
e. Contaminants:	There are no known contaminants in the feed or water available to laboratory animals that would be expected to interfere with this study.
f. Environment:	Target temperature: approximately 20°C ± 3°C. Target relative humidity: approximately 30 - 70%. 12-hour light/dark cycle (regulated automatically), and room ventilation of approximately 10 - 12 air changes per hour.

B. EXPERIMENTAL DESIGN (cont.)

3. Test Substance Administration

a. Preparation of Animals: Animals will be prepared on the day prior to treatment by clipping the dorsal area of the trunk of each animal free of hair to expose an area approximately 8 x 8 cm. Animals with exposure areas free from pre-existing skin irritation or defects will be selected for testing. A single intact exposure site will be selected as the test site with the contralateral intact site to remain as a control site.

b. Reason for Route of Administration: Dermal contact is a potential route of human exposure.

c. Stepwise Exposure of Animals: A single rabbit may be used if it is suspected that the test substance might produce severe irritation/corrosion. Three test patches are applied concurrently or sequentially to the animal. The first patch is removed after 3 min. If no serious skin reaction is observed, the second patch is removed after 1 hour. If observations indicate that exposure can be continued humanely, the third patch is removed after 4 hours and the responses graded. If a corrosive effect is observed after an exposure of up to 4 hours, then further animal testing is not required. If no corrosive effect is observed in one animal after a 4-hour exposure, the test is completed using two additional animals, each with one patch only, for an exposure period of 4 hours. If it is expected that the test substance will not produce severe irritancy or corrosion, the test may be started using three animals, each receiving one patch for an exposure period of 4 hours.

d. Application of Test Substance: On Day 0, 0.5 mL in the case of a liquid test substance, or 0.5 g in the case of solid or semi-solid test substance, will be introduced under a surgical gauze patch measuring 2.5 x 2.5 cm and four single layers thick to a single test site on each animal. Solid test substances will be moistened with deionized water or saline to form a thick paste prior to application and may require a 4-ply 5 x 5 cm gauze patch to cover all of the test substance. In some cases, the test substance may be applied to the gauze patch and the patch placed on the skin. If water or saline cannot be used to moisten the substance, acceptable alternatives are corn oil, glycerol, ethanol and water, mineral oil, aqueous carboxymethyl cellulose and gum arabic. The entire trunk will be covered with a semiocclusive dressing.

e. Control Site: A contralateral area of untreated skin will serve as the control against which the reactions of the treated site are evaluated. No separate control group of animals is used.

f. Removal of Test Substance: After the 4-hour exposure period, the patches and wrappings will be removed and the test substance will be removed as thoroughly as possible using water and/or an appropriate solvent (e.g., non-irritating mineral oil) without irritating the skin. The control site will be treated in a similar manner.

B. EXPERIMENTAL DESIGN (cont.)

4. Observations

a. Dermal Irritation: The animals will be observed and scored for erythema, edema and other signs of dermal irritation or defects 30-60 minutes after the removal of the patches and at 24, 48 and 72 hours after the end of the exposure period (patch removal). If irritation persists through 72 hours, observations will be made every 2 - 4 days thereafter until all reversible irritation subsides (maximum of 14 days). The scoring scale for signs of dermal irritation according to the Draize technique is presented in Appendix A.

b. Other Observations: Observations of any other toxic effects will be recorded.

5. Evaluation of Results: For each animal, all of the erythema and edema scores through 72 hours will be added and the sum divided by 4 (the number of observation periods) to obtain an individual irritation score. The Primary Irritation Index will be determined by calculating the mean of the irritation scores for the three animals and will be used to give the test substance a descriptive rating according to the classifications in Appendix B. Unless otherwise requested by the sponsor, a Toxicity Category will be assigned according to the scores at 72 hours only, as described in Appendix B.

6. Test Substance Accountability: A comprehensive inventory of test substance received and used will be kept. The test substance container(s) will be weighed when received at this facility, and a record of all test substance use will be maintained. Test substance and test substance dosing solutions will be stored in the original containers, or in the equivalent thereof, or in glass containers with Teflon-lined caps.

7. Disposal of Unused Test Substance: Unused test substance will be returned or disposed of at the Sponsor's expense after the termination of the study. The Sponsor should specify return or disposal at the time of study authorization. If returned, the recipient of the test substance will be notified in advance of shipping. The sample will be accompanied by a chain of custody letter describing the sample(s) contained in the shipment.

8. Safety Precautions: General safety precautions required by laboratory SOPs will be followed. The Sponsor will supply basic toxicity data on the test substance to be used. However, since the toxicity of test substances is often not well characterized, this laboratory will be conservative in setting safety procedures. The Sponsor or Sponsor's Representative shall be notified of any exposures requiring a physician's examination or care.

C. DATA MANAGEMENT

1. Records:

The following records will be maintained during the study and transferred to the STILLMEADOW, Inc. archives upon study termination.

a. Protocol and Protocol Amendments (if any).
b. Final report and amendments (if any).
c. Study correspondence.
d. Animal receipt/acclimation data.
e. Test substance receipt, identification as supplied by the Sponsor, preparation, administration, and disposition.
f. Test animal information: number, sex, source, strain.
g. All observations and scores for skin irritation for all time periods.
h. Observations of any other toxic effects.
i. Other pertinent data.

2. Data Storage:

All raw data will be retained at STILLMEADOW, Inc.

3. Data Reporting:

The final report will include all data as described in the Good Laboratory Practice Standards, including:

a. Statement from the Quality Assurance Unit.
b. Signature of the Study Director.
c. A GLP Compliance Statement signed by the Study Director.
d. Names of scientific personnel involved in the study.
e. Dates of study initiation and termination.
f. Identification, description, preparation, and storage of the test substance.
g. All pertinent animal data, animal husbandry, dosing information, and observation methods.
h. Description of the test procedures.
i. Identification and compositions of any vehicles used in adminstering the test substance and justification for their use.
j. Individual observations for erythema, eschar, edema, and any other signs of dermal defects or irritation at all observation periods. Tabulation of dermal irritation data, including onset, duration, and reversibility.
k. Primary dermal irritation score.
l. Descriptive rating for the test substance and a Toxicity Category (unless otherwise requested by Sponsor) for the test substance based on the scores at 72 hours.
m. Observations of any toxic effects.
n. A reference to this Protocol.

4. Report Submission:

A report will be submitted after termination of the in-life portion of the study.

APPENDIX B (cont.)

Appendix A
ACUTE DERMAL IRRITATION STUDY IN RABBITS
Evaluation of Skin Reactions

Primary Dermal Irritation Scoring Scale
(Draize Technique*)

Erythema and Eschar Formation	Score
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Maximum Possible	4

Edema Formation	Score
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4
Maximum Possible	4

Other observations may be made when needed, for example: Staining of the test site skin, necrosis, blanching, desquamation, sloughing, eschar, coriaceousness (leathery texture), atonia, etc.

* - Draize, John H., Woodard, Geoffrey, and Calvery, H.O., "Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes." J. Pharm. & Ther. 82, 377 (1944).

Appendix B
ACUTE DERMAL IRRITATION STUDY IN RABBITS
Classification of Test Substance

Descriptive Rating	Primary Irritation Index
Non-irritating	0.0
Slightly Irritating	0.1 - 1.9
Moderately Irritating	2.0 - 5.0
Severely Irritating	5.1 - 8.0

The primary irritation index is calculated using only the observations through 72 hours.

Dermal Irritation Toxicity Categories (per Proposed Rule, FR Vol. 49, No. 188)

Toxicity Category	Criteria
I	Corrosive
II	Severe irritation at 72 hours
III	Moderate irritation at 72 hours
IV	Non-irritating, mild or slight irritation at 72 hours

ATTACHMENT 38

Acute Eye Irritation Study in Rabbits on Miller 6064

STILLMEADOW
INCORPORATED

VOLUME __ OF __ OF SUBMISSION

Miller 6064

FINAL REPORT

ACUTE EYE IRRITATION STUDY IN RABBITS

OPPTS NO. 870.2400

AUTHOR:

Janice O. Kuhn, Ph.D., D.A.B.T.

STUDY INITIATION DATE: 26 December 2000
STUDY COMPLETION DATE: 26 March 2001

CONDUCTED BY:
STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, TX 77478

LABORATORY STUDY NUMBER:

6209-00

VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 29

SUBMITTED TO:
Miller Chemical & Fertilization Corp.
P.O. Box 333
Hanover, PA 17331

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA § 10 (d) (1) (A), (B) or (C).

Company: Miller Chemical & Fertilization Corp.

Company Agent: _____ Date: _____

_____ _____

Title Signature

These data are the property of Miller Chemical & Fertilization Corp., and as such, are considered to be confidential for all purposes other than compliance with FIFRA § 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other statute or in any other country.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was designed and performed in STILLMEADOW, Inc.'s AAALAC accredited laboratory in compliance with:

- United States Environmental Protection Agency FIFRA: GLP Standards, 40 CFR 160 with exception of Sec. 160.31 (d), and 160.105 (b)(e): stability information was not provided
- United States Environmental Protection Agency TSCA 40 CFR 792 with exception of Sec. 792.31 (d), and 792.105 (b)(e): stability information was not provided
- Organization for Economic Cooperation & Dev. Principles of GLP, Annex 2, C(97)186 with exception of Sec. 6.2 (4): stability information was not provided
- Japan Ministry of Agriculture, Forestry & Fisheries, Notification No. 59 Nohsan 3850, Director-General of Agricultural Prod. Bureau, 10 Aug 84 with exception of Art. 5 (2)(9), and 21 (3): stability information was not provided

Janice O. Kuhn, Ph.D., D.A.B.T.
Study Director, STILLMEADOW, Inc.

26 Mar 01
Date

Signature of Agent of Sponsor

Date

Agent Name
Sponsor: Miller Chemical & Fertilization Corp.

Signature of Agent of Submitter

Date

Agent Name
Submitter: Mandava Associates

TABLE OF CONTENTS

QUALITY ASSURANCE STATEMENT

Study Number: 6209-00
Test Substance: Miller 6064
Study Title: Acute Eye Irritation Study in Rabbits

The study report and data have been audited in accordance with STILLMEADOW, Inc. Standard Operating Procedures (SOPs). The findings from the inspection and audit were reported to the Study Director and management as follows:

Phase of Study Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
Dosing	2 Jan 01	2 Jan 01	2 Jan 01
Report/Data Audit	26 Jan 01	26 Jan 01	26 Jan 01

Darrell Zeno

Darrell Zeno, M.S.
Quality Assurance Unit, STILLMEADOW, Inc.

26 Mar 01

Date

SUMMARY

An acute eye irritation study was conducted on six albino rabbits using test substance Miller 6064. The undiluted test substance (0.1 mL) was placed into the conjunctival sac of the right eye of each animal selected for testing. All treated eyes were washed with room temperature deionized water for one minute immediately after recording the 24 hour observation.

The number of animals testing "positive" for each parameter (according to the Legend to Table 1) over the number of animals tested is presented below.

	Time After Treatment Hours			
	1	24	48	72
Cornea				
Opacity	0/6	0/6	0/6	0/6
Iritis	0/6	0/6	0/6	0/6
Conjunctivae				
Redness	0/6	0/6	0/6	0/6
Chemosis	0/6	0/6	0/6	0/6

There were no "positive" effects exhibited in any eyes at any time during the study. The test substance is rated minimally irritating and assigned to Toxicity Category IV.

INTRODUCTION

The objective of this study was to assess the relative level of eye irritation following a single exposure of the test substance to rabbits in accordance with US EPA OPPTS 870.2400, which is intended to meet testing requirements of FIFRA 7 USC 136, et seq, and TSCA 15 USC 2601. This study was conducted for Miller Chemical & Fertilization Corp., according to the approved protocol and STILLMEADOW, Inc. SOPs. There were no deviations from the protocol which affected the quality or outcome of the study. All procedures used in this study are in compliance with Animal Welfare Act Regulations. The protocol, raw data and this report are archived at STILLMEADOW, Inc. The animals were treated with the test substance between 1402 and 1404 on 2 Jan 01. The in-life portion of the study was terminated on 5 Jan 01.

TEST SUBSTANCE

Identification:	Miller 6064
Date & Quantity Received:	19 Dec 00; 2 x 1 gal
Physical Description:	Amber liquid
Storage:	Room temperature
Purity & Composition:	Refer to Certificate of Analysis (Appendix A)
Stability:	Not provided by sponsor

Records pertaining to stability, characterization and verification of test substance identity are the responsibility of the sponsor. A copy of the Certificate of Analysis is included in the report.

TEST SYSTEM

Experimental Animals

Species & Strain:	Albino rabbit; New Zealand White
Justification of Species:	The rabbit is preferred by the various regulatory agencies for use in eye irritation testing.
Source:	Ray Nichols Rabbitry, Lumberton, TX
Date Received:	28 Dec 00
Quarantine Period:	5 days
Quantity & Sex:	3 males and 3 females
Group Identification:	Cage cards
Animal Identification:	Ear tag
Initial Body Weight:	Males: 2.075-2.475 kg; Females: 2.125-2.300 kg
Date of Birth:	8 Oct 00

Animal Husbandry

Cage Type:	Suspended, wire bottom, stainless steel
Housing:	1 per cage
Environmental Controls Set To Maintain:	· Temperature Range 20°C± 3° · Humidity Range 30-70% · 12-hour light/dark cycle · 10-12 air changes/hour
Food:	PMI Feeds, Inc.™ Lab Rabbit Diet #5321, in measured amounts
Water:	Municipal water supply analyzed by TNRCC Water Utilities Division; available *ad libitum* from automatic water system.

Animal husbandry and housing at STILLMEADOW, Inc. comply with Animal Welfare Act Regulations. No contaminants were expected to have been present in the feed or water which would have interfered with or affected the results of the study.

PROCEDURES

Test Substance Administration

Prior to starting the study, the pH of the test substance was determined to be 7.13. Healthy albino rabbits were released from quarantine. Both eyes of each animal were carefully examined within 24 hours prior to treatment with a fluorescein sodium ophthalmic solution, and cobalt-filtered light. Both eyes of each animal were again carefully examined just prior to treatment, but without the fluorescein sodium ophthalmic solution. Only those animals without eye defects or irritation were selected for testing.

On Day 0, a dose of 0.1 mL of the undiluted test substance was placed into the conjunctival sac of the right eye of each animal by gently pulling the lower lid away from the eyeball to form a cup into which the test substance was dropped. The lids were gently held together for one second to prevent loss of material. The untreated left eyes served as comparative controls.

Observations

The treated eyes of all animals were examined without magnification under white room lighting provided by daylight-type fluorescent ceiling fixtures and an additional source of white light present on the examining table. The grades of ocular reaction were recorded at 1, 24, 48 and 72 hours after treatment. The corneas of all treated eyes were examined immediately after the 24-hour observation with a fluorescein sodium ophthalmic solution. A Finoff ocular transilluminator with cobalt blue filter (Welch Allyn, Skaneateles Falls, NY) was utilized to enhance visualization of fluorescein staining. Any of the corneas which exhibited fluorescein staining at the 24-hour observation were re-examined with the fluorescein sodium ophthalmic solution at each consecutive observation until fluorescein staining of the cornea no longer occurred. All treated eyes were washed with room temperature deionized water for one minute immediately after recording the 24-hour observation.

Irritation Scoring Method

Individual irritation scores for each animal at each scheduled observation were determined using the grading scale given in the Legend to Table 1. An average irritation score for each scheduled observation was then determined, based on the number of animals tested. A maximum average irritation score was derived from the observation yielding the highest average irritation score. The maximum average irritation score was used to rate the test substance according to the ratings presented in the Legend to Table 2. The scale used to categorize the test substance is also presented in the Legend to Table 2. Any corneal involvement or iridic irritation with a score of 1 or more is considered positive. Any conjunctival irritation (redness or chemosis) with a score of 2 or more is considered positive.

RESULTS AND DISCUSSION

Evaluation

The number of animals with "positive" findings at each observation period is presented in the summary section of this report. Ocular reactions are presented in Table 1. A summary of irritation scores is presented in Table 2.

The maximum average irritation score of 6.0, obtained at 1 hour after treatment, was used to rate Miller 6064 minimally irritating. Fluorescein staining did not occur in any of the eyes.

Toxicity categories are determined by the presence and duration of corneal involvement, iridic irritation, and "positive" conjunctival irritation. Any corneal involvement or iridic irritation with a score of 1 or more is considered positive. Any conjunctival irritation (redness or chemosis) with a score of 2 or more is considered positive.

CONCLUSION

Based on the Maximum Average Irritation Score of 6.0, the test substance Miller 6064 is rated minimally irritating. Since there were no "positive" effects observed during the study, the test substance is assigned to Toxicity Category IV. No irritation was observed in any eyes at 24 hours.

Study Director: Janice O Kuhn — 26 Mar 01

Janice O. Kuhn, Ph.D., D.A.B.T.
Senior Toxicologist, STILLMEADOW, Inc.

Date

STUDY PERSONNEL

Technical Staff: Carol Morris, B.A.
Hector Fuentes
Michelle Gantt, B.S.

Data Services: Connie Pavatte, Report Preparation

LEGEND TO TABLE 1
ACUTE EYE IRRITATION STUDY IN RABBITS
Grading Scale

I. Cornea

A. Opacity - degree (area most dense taken for reading)

No opacity	0
Slight dulling of normal luster	+
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible	1*
Easily discernible translucent area, details of iris slightly obscured	2*
Nacreous area, no details of iris visible, size of pupil barely discernible	3*
Opaque cornea, iris not discernible through the opacity	4*

B. Area of cornea involved

One quarter (or less), but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4

C. Fluorescein Staining - appearance of yellow-green staining of cornea

Cornea not examined with fluorescein	-
No fluorescein staining	0
Positive fluorescein staining	P
Area of cornea involved	
One quarter (or less), but not zero	A
Greater than one quarter, but less than half	B
Greater than half, but less than three quarters	C
Greater than three quarters, up to whole area	D

D. Stippling - appearance of pinpoint roughening

No stippling	0
Presence of stippling	S
Area of cornea involved	
One quarter (or less), but not zero	A
Greater than one quarter, but less than half	B
Greater than half, but less than three quarters	C
Greater than three quarters, up to whole area	D

A X B X 5 Total Maximum = 80

* - Reaction indicates a positive effect.

Reference: Draize, John H., Woodard, Geoffrey, and Calvery, Herbert O., Journal of Pharmacol. Exp. Ther., 82, 377-390 (1944).

LEGEND TO TABLE 1 (cont.)
ACUTE EYE IRRITATION STUDY IN RABBITS
Grading Scale

II. Iris

A. Grades

Normal	0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperemia or injection (any of these or combination thereof), iris still reacting to light (sluggish reaction is positive)	1*
No reaction to light, hemorrhage, gross destruction (any or all of these)	2*

A X 5 Total Maximum = 10

III. Conjunctivae

A. Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)

Blood vessels normal	0
Some blood vessels definitely hyperemic (injected)	1
Diffuse, crimson color, individual vessels not easily discernible	2*
Diffuse beefy red	3*

B. Chemosis: lids and/or nictitating membrane

No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2*
Swelling with lids about half closed	3*
Swelling with lids more than half closed	4*

C. Discharge

No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3

D. Necrosis or Ulceration of the palpebral and bulbar conjunctivae or nictitating membrane

No necrosis or ulceration	0
Presence of necrosis or ulceration	N

(A + B + C) X 2 Total Maximum = 20

The total score for the eye is the sum of all scores obtained for the cornea, iris, and conjunctivae with the possible maximum total score for the eye being equal to 110.

* - Reaction indicates a positive effect.

LEGEND TO TABLE 2
ACUTE EYE IRRITATION STUDY IN RABBITS
Rating of Test Substance Based on Eye Irritation[1]

Rating	Maximum Average Score	Definition
Non-Irritating	0.0-0.5	To maintain this rating, all scores at the 24-hour reading must be zero; otherwise, increase rating one level.
Practically Non-Irritating	>0.5-2.5	To maintain this rating, all scores at the 24-hour reading must be zero; otherwise, increase rating one level.
Minimally Irritating	>2.5-15.0	To maintain this rating, all scores at the 72-hour reading must be zero; otherwise, increase rating one level.
Mildly Irritating	>15.0-25.0	To maintain this rating, scores at the 7-day reading must be zero; otherwise, increase rating one level.
Moderately Irritating	>25.0-50.0	To maintain this rating, scores at the 7-day reading must be less than or equal to 10 for 60% or more of the animals. Also, the 7-day mean score must be less than or equal to 20. If the 7-day mean score is less than or equal to 20, but less than 60% of the animals show scores less than 10, then no animal among those showing scores greater than 10 can exceed a score of 30 if rating is to be maintained; otherwise, increase rating one level.
Severely Irritating	>50.0-80.0	To maintain this rating, scores at the 7-day reading must be less than or equal to 30 for 60% or more of the animals. Also, the 7-day mean score must be less than or equal to 40. If the 7-day mean score is less than or equal to 40, but less than 60% of the animals show scores less than or equal to 30, then no animal among those showing scores greater than 30 can exceed a score of 60 if rating is to be maintained; otherwise, increase rating one level.
Extremely Irritating	>80.0-110.0	

NOTE: The rating of the test substance is not to be increased more than one level above its maximum average score.

[1]Slightly modified from Kay, J.H. and Calandra, J.C. (1962) Interpretation of Eye Irritation Tests. J. Soc. Cosmetic Chemists 13:281-289

LEGEND TO TABLE 2 (cont.)
ACUTE EYE IRRITATION STUDY IN RABBITS
Criteria of Eye Irritation for Classification into Toxicity Categories [1]

Category	Criteria
I	Corrosive (irreversible destruction of ocular tissue) or corneal involvement or "positive" conjunctival irritation persisting through Day 21.
II	Corneal involvement or "positive" conjunctival irritation clearing in 8-21 days.
III	Corneal involvement or "positive" conjunctival irritation clearing in 7 days or less.
IV	Minimal effects clearing in less than 24 hours. No "positive" effects at 24 hours.

[1] Per Proposed Rule, FR Vol. 49, No. 188

TABLE 1
ACUTE EYE IRRITATION STUDY IN RABBITS
Ocular Reactions
Test Substance: Miller 6064

	Rabbit No. 2290-M										Rabbit No. 2291-F									
	Hrs. After Treatment				Days After Treatment						Hrs. After Treatment				Days After Treatment					
	1	24	48	72	4	7	10	14	17	21	1	24	48	72	4	7	10	14	17	21
I. Cornea																				
A. Opacity	0	0	0	0							0	0	0	0						
B. Area	0	0	0	0							0	0	0	0						
C. Fluorescein Staining	-	0	-	-							-	0	-	-						
D. Stippling	0	0	0	0							0	0	0	0						
SCORE	0	0	0	0							0	0	0	0						
II. Iris																				
A. Grade	0	0	0	0							0	0	0	0						
SCORE	0	0	0	0							0	0	0	0						
III. Conjunctivae																				
A. Redness	1	0	0	0							1	0	0	0						
B. Chemosis	1	0	0	0							1	0	0	0						
C. Discharge	1	0	0	0							1	0	0	0						
D. Necrosis or Ulceration	0	0	0	0							0	0	0	0						
SCORE	6	0	0	0							6	0	0	0						
TOTAL SCORE	6	0	0	0							6	0	0	0						

M – Male; F – Female

Duration of Study: 72 Hours

TABLE 1 (cont.)
ACUTE EYE IRRITATION STUDY IN RABBITS
Ocular Reactions
Test Substance: Miller 6064

	Rabbit No. 2292-M										Rabbit No. 2293-F									
	Hrs. After Treatment				Days After Treatment						Hrs. After Treatment				Days After Treatment					
	1	24	48	72	4	7	10	14	17	21	1	24	48	72	4	7	10	14	17	21
I. Cornea																				
A. Opacity	0	0	0	0							0	0	0	0						
B. Area	0	0	0	0							0	0	0	0						
C. Fluorescein Staining	-	0	-	-							-	0	-	-						
D. Stippling	0	0	0	0							0	0	0	0						
SCORE	0	0	0	0							0	0	0	0						
II. Iris																				
A. Grade	0	0	0	0							0	0	0	0						
SCORE	0	0	0	0							0	0	0	0						
III. Conjunctivae																				
A. Redness	1	0	0	0							1	0	0	0						
B. Chemosis	1	0	0	0							1	0	0	0						
C. Discharge	1	0	0	0							1	0	0	0						
D. Necrosis or Ulceration	0	0	0	0							0	0	0	0						
SCORE	6	0	0	0							6	0	0	0						
TOTAL SCORE	6	0	0	0							6	0	0	0						

M – Male; F – Female
Duration of Study: 72 Hours

TABLE 1 (cont.)
ACUTE EYE IRRITATION STUDY IN RABBITS
Ocular Reactions
Test Substance: Miller 6064

	Rabbit No. 2294-M										Rabbit No. 2295-F									
	Hrs. After Treatment				Days After Treatment						Hrs. After Treatment				Days After Treatment					
	1	24	48	72	4	7	10	14	17	21	1	24	48	72	4	7	10	14	17	21
I. Cornea																				
A. Opacity	0	0	0	0							0	0	0	0						
B. Area	0	0	0	0							0	0	0	0						
C. Fluorescein Staining	-	0	-	-							-	0	-	-						
D. Stippling	0	0	0	0							0	0	0	0						
SCORE	0	0	0	0							0	0	0	0						
II. Iris																				
A. Grade	0	0	0	0							0	0	0	0						
SCORE	0	0	0	0							0	0	0	0						
III. Conjunctivae																				
A. Redness	1	0	0	0							1	0	0	0						
B. Chemosis	1	0	0	0							1	0	0	0						
C. Discharge	1	0	0	0							1	0	0	0						
D. Necrosis or Ulceration	0	0	0	0							0	0	0	0						
SCORE	6	0	0	0							6	0	0	0						
TOTAL SCORE	6	0	0	0							6	0	0	0						

M – Male; F – Female

Duration of Study: 72 Hours

TABLE 2
ACUTE EYE IRRITATION STUDY IN RABBITS
Scores and Score Summary
Test Substance: Miller 6064

Time After Treatment	Rabbit Number						Average Score
	2290-M	2291-F	2292-M	2293-F	2294-M	2295-F	
Hour 1	6	6	6	6	6	6	6.0
Hour 24	0	0	0	0	0	0	0.0
Hour 48	0	0	0	0	0	0	0.0
Hour 72	0	0	0	0	0	0	0.0
Day 4							
Day 7							
Day 10							
Day 14							
Day 17							
Day 21							
Maximum Average Score:				6.0	Minimally Irritating		
Toxicity Category:				IV			
M – Male; F – Female							
Duration of Study: 72 Hours							

APPENDIX A

MILLER CHEMICAL & FERTILIZER CORPORATION

P.O. BOX 333, 120 RADIO ROAD
HANOVER, PENNSYLVANIA 17331 U.S.A.
TELEPHONE: 717-632-8921
FAX NO.: 717-632-4561

1/18/01

CERTIFICATE OF ANALYSIS

Product Name: MILLER 6064
Quantity Produced: 7.4 kilograms
Batch Number: 150386
Date of Formulation: 12/13/00

Appearance: Yellow to Amber Liquid
Odor: Terpenic
Moisture: Negligble (non-aqueous)
Specific Gravity: 0.92 - 0.94 @ 20c
Viscosity @ 20c: 100 to 500 cps
pH(1% solution): 6.0 to 8.0
Solubility in Water: Insoluble (Emulsifies)
Flash Point: over 200 f
Purity: 100%

Signature:

Name: Andrew G. Smith
Title: Compliance Officer

APPENDIX B

STILLMEADOW
INCORPORATED

PROTOCOL FOR STUDY 6209-00

Study Title: ACUTE EYE IRRITATION STUDY IN RABBITS
(OPPTS 870.2400)

Test Substance: MILLER 6064

Test Facility: STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, Texas 77478

Approved: _____ 26 Dec 00
Janice O. Kuhn, Ph.D., D.A.B.T. Date
Study Director
STILLMEADOW, Inc.

Approved: _____ 6 Dec 00
Mark S. Holbert Date
Vice President
STILLMEADOW, Inc.

Reviewed: _____ 6 Dec. 2000
Vicki S. Crutchfield, RQAP Date
Director, Quality Assurance Unit
STILLMEADOW, Inc.

Sponsor: Miller Chemical and Fertilization Corp.
P.O. Box 333
Hanover, PA 17331

Sponsor Representative: Mandava Associates
1730 M Street, N.W., Suite 906
Washington, DC 20036

Approved: _____ December 26, 2000
N. Bhushan Mandava Date
Agent to Miller Chemical and Fertilization Corp.

PROTOCOL FOR STUDY 6209-00

A. GENERAL

1. Study Title: ACUTE EYE IRRITATION STUDY IN RABBITS

2. Purpose: To assess the relative level of irritation produced following a single exposure of a test substance to one eye of albino rabbits.

3. Regulatory Compliance: This study will be conducted according to OPPTS 870.2400, which is intended to meet testing requirements of both FIFRA (7 U.S.C. 136, *et seq.*) and TSCA (15 U.S.C. 2601).

This study will be conducted in compliance with Good Laboratory Practice Standards:
1. EPA FIFRA: 40 CFR 160
2. EPA TSCA: 40 CFR 792
3. OECD: C(97)186
4. Japanese MAFF

All procedures in this protocol are in compliance with Animal Welfare Act Regulations. All methods can be found in STILLMEADOW, Inc. Standard Operating Procedures (SOPs).

4. Quality Assurance: The Protocol is reviewed by the Quality Assurance Unit (QAU). The study information will be entered into the Master Schedule. The study will be inspected at least once during its progress. Further inspections may be scheduled as needed to ensure the integrity of the study. Any deviations from SOPs, the Protocol, or Good Laboratory Practice Standards will be immediately reported to the Study Director and Management. The report will be audited and a statement prepared and signed which shall specify the dates inspections were made and findings reported to Management and to the Study Director.

5. Test Substance: MILLER 6064. Test substance identification should include the name, batch number, and purity. Information regarding safety, stability, storage conditions, and disposal should also be provided by the Sponsor. The Sponsor assumes responsibility for purity and stability determinations. Analysis and archiving of reserve samples will be the responsibility of the Sponsor.

6. Proposed Schedule: Testing will begin within approximately three weeks of receipt of the test substance and authorization to conduct the study.

Proposed Start Date: 18 Dec 00
Proposed End Date: 08 Jan 01

If ocular effects are resolved prior to 21 days after treatment, the study may end as early as 72 hours after treatment. The period of observation will not exceed 21 days.

A. GENERAL (cont.)

7. Study Director:	Janice O. Kuhn, Ph.D., D.A.B.T.
8. Experimental Summary:	One eye of each of six rabbits will be treated with the test substance. Eye irritation scores will be determined at 1, 24, 48 and 72 hours after treatment. If irritation persists at the 72-hour reading, observations will be made at 4 and 7 days and every 2 - 4 days thereafter until the eyes are clear or for a maximum of 21 days. These scores will be used to determine a descriptive rating for the test substance.
9. Protocol Amendments:	Any alteration in the Protocol will be justified, approved by the Study Director, and recorded in writing.
10. Sponsor Audits:	The Sponsor may send an authorized representative to inspect the test system and/or data on the STILLMEADOW, Inc. premises during normal working hours.

B. EXPERIMENTAL DESIGN

1. Animals

a.	Species:	Albino rabbit
b.	Strain/Source:	New Zealand White; Ray Nichols Rabbitry, Lumberton, TX (or other suitable source)
c.	Justification of Species:	The rabbit is conventionally used in primary eye irritation studies to furnish information on which human hazard can be judged, and is preferred by the regulatory agencies.
d.	Quantity and Sex:	Six rabbits. Both sexes will be represented on test.
e.	Age/Weight at Initiation:	Young adult (12 weeks - 6 months); approximately 2 - 4 kg
f.	Identification:	Ear tag
g.	Acclimation and Health Status:	Animals will be acclimated for at least five days prior to testing. Normal weight gain, appearance, behavior, and a negative pretest eye examination will be factors used to select healthy animals for testing. Only naive animals will be selected.

2. Animal Husbandry

a.	Cages:	Stainless steel, suspended, wire bottom. The housing will be maintained to exclude sawdust, woodchips, and other extraneous substances that might produce eye irritation.
b.	Number per Cage:	Animals will be individually housed.
c.	Food:	A measured amount of PMI Feeds, Inc.™ Laboratory Rabbit Diet #5321. The food is routinely analyzed by the manufacturer for nutritional components and environmental contaminants.
d.	Water:	Tap water; available *ad libitum* (automatic system). Municipal water supply analyzed by Texas Natural Resource Conservation Commission (TNRCC) Water Utilities Division.
e.	Contaminants:	There are no known contaminants in the feed or water available to laboratory animals that would be expected to interfere with this study.
f.	Environment:	Target temperature: approximately 20°C ± 3°C Target relative humidity: approximately 30 - 70% 12-hour light/dark cycle (regulated automatically) Room ventilation of approximately 10 - 12 air changes per hour.

B. EXPERIMENTAL DESIGN (cont.)

3. Test Substance Administration

a. Pretest Considerations: Ideally, the primary skin irritation potential of the test substance will be determined prior to the eye irritation test; however, this is not a requirement of this Protocol.

Any test substance with a pH of ≤2 or ≥11.5 will not be tested in the rabbit eye for irritation without consulting with the Sponsor's Representative. pH will be measured on powders (placed in aqueous solution) only if requested. pH measurement may not be appropriate for non-aqueous liquids.

Any test substance with a known Primary Irritation Index (PII) ≥5.0 will not be tested in the rabbit eye without consulting with the Sponsor's Representative.

b. Preparation of Animals: Both eyes of each animal will be examined using a fluorescein sodium ophthalmic solution within 24 hours prior to treatment. Only eyes without defects or irritation will be selected for testing.

c. Reason for Route of Administration: Ocular contact is a potential route of human exposure.

d. Application of Test Substance: On Day 0, a dose of 0.1 mL in the case of liquids or 0.1 mL by volume (with a weight of not more than 100 mg) in the case of solids or pastes, flakes, granules, powders, or other particulate forms, will be applied at room temperature to each test eye. If the test substance is solid or granular, it will be ground to a fine dust. If it is believed that the test substance may cause extreme pain, a local anesthetic may be used in both test and control eyes prior to the instillation of the test substance. The test substance will be placed in the selected eye of each animal by gently pulling the lower eyelid away from the eyeball to form a cup into which the test substance will be dropped. To prevent loss of material the eyelids will then be gently held together for approximately one second before releasing. The other eye remains untreated and serves as a control.

If the test substance is contained in a pressurized aerosol container, the eye will be held open and the test substance administered in a single burst of about one second from a distance of 10 cm directly in front of the eye.

After the twenty-four hour observation, the treated eye of each animal will be washed for one minute with room temperature deionized water.

B. EXPERIMENTAL DESIGN (cont.)

4. Ocular Observations

The treated eyes of all animals will be examined (magnification may be used as an aid) and the grades for ocular reactions will be recorded at 1, 24, 48 and 72 hours after treatment. If irritation or injury persists at the 72-hour observations, observations will be made on Days 4 and 7 and every 2 - 4 days thereafter until the eyes are clear, or for a maximum of 21 days. The study will be terminated when all animals on the study are clear of eye irritation. Fluorescein sodium ophthalmic solution will be used as an aid at the 24-hour observation. Any of the corneas which exhibit positive fluorescein staining at the 24-hour observation will be re-examined at each successive observation time until fluorescein staining is no longer present. The visualization of fluorescein staining will be aided by using a Finoff ocular transilluminator with cobalt blue filter (Wellch-Allyn, Skaneateles Falls, N.Y.).

Irritation will be graded and scored using the Draize technique. The grading scale is presented in Appendix A. All animals that have a damaged eye producing undue stress or discomfort will be sacrificed for humane reasons after consulting with the Sponsor.

5. Non-ocular Effects

Any non-ocular effects observed following treatment will be recorded.

6. Evaluation of Results:

An average irritation score will be determined for each observation time based on the number of animals scored. A maximum average irritation score will be derived from the observation period yielding the highest average irritation score. The maximum average irritation scores will be used to rate the test substance according to the ratings in Appendix B. In addition, the number of eyes with positive findings at each time period will be noted, and a determination of irritation reversibility will be made (no positive findings). Unless otherwise requested by the Sponsor, a determination of the toxicity category will be made according to the criteria in Appendix C.

7. Test Substance Accountability:

A comprehensive inventory of test substance received and used will be kept. The test substance container(s) will be weighed when received at this facility, and a record of all test substance use will be maintained. Test substance and test substance dosing solutions will be stored in the original containers, or in the equivalent thereof, or in glass containers with Teflon-lined caps.

8. Disposal of Unused Test Substance:

Unused test substance will be returned or disposed of at the Sponsor's expense after the termination of the study. The Sponsor should specify return or disposal at the time of study authorization. If returned, the recipient of the test substance will be notified in advance of shipping. The sample will be accompanied by a chain of custody letter describing the sample(s) contained in the shipment.

9. Safety Precautions:

General safety precautions required by laboratory SOPs will be followed. The Sponsor will supply basic toxicity data on the test substance to be used. However, since the toxicity of test substances is often not well characterized, this laboratory will be conservative in setting safety procedures. The Sponsor or Sponsor's Representative shall be notified of any exposures requiring a physician's examination or care.

C. DATA MANAGEMENT

1. Records:

The following records will be maintained during the study and transferred to the STILLMEADOW, Inc. archives upon study termination.

a. Protocol and Protocol Amendments (if any).
b. Final report and amendments (if any).
c. Study correspondence.
d. Animal receipt/acclimation data.
e. Test substance receipt, identification as supplied by Sponsor, preparation, administration, and disposition.
f. Test animal information: number, sex, source, strain.
g. Observation data for ocular irritation or injury.
h. Other pertinent data.

2. Data Storage:

All raw data will be retained at STILLMEADOW, Inc.

3. Data Reporting:

The final report will include all data as described in the Good Laboratory Practice Standards, including:

a. Statement from the Quality Assurance Unit.
b. Signature of the Study Director.
c. A GLP Compliance Statement signed by the Study Director.
d. Names of scientific personnel involved in the study.
e. Dates of study initiation and termination.
f. Identification, description, preparation, and storage of the test substance.
g. Identification and description of any vehicles, anesthetics or other materials used in the study.
h. All pertinent animal data, animal husbandry, acclimation information, dosing information.
i. Description of the method used to score irritation.
j. Description of any non-ocular effects noted.
k. Individual observations for treated eyes.
l. The number of eyes with positive findings at each time period; determination when irritation was reversible (no positive findings).
m. Individual eye irritation scores for each time period for each animal.
n. Maximum average irritation scores.
o. Rating and Toxicity Category (unless otherwise requested by the Sponsor) of the test substance.
p. A reference to this Protocol.

4. Report Submission:

A report will be submitted after termination of the in-life portion of the study.

Appendix A
ACUTE EYE IRRITATION STUDY IN RABBITS
Grading Scale

I. Cornea

A. Opacity - degree (area most dense taken for reading)

No opacity 0
Slight dulling of normal luster ..... +
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible 1*
Easily discernible translucent areas, details of iris slightly obscured 2*
Opalescent area, no details of iris visible, size of pupil barely discernible 3*
Opaque cornea, iris not discernible through the opacity 4*

B. Area of cornea involved

One quarter (or less), but not zero 1
Greater than one quarter, but less than half 2
Greater than half, but less than three quarters 3
Greater than three quarters, up to whole area 4

C. Fluorescein Staining - appearance of yellow-green staining of cornea

Cornea not examined with fluorescein ..... -
No fluorescein staining 0
Positive fluorescein staining ..... P
Area of cornea involved
One quarter (or less), but not zero ..... A
Greater than one quarter, but less than half ..... B
Greater than half, but less than three quarters C
Greater than three quarters, up to whole area ..... D

D. Stippling - appearance of pinpoint roughening

No stippling 0
Presence of stippling ..... S
Area of cornea involved
One quarter (or less), but not zero ..... A
Greater than one quarter, but less than half ..... B
Greater than half, but less than three quarters C
Greater than three quarters, up to whole area ..... D

A X B X 5 Total Maximum = 80

* - Reaction indicates a positive effect.

Reference: Draize, John H., Woodard, Geoffrey, and Calvery, Herbert O., Journal of Pharmacol. Exp. Ther., 82, 377-390 (1944).

Appendix A (cont.)
ACUTE EYE IRRITATION STUDY IN RABBITS
Grading Scale

II. Iris
A. Grades
Normal 0
Folds above normal, congestion, swelling, moderate circumcorneal hyperemia or injection (any of these or combination thereof), iris still reacting to light (sluggish reaction is positive) 1*
No reaction to light, hemorrhage, gross destruction (any or all of these) 2*

A X 5 Total Maximum = 10

III. Conjunctivae
A. Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)
Blood vessels normal 0
Some blood vessels definitely hyperemic (injected) 1
Diffuse, crimson color, individual vessels not easily discernible 2*
Diffuse beefy red 3*

B. Chemosis: lids and/or nictitating membrane
No swelling 0
Any swelling above normal (includes nictitating membrane) 1
Obvious swelling with partial eversion of lids 2*
Swelling with lids about half closed 3*
Swelling with lids more than half closed 4*

C. Discharge
No discharge 0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals) 1
Discharge with moistening of the lids and hairs just adjacent to lids 2
Discharge with moistening of the lids and hairs, and considerable area around the eye 3

D. Necrosis or Ulceration of the palpebral and bulbar conjunctivae or nictitating membrane
No necrosis or ulceration 0
Presence of necrosis or ulceration ..... N

(A + B + C) X 2 Total Maximum = 20

The total score for the eye is the sum of all scores obtained for the cornea, iris, and conjunctivae with the possible maximum total score for the eye being equal to 110.

* - Reaction indicates a positive effect.

Appendix B
ACUTE EYE IRRITATION STUDY IN RABBITS
Rating of Test Substance Based on Eye Irritation

Rating	Maximum Average Score	Definition
Non-Irritating	0.0 - 0.5	To maintain this rating, all scores at the 24-hour reading must be zero; otherwise, increase rating one level.
Practically Non-Irritating	> 0.5 - 2.5	To maintain this rating, all scores at the 24-hour reading must be zero; otherwise, increase rating one level.
Minimally Irritating	> 2.5 - 15.0	To maintain this rating, all scores at the 72-hour reading must be zero; otherwise, increase rating one level.
Mildly Irritating	> 15.0 - 25.0	To maintain this rating, scores at the 7-day reading must be zero; otherwise, increase rating one level.
Moderately Irritating	> 25.0 - 50.0	To maintain this rating, scores at the 7-day reading must be less than or equal to 10 for 60% or more of the animals. Also, the 7-day mean score must be less than or equal to 20. If the 7-day mean score is less than or equal to 20, but less than 60% of the animals show scores less than 10, then no animal among those showing scores greater than 10 can exceed a score of 30 if rating is to be maintained; otherwise, increase rating one level.
Severely Irritating	> 50.0 - 80.0	To maintain this rating, scores at the 7-day reading must be less than or equal to 30 for 60% or more of the animals. Also, the 7-day mean score must be less than or equal to 40. If the 7-day mean score is less than or equal to 40, but less than 60% of the animals show scores less than or equal to 30, then no animal among those showing scores greater than 30 can exceed a score of 60 if rating is to be maintained; otherwise, increase rating one level.
Extremely Irritating	> 80.0 - 110.0	

NOTE: The rating of the test material is not to be increased more than one level above its maximum average score.

Reference: Modification of Classification System of John H. Kay, Ph.D., and Joseph C. Calandra, Ph.D., Interpretation of Eye Irritation Tests, Journal of the Society of Cosmetic Chemists, p. 286.

Appendix C
ACUTE EYE IRRITATION STUDY IN RABBITS
Criteria of Eye Irritation for Classification into Toxicity Categories

Category	Criteria
I	Corrosive (irreversible destruction of ocular tissue) or corneal involvement or "positive" conjunctival irritation persisting through Day 21.
II	Corneal involvement or "positive" conjunctival irritation clearing in 8-21 days.
III	Corneal involvement or "positive" conjunctival irritation clearing in 7 days or less.
IV	Minimal effects clearing in less than 24 hours. No "positive" effects at 24 hours.

ATTACHMENT 39

Skin Sensitization Study
(Guinea Pig Maximization Test for Topically Applied Test Substance)
on Miller 6064

STILLMEADOW
INCORPORATED

VOLUME __ OF __ OF SUBMISSION

Miller 6064

FINAL REPORT

GUINEA PIG MAXIMIZATION TEST FOR TOPICALLY APPLIED TEST SUBSTANCE

OPPTS NO. 870.2600

AUTHOR - Janice O. Kuhn, Ph.D., D.A.B.T.

STUDY INITIATION DATE: 26 December 2000
STUDY COMPLETION DATE: 27 March 2001

CONDUCTED BY:
STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, TX 77478

LABORATORY STUDY NUMBER

6211-00

VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 29

SUBMITTED TO:
Miller Chemical & Fertilizer Corp.
P.O. Box 333, Radio Road
Hanover, PA 17331

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA § 10 (d) (1) (A), (B) or (C).

Company: Miller Chemical & Fertilizer Corp.

Company Agent: _____ Date: _____

_____ _____
Title Signature

These data are the property of Miller Chemical & Fertilizer Corp., and as such, are considered to be confidential for all purposes other than compliance with FIFRA § 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other statute or in any other country.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was designed and performed in STILLMEADOW, Inc.'s AAALAC accredited laboratory in compliance with:

- United States Environmental Protection Agency FIFRA: GLP Standards, 40 CFR 160 with exception of Sec. 160.31 (d) and 160.105 (b)(e): stability information was not provided
- United States Environmental Protection Agency TSCA: 40 CFR 792, with exception of Sec. 792.31 (d) and 792.105 (b)(e): stability information was not provided
- Organization for Economic Cooperation & Dev. Principles of GLP, Annex 2, C(97)186, with exception of Sec. 6.2 (4): stability information was not provided
- Japan Ministry of Agriculture, Forestry & Fisheries, Notification No. 59 Nohsan 3850, Director-General of Agricultural Prod. Bureau, 10 Aug 84, with exception of Art. 5 (2)(9) and 22 (3): stability information was not provided

Janice O. Kuhn, Ph.D., D.A.B.T.
Study Director, STILLMEADOW, Inc.

27 Mar 01
Date

Signature of Agent of Sponsor

Date

Agent Name
Sponsor: Miller Chemical & Fertilizer Corp.

Signature of Agent of Submitter

Date

Agent Name
Submitter: Mandava Associates

TABLE OF CONTENTS

QUALITY ASSURANCE STATEMENT

Study Number: 6211-00
Test Substance: Miller 6064
Study Title: Guinea Pig Maximization Test For Topically Applied Test Substance

The study report and data have been audited in accordance with STILLMEADOW, Inc. Standard Operating Procedures (SOP). The findings from inspection and audit were reported to study Director and management as follows:

Phase of Study Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
Dosing	30 Jan 01	31 Jan 01	31 Jan 01
Report/Data Audit	7 Mar 01	7 Mar 01	7 Mar 01

B. Lynn Murphy, B.S.
Quality Assurance Unit, STILLMEADOW, Inc.

27 Mar 01
Date

SUMMARY

A maximization test for topically applied test substances was conducted on 30 short-haired male and female albino guinea pigs to determine if the test substance, Miller 6064, produced a sensitizing reaction. Group I animals, the test group (10/sex), each received three pairs of intradermal injections (adjuvant, a solution of test substance in deionized water, and a 50:50 mixture of adjuvant and the test substance solution) followed one week later by a single topical application of undiluted test substance. Ten additional animals (5/sex) served as a control group (Group II). Control animals were treated at the same time periods and locations but with the vehicle used in place of the test substance/solution. Two weeks after the topical application, the test animals were challenged with a second topical application of undiluted test substance at a virgin test site. Control animals were also given a topical application of undiluted test substance. The percentage of animals exhibiting erythema with or without edema after the challenge treatment was used to assign the test substance a sensitization potency rating. Since 0% of the test animals exhibited scores greater than zero, Miller 6064 was given a sensitization potency rating of non-sensitizer.

INTRODUCTION

The objective of this study was to determine the sensitizing potential of the test substance, using the methods of Magnusson and Kligman (J. Invest. Dermat 52: 268-276 (1969)). This study was conducted for Miller Chemical & Fertilizer Corp., according to the approved protocol and STILLMEADOW, Inc. SOP's. There were no deviations from the protocol that affected the quality or outcome of the study. All procedures used in this study are in compliance with Animal Welfare Act Regulations. The protocol, raw data and this report are archived at STILLMEADOW, Inc. The treatment schedule was as follows, and the study was terminated on 2 Feb 01:

	Treatments		
	Intradermal Injections	Topical Applications	
Groups	Initial Treatment	Second Insult	Challenge
Test and Control	9 Jan 01	16 Jan 01	30 Jan 01

TEST SUBSTANCE

Label:	Miller 6064
Quantity & Date Received:	19 Dec 00; 2 x 1 gal
Physical Description:	Amber liquid
Storage:	Room temperature
Purity & Composition:	See Certificate of Analysis (App. B)
Stability:	Not provided by sponsor
Concentrations Administered:	3% v/v solution of test substance in vehicle with and without adjuvant (50:50 v/v) for intradermal injections; 0.5 mL undiluted for topical and challenge applications
Vehicle:	Deionized water

Records pertaining to stability, characterization, and verification of test substance identity are the responsibility of the sponsor. A copy of the Certificate of Analysis is included in the report.

ADJUVANT

Label:	FREUND'S ADJUVANT Complete Lot # 20K8933
Manufacturer:	Sigma Chemical
Physical Description:	Clear liquid
Storage:	Store in a cool, dark place at 0 to 5°C, do not freeze
Concentration Administered:	Diluted at 50% v/v in 0.9% saline
Purity & Composition:	Available from manufacturer
Stability:	Expiration – Mar 03

VEHICLE AND/OR OTHER MATERIALS

Label:	0.9% Sodium Chloride Lot # 50-164-JT
Manufacturer:	Abbott Labs
Expiration Date:	Mar 01

TEST SYSTEM

Experimental Animals

Species & Strain:	Guinea Pig; Hartley-Albino
Justification of Species:	The guinea pig is conventionally used in skin sensitization studies to provide information on which human hazard can be judged.
Source:	Charles River Laboratories, Wilmington, MA
Quantity & Sex:	15 males and 15 females
Quarantine Period:	5 days
Date Received:	2 Jan 01
Animal Identification:	Ear punch
Weight When Tested:	Males (328-406 g); Females (323-368 g)

Animal Husbandry

Cage Type:	Suspended, wire bottom, stainless steel
Housing:	Housed individually for 3 days (during wrapping) 1-4 animals per cage (males separate from females)
Environmental Controls Set to Maintain:	·Temperature Range 20°C ± 3° ·Humidity Range 30-80% ·12-hour light/dark cycle ·10-12 air changes/hour
Food:	PMI Feeds, Inc.™ Guinea Pig Diet #5025 available *ad libitum*
Water:	Municipal water supply analyzed by TNRCC Water Utilities Division; available *ad libitum* from water bowls or automatic system

Animal husbandry and housing at STILLMEADOW, Inc. comply with Animal Welfare Act Regulations. No contaminants were expected to have been present in the feed or water which would have interfered with or affected the results of the study.

POSITIVE CONTROL INFORMATION

Positive Control Material

Label:	1-Chloro-2,4-Dinitro-Benzene Min. 98% [97-00-7] EEC No. 202-551-4 C-6396 Lot 87H0799
Manufacturer:	Sigma Chemical
Physical Description:	Light yellow crystals
Concentrations Administered:	0.5% w/v solution of test substance in cottonseed oil with and without adjuvant (50:50 v/v) for intradermal injections; 2.0% w/w concentration of test substance in petrolatum for topical insult and challenge application
Purity, Composition & Stability:	Available from manufacturer

POSITIVE CONTROL INFORMATION (cont.)

Positive Control Testing
The sensitivity of guinea pigs to a positive control material is confirmed in this laboratory periodically. The positive control animals used to conduct this study were supplied by Charles River Laboratory, and were tested according to the Magnusson and Kligman (J. Invest. Dermat 52: 268-276 (1969)).

STILLMEADOW, Inc. Study No. 5782-00
In-life start: 18 Apr 00; In-life completed: 12 May 00

Results: Data from this study are presented in Appendix A. Since all 20 animals of the test group exhibited patchy to intense erythema after the challenge treatment, and only three of the 10 animals of the naive control group exhibited patchy to moderate erythema after the challenge treatment, the test substance is considered an extreme sensitizer and confirmed the sensitivity of guinea pigs to the positive control material.

PROCEDURES

Test Substance Preparation
A 3% v/v solution of test substance in deionized water was selected for intradermal injection, and 0.5 mL of undiluted test substance was selected for the topical applications (induction and challenge).

Healthy, short-haired, albino guinea pigs (males and females) were released from quarantine prior to testing. Five animals per sex were assigned to a control group (Group II). Ten animals per sex were assigned to the test group (Group I). On the day prior to each treatment, the animals were prepared by clipping the appropriate exposure areas free of hair. Individual body weights were recorded on Days -1 and 24. The animals were treated on Days 0, 7, and 21.

Test Substance Application
Induction: Intradermal Injections: The animals were treated on Day 0 by making three pairs of symmetrical intradermal injections on the upper back of each animal within a 4 x 6 cm exposure area running laterally across the shoulders. For the test animals (Group I), the first pair of injections (one on each side of the spinal column and approximately 3.5 cm apart), consisting of Freund's Complete Adjuvant diluted to 50% v/v in saline, was made at the anterior edge of the exposure area. The second pair of injections, consisting of 3% v/v test substance in deionized water, was made approximately 0.5 cm behind the first pair. The third pair of injections, consisting of a 50:50 mixture of Freund's Complete Adjuvant (diluted to 50% v/v in saline) and a solution of 3% v/v test substance in deionized water was made approximately 0.5 cm behind the second pair of injections. Group II control animals received the same injections with the vehicle substituted for the test substance in the second and third pairs of injections. All injections were within a 2 x 4 cm area of the 4 x 6 cm exposure area. A volume of 0.1 mL was administered at each site.

PROCEDURES (cont.)

Induction: Topical Applications: On Day 7, 0.5 mL of undiluted test substance was applied to the exposure area of each test group animal (Group I) to cover the intradermal injection sites. A 5 cm round patch of filter paper was used to cover the dose site. The patch was then occluded with an adhesive masking tape and secured in place with an elastic adhesive wrap wound around the torso of the animal. Control group animals (Group II) received 0.5 mL of vehicle and a 5 cm round patch of filter paper was placed over the dose sites. The wrappings and patches were removed after 48 hours. Test sites 3 and 4 were observed for dermal irritation on Day 10.

Challenge: Test and Control Animals: On Day 21, a 5 x 5 cm area was clipped on both the left and right flanks of each test and control animal. For the challenge treatment, 0.5 mL of undiluted test substance was applied topically to the right flank of each animal in a manner identical to the Day 7 treatment. A 5 cm round patch of filter paper was used to cover the dose site. A dry 5 cm round patch of filter paper was applied topically to the left flank of each animal. Patches were secured as above.

Observations and Scoring Methods

On Day 22 (24 hours after challenge), the wrappings and patches were removed. On Day 23 (24 hours after unwrapping), the test sites were observed for skin reactions, and again observed on Day 24 (48 hours after unwrapping). Observations were made of right and left flanks of each animal in Groups I and II. The scoring scale used for grading skin reactions after both intradermal and topical exposures is presented in the Legend to Table 1. After both induction treatments (intradermal injections and topical applications) and the challenge exposure, average skin reaction scores were calculated. These data appear in Table 1.

Any Group I animals which exhibited scores greater than 0 for erythema with or without edema for the treated right flank after the challenge treatment were considered possibly sensitized. However, the skin reactions of the left flank treated with patch alone, and the skin reactions of the naive controls were also evaluated. The test substance was graded and rated for sensitization potency based upon the percentage of animals sensitized using the scoring scale presented below:

% Sensitized	Grade	Rating
0	0	Non-sensitizer
1-8	I	Weak
9-28	II	Mild
29-64	III	Moderate
65-80	IV	Strong
81-100	V	Extreme

RESULTS AND DISCUSSION

Skin reaction scores and average skin reaction scores are presented in Table 1. Body weights are presented in Table 2. Body weight gain was unaffected by the administration of the test substance. The challenge treatments with either patch alone or test substance produced no erythema in any test or control group animals.

CONCLUSION

Since 0% of the test animals exhibited scores greater than zero, Miller 6064 was given a sensitization potency rating of non-sensitizer.

Study Director: Janice O. Kuhn — 27 Mar 01
Janice O. Kuhn, Ph.D., D.A.B.T. — Date
Senior Toxicologist, STILLMEADOW, Inc.

STUDY PERSONNEL

Technical Staff: Carol Morris, B.A.
Hector Fuentes
Michelle Gantt, B.S.

Data Services: Connie Pavatte, Report Preparation

TABLE 1
GUINEA PIG MAXIMIZATION TEST FOR TOPICALLY APPLIED TEST SUBSTANCE
Skin Reaction Scores and Averages
Test Substance: Miller 6064
Group I – Test

	Intradermal Induction						Topical Induction		Challenge			
Animal Number	Scores for each site on Day 6						Day 10 Scores		Day 23		Day 24	
	1	2	3	4	5	6	Site 3	Site 4	LF	RF	LF	RF
1-M	2	2	1	1	1	1	1	1	0	0	0	0
2-M	2	2	1	2	1	2	1	1	0	0	0	0
3-M	2	2	1	1	1	1	1	1	0	0	0	0
* 4-M	2	2	1	1	1	2	0	1	0	0	0	0
5-M	2	2	1	1	1	1	0	0	0	0	0	0
6-M	2	2	1	1	1	2	1	1	0	0	0	0
7-M	2	2	1	1	1	1	1	1	0	0	0	0
8-M	1	1	1	1	1	1	1	1	0	0	0	0
9-M	2	2	0	0	1	1	1	1	0	0	0	0
10-M	2	2	1	1	1	2	1	1	0	0	0	0
11-F	2	2	1	1	2	2	1	1	0	0	0	0
12-F	2	2	1	1	2	2	1	1	0	0	0	0
13-F	1	2	1	1	1	2	0	1	0	0	0	0
14-F	2	1	1	1	2	1	0	1	0	0	0	0
15-F	2	1	1	1	1	1	0	0	0	0	0	0
16-F	1	1	1	1	1	1	1	1	0	0	0	0
17-F	2	2	1	1	1	2	1	1	0	0	0	0
18-F	1	1	1	1	1	1	1	1	0	0	0	0
19-F	1	1	1	1	1	1	1	1	0	0	0	0
20-F	1	1	1	0	1	1	0	0	0	0	0	0
Mean:	1.7	1.7	1.0	1.0	1.2	1.4	0.8		0.0	0.0	0.0	0.0
	1.3						Incidence of Reactions:		0/20		0/20	

% Sensitized: 0% Sensitization Potency Rating: Non-sensitizer

Injection Sites:
Sites 1 & 2 - Freund's Complete Adjuvant - 50% v/v in saline
Sites 3 & 4 - Test Substance in deionized water
Sites 5 & 6 - Mixture (50:50) of Freund's Complete Adjuvant in saline and Test Substance in deionized water

Induction topical and Challenge Treatments: 100% Test Substance
LF - Left Flank (dosed w/dry patch); RF - Right Flank (dosed w/Test Substance)

M - Male; F - Female

TABLE 1 (cont.)
GUINEA PIG MAXIMIZATION TEST FOR TOPICALLY APPLIED TEST SUBSTANCE
Skin Reaction Scores and Averages
Test Substance: Miller 6064
Group II - Control

	Intradermal Induction						Topical Induction		Challenge			
Animal Number	Scores for each site on Day 6						Day 10 Scores		Day 23		Day 24	
	1	2	3	4	5	6	Site 3	Site 4	LF	RF	LF	RF
21-M	2	2	0	0	0	0	0	0	0	0	0	0
22-M	2	2	0	0	0	0	0	0	0	0	0	0
23-M	2	2	0	0	0	0	0	0	0	0	0	0
24-M	2	2	0	0	0	0	0	0	0	0	0	0
25-M	2	2	0	0	0	0	0	0	0	0	0	0
26-F	1	1	0	0	0	0	0	0	0	0	0	0
27-F	2	2	0	0	0	0	0	0	0	0	0	0
28-F	2	2	0	0	0	0	0	0	0	0	0	0
29-F	1	1	0	0	0	0	0	0	0	0	0	0
30-F	1	1	0	0	0	0	0	0	0	0	0	0
Mean:	1.7	1.7	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0
	0.6						Incidence of Reactions:		0/10		0/10	

Injection Sites:
Sites 1 & 2 - Freund's Complete Adjuvant - 50% v/v in saline
Sites 3 & 4 – Vehicle (deionized water)
Sites 5 & 6 - Mixture (50:50) of Freund's Complete Adjuvant in saline and vehicle

Induction Topical Treatments: 0.5 mL of vehicle

Challenge Treatments: 100% Test Substance
LF - Left Flank (dosed w/dry patch); RF - Right Flank (dosed w/Test Substance)

M - Male; F - Female

LEGEND TO TABLE 1
GUINEA PIG MAXIMIZATION TEST FOR TOPICALLY APPLIED TEST SUBSTANCE
Evaluation of Skin Reactions

Magnusson and Kligman Grading Scale for the Evaluation of Challenge Patch Test Reactions*

Erythema Formation	Score
No visible change	0
Slightly patchy erythema	±
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

* - OECD Guidelines for the Testing of Chemicals, Volume 2, Section 4, Number 406, Skin Sensitization, Paragraph 23, page 4/9, Adopted 17 Jul 92

APPENDIX A
GUINEA PIG MAXIMIZATION TEST FOR TOPICALLY APPLIED TEST SUBSTANCE
Positive Control Table 1
Skin Reaction Scores and Averages
Positive Control Substance: DNCB C-6396 Lot 87H0799
Study Number: 5782-00
Group I – Test

	Intradermal Induction						Topical Induction		Challenge			
Animal Number	Scores for each site on Day 6						Day 10 Scores		Day 23		Day 24	
	1	2	3	4	5	6	Site 3	Site 4	LF	RF	LF	RF
261-M	0	0	0	0	0	0	1	2	0	2	0	2
262-M	0	±	0	0	0	0	3	3	0	2	0	2
263-M	0	0	±	±	0	0	2	2	0	3	0	2
264-M	0	0	±	0	0	0	2	3	0	3	0	2
265-M	0	0	0	1	0	0	2	2	0	3	0	2
266-M	0	0	0	±	1	0	3	2	0	3	0	2
267-M	±	±	0	0	0	0	3	3	0	3	0	1
268-M	0	0	0	0	±	±	1	1	0	3	0	2
269-M	±	±	0	0	0	±	3	3	0	1	0	2
270-M	±	±	0	±	0	0	3	3	0	2	0	1
271-F	±	±	0	0	0	0	3	3	0	1	0	1
272-F	±	±	0	±	±	0	3	3	0	3	0	2
273-F	0	0	0	0	0	±	3	3	0	3	0	3
274-F	±	±	0	0	0	±	3	3	0	3	0	1
275-F	±	±	0	0	0	0	2	3	0	1	0	1
276-F	1	±	0	0	0	0	3	3	0	3	0	2
277-F	0	0	0	0	0	0	2	3	0	1	0	1
278-F	0	±	0	0	0	0	3	3	0	3	0	2
279-F	1	±	0	0	0	0	2	3	0	1	0	3
280-F	0	±	0	0	0	0	3	2	0	2	0	2
Mean:	0.1	0.0	0.0	0.1	0.1	0.0	2.6		0.0	2.3	0.0	1.8
	0.1						Incidence of Reactions:		20/20		20/20	

% Sensitized: 100% Sensitization Potency Rating: Extreme Sensitizer

Injection Sites:
Sites 1 & 2 - Freund's Complete Adjuvant - 50% v/v in saline
Sites 3 & 4 - Test Substance 0.5% w/v in cottonseed oil
Sites 5 & 6 - Mixture (50:50) of Freund's Complete Adjuvant in saline and Test Substance (0.5% w/v)

Induction Topical and Challenge Treatments: 2% w/w concentration of Test Substance in petrolatum
LF - Left Flank (dosed w/vehicle); RF - Right Flank (dosed w/Test Substance in vehicle)

M - Male; F - Female

APPENDIX A (cont.)
GUINEA PIG MAXIMIZATION TEST FOR TOPICALLY APPLIED TEST SUBSTANCE
Positive Control Table 1 (cont.)
Skin Reaction Scores and Averages
Positive Control Substance: DNCB C-6396 Lot 87H0799
Study Number: 5782-00
Group II - Control

	Intradermal Induction						Topical Induction		Challenge			
Animal Number	Scores for each site on Day 6						Day 10 Scores		Day 23		Day 24	
	1	2	3	4	5	6	Site 3	Site 4	LF	RF	LF	RF
281-M	±	±	0	0	0	0	0	2	0	2	0	1
282-M	±	±	0	0	0	0	0	0	0	0	0	0
283-M	±	±	0	0	0	0	0	0	0	1	0	1
284-M	±	±	0	0	0	0	0	0	0	0	0	0
285-M	±	±	0	0	0	0	0	0	0	0	0	0
286-F	±	±	0	0	±	0	0	0	0	0	0	0
287-F	±	±	0	0	0	0	0	0	0	1	0	1
288-F	±	±	0	0	0	±	0	0	0	0	0	0
289-F	±	±	0	0	0	0	0	0	0	0	0	0
290-F	0	±	0	0	0	0	0	0	0	0	0	0
Mean:	0.0	0.0	0.0	0.0	0.0	0.0	0.1		0.0	0.4	0.0	0.3
	0.0						Incidence of Reactions:		3/10		3/10	

Injection Sites:
Sites 1 & 2 - Freund's Complete Adjuvant - 50% v/v in saline
Sites 3 & 4 – Cottonseed oil
Sites 5 & 6 - Mixture (50:50) of Freund's Complete Adjuvant in saline and Cottonseed oil

Induction Topical Treatments: 0.5 g of petrolatum

Challenge Treatments: 2% w/w concentration of Test Substance in petrolatum
LF - Left Flank (dosed w/vehicle); RF - Right Flank (dosed w/Test Substance in vehicle)

APPENDIX B

MILLER® CHEMICAL & FERTILIZER CORPORATION

P.O. BOX 333, 120 RADIO ROAD
HANOVER, PENNSYLVANIA 17331 U.S.A.
TELEPHONE: 717-632-8921
FAX NO.: 717-632-4561

1/18/01

CERTIFICATE OF ANALYSIS

Product Name: MILLER 6064
Quantity Produced: 7.4 kilograms
Batch Number: 150386
Date of Formulation: 12/13/00

Appearance: Yellow to Amber Liquid
Odor: Terpenic
Moisture: Negligible (non-aqueous)
Specific Gravity: 0.92 - 0.94 @ 20c
Viscosity @ 20c: 100 to 500 cps
pH(1% solution): 6.0 to 8.0
Solubility in Water: Insoluble (Emulsifies)
Flash Point: over 200 f
Purity: 100%

Signature:

Name: Andrew G. Smith
Title: Compliance Officer

APPENDIX C

STILLMEADOW
INCORPORATED

PROTOCOL FOR STUDY 6211-00

Study Title: GUINEA PIG MAXIMIZATION TEST FOR TOPICALLY APPLIED TEST SUBSTANCE (OPPTS 870.2600)

Test Substance: MILLER 6064

Test Facility: STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, Texas 77478

Approved: _____ 26 Dec 00
Janice O. Kuhn, Ph.D., D.A.B.T. Date
Study Director
STILLMEADOW, Inc.

Approved: _____ 6 Dec 00
Mark S. Holbert Date
Vice President
STILLMEADOW, Inc.

Reviewed: _____ 6 Dec. 2000
Vicki S. Crutchfield, RQAP Date
Director, Quality Assurance Unit
STILLMEADOW, Inc.

Sponsor: Miller Chemical & Fertilization Corp.
P.O. Box 333
Hanover, PA 17331

Sponsor Representative: Mandava Associates
1730 M Street, N.W., Suite 906
Washington, DC 20036

Approved: _____ December 26, 2000
N. Bhushan Mandava Date
Agent to Miller Chemical & Fertilization Corp.

APPENDIX C (cont.)

PROTOCOL FOR STUDY 6211-00

A. GENERAL

1. Study Title: GUINEA PIG MAXIMIZATION TEST FOR TOPICALLY APPLIED TEST SUBSTANCE

2. Purpose: To determine the skin sensitization potential of the test substance in guinea pigs. This protocol follows the recommendations of the "maximization" method of Magnusson B. and Kligman A.M. (*Journal of Investigative Dermatology*. 52: 268-276 (1969)) concerning the evaluation of the dermal sensitizing potential in guinea pigs.

3. Regulatory Compliance: This study meets or exceeds the requirements of OECD Guideline 406 and EPA OPPTS Health Effects Test Guidelines 870.2600.

This study will be conducted in compliance with Good Laboratory Practice Standards:

1. EPA FIFRA: 40 CFR 160
2. EPA TSCA: 40 CFR 792
3. OECD: C(97)186
4. Japanese MAFF

All procedures in this protocol are in compliance with Animal Welfare Act Regulations. All methods can be found in STILLMEADOW, Inc. Standard Operating Procedures (SOPs).

4. Quality Assurance: The protocol is reviewed by the Quality Assurance Unit (QAU). The study information will be entered into the Master Schedule. The study will be inspected at least once during its progress. Further inspections may be scheduled as needed to ensure the integrity of the study. Any deviations for SOPs, the Protocol, or Good Laboratory Practice Standards will be immediately reported to the Study Director and Management. The report will be audited and a statement prepared and signed which shall specify the dates inspections were made and findings reported to Management and to the Study Director.

5. Test Substance: MILLER 6064. Test substance identification should include the name, batch number and purity. Information regarding safety, stability, storage conditions and disposal should also be provided by the Sponsor. The Sponsor assumes responsibility for purity and stability determinations. Analysis and archiving of reserve samples will be the responsibility of the Sponsor.

6. Positive Control Substance: 2,4-Dinitrochlorobenzene (DNCB - CAS No. 97-00-7) or other suitable positive control substance such as α-hexylcinnamaldehyde (CAS No. 101-86-0); tested periodically in this laboratory to confirm sensitization potential of the animals used and validate procedures. Results of a separate positive control study will be referenced in the final report.

APPENDIX C (cont.)

A. GENERAL (cont.)

7. Proposed Schedule:	Testing will begin within approximately three weeks of receipt of test substance and authorization to conduct the study. Proposed Start Date: 20 Dec 00 Proposed End Date: 26 Jan 01 In-life portion of the study: 25 days; if equivocal results are obtained, a rechallenge will be conducted 7 days later.
8. Study Director:	Janice O. Kuhn, Ph.D., D.A.B.T.
9. Experimental Summary:	Test group guinea pigs will be given three pairs of intradermal injections (adjuvant, test substance, and a mixture of adjuvant and test substance) followed one week later by a single topical application of the test substance in the same exposure area. (The intradermal and topical concentrations of the test substance that produce no more than moderate irritation will be determined from a range-finding test and used for the induction treatments.) Two weeks after the topical application, the animals will be challenged by a second topical application of the test substance at a virgin site using the maximum non-irritating concentration as determined from the range-finding. Control group guinea pigs will be treated at the same times and exposure areas, but with no test substance. If equivocal results are obtained at the challenge, the animals will be rechallenged after seven days. The percentage of animals exhibiting erythema with or without edema after the challenge treatment will be used to assign the test substance a sensitization rating.
10. Protocol Amendments:	Any alteration in the Protocol will be justified, approved by the Study Director, and recorded in writing.
11. Sponsor Audits:	The Sponsor may send an authorized representative to inspect the test system and/or data on the STILLMEADOW, Inc. premises during normal working hours.

APPENDIX C (cont.)

B. EXPERIMENTAL DESIGN

1. Animals

a. Species:	Guinea Pig
b. Strain/Source:	Hartley Albino (Harlan Sprague Dawley, Inc., Houston, Texas or other suitable supplier)
c. Justification of Species:	The guinea pig is conventionally used in skin sensitization studies to provide information on which human hazard can be judged, and is the preferred species in the Guidelines.
d. Quantity and Sex:	Test Group: 20 (10/sex) (females nulliparous and non-pregnant) Control Group: 10 (both sexes will be represented) (females nulliparous and non-pregnant) Several additional animals will be used for a preliminary range-finding study. Additional animals may be required if a rechallenge is necessary.
e. Age/Weight:	Young adult; approximately 300 - 500 grams
f. Identification:	Ear punch
g. Acclimation and Health Status:	Animals will be acclimated for at least five days prior to testing. Range-finding may be conducted during the acclimation period. Normal weight gain, appearance, and behavior will be factors used to select healthy animals for testing.

2. Animal Husbandry

a. Cages:	Stainless steel, suspended, wire bottom
b. Number per Cage:	Animals will be housed 1 - 4 per cage (and individually during the topical application exposure periods).
c. Food:	PMI Feeds, Inc.™ Guinea Pig Diet #5025; available *ad libitum*. Analyzed by manufacturer.
d. Water:	Tap water; available *ad libitum*. Water bowl or automatic system. Municipal water supply analyzed by Texas Natural Resource Conservation Commission (TNRCC) Water Utilities Division.
e. Contaminants:	There are no known contaminants in the feed or water available to laboratory animals that would be expected to interfere with this study.
f. Environment:	Environmental controls for the animal room will be set to maintain a temperature of 20°C ± 3°C, a relative humidity range of 30 - 80%, a 12-hour light/dark cycle (regulated automatically), and room ventilation of approximately 10 - 12 air changes per hour.

B. EXPERIMENTAL DESIGN (cont.)

3. Test Substance Administration

a. Route of Administration: Intradermal injection and application of a topical patch will be employed.

b. Justification for Route of Administration: Dermal exposure is a potential route of human exposure; intradermal injection in the presence of an adjuvant is intended to maximally stimulate the immune response.

c. Positive Control Substance: 2,4-Dinitrochlorobenzene (DNCB - CAS No. 97-00-7) or other suitable positive control substance such as α-hexylcinnamaldehyde (CAS No. 101-86-0); tested periodically in this laboratory by this method. Test is done within six months of the definitive study to confirm sensitization potential of the animals used and validate procedures. The date the test was performed and the results will be reported in the final report.

d. Range-finding: Determination of the Maximum Irritating Concentration (MIC) by the Intradermal Route:

The day before treatment, the dorsal region of the animals will be clipped. The test substance will be diluted or suspended in an appropriate vehicle as necessary. Intradermal injection of the test substance at a volume of 0.1 mL at increasing concentrations (4 concentrations per animal) will be made in order to determine the maximum concentration which causes no more than moderate irritation without necrosis or ulceration. Typically, concentrations will range from 1 - 5% w/v or v/v. Cutaneous reactions will be evaluated 24 and 48 hours after the injections. Observations will be made using the scoring scale presented in Appendix A.

Determination of the Maximum Irritating Concentration (MIC) and the Maximum Non-Irritating Concentration (MNIC) by the External cutaneous route:

The day before treatment, the dorsal region of the animals will be clipped. Liquids will be tested undiluted, if possible, as well as diluted in an appropriate vehicle as necessary. Solids will be finely pulverized and mixed with petrolatum to a maximum concentration of 25% w/v. 500 mg or 0.5 mL of each concentration (2 concentrations per animal) will be applied under a 2 x 2 cm filter paper patch and held in place by an occlusive dressing for 24 hours. Cutaneous reactions will be evaluated 24 hours after removal of the patches to determine the topical MIC (maximum topical concentration that produces no more than moderate irritation without necrosis or ulceration) and topical MNIC (maximum non-irritating concentration).

Further screening tests may be necessary if results obtained do not adequately define the MIC and MNIC for the external cutaneous treatments for the induction on Day 7 and the challenge on Day 21, respectively.

B. EXPERIMENTAL DESIGN (cont.)

3. Test Substance Administration (cont.)

e. Preparation of Animals: The animals will be prepared on the day prior to each treatment by clipping the exposure area on the back (4 x 6 cm across the shoulders or 5 x 5 cm on each flank) with animal clippers. This procedure may be repeated as necessary.

f. Sensitization by Intradermal and Cutaneous Routes:

Induction: Intradermal Injections

On Day -1, each animal will be clipped to expose a 4 x 6 cm area running laterally across the shoulders. On Day 0, three pairs of symmetrical intradermal injections of 0.1 mL will be made in a 2 x 4 cm area within the larger exposed area. Injections 1 & 2 will be given close together and nearest the head. The third pair of injections will be given towards the caudal part of the test area.

1) Treated Group

- *Injection 1* - Freund's Complete Adjuvant (FCA) diluted at 50% in injectable isotonic saline (NaCl 0.9%) (hereafter referred to as FCA/saline)
- *Injection 2* - Test substance in the vehicle, diluted as indicated by the range-finding study to the maximum concentration to obtain no more than moderate irritation.
- *Injection 3* - Test substance at the selected concentration and FCA/saline, 50/50 (v/v). If the test substance is water soluble, it is first dissolved in the saline and then mixed thoroughly with the adjuvant. If the test substance is not water soluble, it is first mixed with FCA and then diluted with saline.

2) Control Group

- *Injection 1* - FCA/saline
- *Injection 2* - Vehicle alone
- *Injection 3* - A mixture of FCA/saline and vehicle, 50/50 (v/v)

Observations for reactions to the intradermal injections will be made on Day 6. Prior to observations, each animal will be reclipped, if necessary, to expose a 4 x 6 cm area running laterally across the shoulders.

If the Day 6 observations indicate that the test substance was not a skin irritant, the exposure area of each animal in both treated and control groups will be treated (Day 6) with 0.5 mL of a 10% w/w mixture of sodium lauryl sulfate in petrolatum in order to create a local irritation. The mixture will be gently massaged into the skin using a glass rod. The area will not be occluded. If the Day 6 observations indicate that the test substance was a skin irritant, there will be no further treatment on Day 6.

APPENDIX C (cont.)

B. EXPERIMENTAL DESIGN (cont.)

3. Test Substance Administration (cont.)

f. Sensitization by Intradermal and Cutaneous Routes (cont.):

Induction: Topical Application

On Day 7, one week after the intradermal induction treatment, the test substance will be applied topically to the same exposure area on the shoulders at the maximum topical concentration that produced no more than moderate irritation in the range-finding test (topical MIC).

Treated Group - On Day 7, a 4.5 cm circle of filter paper will be saturated with the liquid test substance (0.5 mL of the topical MIC as indicated by the range-finding study) and placed over the exposure area to cover the three initial pairs of injections. Alternatively, a solid test substance (topical MIC as indicated by the range-finding study) will be suspended in petrolatum, and 500 mg of the mixture will be spread thickly on the patch before it is applied to the exposure area. The patch will then be occluded with non-irritating adhesive tape and secured in place with an elastic adhesive bandage wound around the torso of the animal. The exposure will last for 48 hours.

Control Group - On Day 7, application of 0.5 mL of the vehicle alone on a filter paper patch, or a dry patch of filter paper if no vehicle is used, will be made as above. The exposure will last for 48 hours.

All animals - On Day 10, 24 hours after unwrapping, observations for dermal irritation will be made.

g. Challenge Treatment (both Treated and Control Groups): For the challenge treatment, 5 x 5 cm areas will be clipped on both the left and right flanks on Day 20. On Day 21, a 2 x 2 cm patch of filter paper, dry, or saturated with 0.5 mL of the vehicle (if applicable) will be applied to the left flank as a control. As determined from the range-finding test, the maximum non-irritating concentration of the test substance (topical MNIC) will be applied topically as for the topical induction, but application will be to the right flank. The patches will be sealed with non-irritating adhesive tape and secured in place with an elastic adhesive bandage wound around the torso of the animal. After a 24-hour exposure period, the wrappings and patches will be removed. Animals will be clipped 21 hours after patch removal.

4. Observations for Dermal Irritation: Observations for erythema with or without edema and other signs of dermal irritation will be made on Days 6, 10, 23, and 24. Observations will be made using the scoring scale presented in Appendix A.

5. Other Observations: Any unusual systemic reactions or any other unusual findings will be observed and recorded.

APPENDIX C (cont.)

B. EXPERIMENTAL DESIGN (cont.)

6. Body Weights: Body weights will be taken on Days -1 and 24. If rechallenge is conducted, body weights will be taken at study termination.

7. Study Design:: The following schedule will be followed during the study.

Day -1: A 4 x 6 cm exposure area will be clipped free of hair on each animal. The exposure area will run laterally across the shoulders. Body weights will be taken.

Day 0: Each animal will be treated with three pairs of symmetrical intradermal injections.

Day 6: Exposure areas clipped, if necessary. Observations for dermal irritation after intradermal injections. If no skin irritation observed, dermal administration of sodium lauryl sulfate.

Day 7: Each animal will be treated with the test substance or vehicle by topical application, and the exposure area will be occluded for 48 hours.

Day 9: All wrappings and patches will be removed.

Day 10: Observations for dermal irritation after topical application.

Day 20: A 5 x 5 cm exposure area will be clipped free of hair on the right and left flanks of each animal.

Day 21: Each animal will be treated with the test substance on the right flank and vehicle on the left flank, by topical application, and the exposure areas will be occluded for 24 hours.

Day 22: All wrappings and patches will be removed.

Day 23: The exposure areas will be observed for skin reactions 24 hours after removing the patches. If necessary, animals will be clipped at least two hours prior to observations.

Day 24: The exposure areas will be observed for skin reactions 24 hours after the Day 23 observations. Body weights will be recorded.

8. Rating of Sensitization: Any test group animal that exhibits scores greater than zero for erythema or edema and/or greater than control group animals' reactions after the challenge treatment will be considered sensitized.

% Sensitized	Grade	Rating
0	0	Non-Sensitizer
1-8	I	Weak
9-28	II	Mild
29-64	III	Moderate
65-80	IV	Strong
81-100	V	Extreme

B. EXPERIMENTAL DESIGN (cont.)

9. Rechallenge:	If it is necessary to clarify the results obtained in the first challenge, a second challenge (a rechallenge), where appropriate with a new control group, should be conducted approximately one week after the first one. A rechallenge may also be performed on the original control group. Body weights will be recorded at study termination.
10. Necropsy:	At the end of the study, the animals will be weighed and sacrificed by CO_2 inhalation in excess. For animals found dead during the study, a macroscopic examination of the main organs will be performed and abnormalities recorded.
11. Histopathology:	No histopathologic examination will be performed routinely. Cutaneous samples showing "doubtful" macroscopic reactions will be examined microscopically only after an agreement from the Sponsor and the Study Director. Increased vascularity, edema, and accumulations of plasma cells, mast cells and/or lymphocytes will be considered indicative of a sensitization reaction.
12. Test Substance Accountability:	A comprehensive inventory of test substance received and used will be kept. The test substance container(s) will be weighed when received at this facility, and a record of all test substance use will be maintained. Test substance and test substance dosing solutions will be stored in the original containers, or in the equivalent thereof, or in glass containers with Teflon-lined caps.
13. Disposal of Unused Test Substance:	Unused test substance will be returned after the termination of the study to the Sponsor or Sponsor's Representative. The Sponsor should specify return or disposal at the time of study authorization. If returned, the recipient of the test substance will be notified in advance of shipping. The sample will be accompanied by a chain of custody letter describing the sample(s) contained in the shipment.
14. Safety Precautions:	General safety precautions required by laboratory SOPs will be followed. The Sponsor will supply basic toxicity data on the test substance to be used. However, since the toxicity of test substances is often not well characterized, this laboratory will be conservative in setting safety procedures. The Sponsor or Sponsor's Representative shall be notified of any exposures requiring a physician's examination or care.

APPENDIX C (cont.)

C. DATA MANAGEMENT

1. Records:

The following records will be maintained during the study and transferred to the STILLMEADOW, Inc. archives upon study termination.

a. Protocol and Protocol Amendments (if any).
b. Final report and amendments (if any).
c. Study correspondence.
d. Animal receipt/acclimation data.
e. Test substance receipt, identification as provided by the Sponsor, preparation, administration, and disposition. Data on the vehicle used in dilutions, range-finding, or administration.
f. Test animal information: number, species, strain, age, source and sex.
g. Body weight data.
h. Range-finding study information.
i. Individual scores for dermal reactions and any other irritation.
j. Observations for unusual systemic reactions, if any.
k. Records from an appropriate positive control study conducted within six months of the definitive study.
l. Other pertinent data.

2. Data Storage:

All raw data will be retained at STILLMEADOW, Inc.

3. Data Reporting:

The final report will include all data as described in the Good Laboratory Practice Standards, including:

a. Statement from the Quality Assurance Unit.
b. Signature of the Study Director.
c. A GLP Compliance Statement signed by the Study Director.
d. Names of scientific personnel involved in the study.
e. Dates of study initiation and termination.
f. Identification, description, and storage of the test substance, and identification of the vehicle used in dilutions.
g. All pertinent animal data, animal husbandry, dosing information, and observation methods.
h. Description of the test procedures.
i. Determination of whether or not the test substance was a sensitizing agent.
j. Individual observations for dermal reactions and any other irritation.
k. Mean and individual skin irritation scores for each group for each time period.
l. Individual body weight data.
m. Results of pretest screening.
n. Observations of unusual systemic reactions or any other unusual findings.
o. Results from an appropriate positive control study conducted within six months of the definitive study.
p. A reference to this Protocol.

4. Report Submission:

A report will be submitted after termination of the in-life portion of the study.

APPENDIX C (cont.)

Appendix A
GUINEA PIG MAXIMIZATION TEST FOR TOPICALLY
APPLIED NON-IRRITATING TEST SUBSTANCE
Evaluation of Skin Reactions

MAGNUSSON AND KLIGMAN GRADING SCALE FOR THE EVALUATION OF CHALLENGE PATCH TEST REACTIONS*

Observation	Score
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

* - OECD Guidelines for the Testing of Chemicals, Volume 2, Section 4, Number 406, Skin Sensitization, Paragraph 23, page 4/9, Adopted 17 Jul 92

ATTACHMENT 40

Bluegill Sunfish (*Lepomis macrochirus*)
Static 96-Hour Acute Toxicity Test
on Miller 6064

STILLMEADOW
INCORPORATED

VOLUME __ OF __ OF SUBMISSION

Miller 6064

AMENDED FINAL REPORT

BLUEGILL SUNFISH (*Lepomis macrochirus*) STATIC 96-HOUR ACUTE TOXICITY TEST

OPPTS No. 850.1075

AUTHOR:

Neil A. Rodrigue, M.S.

STUDY INITIATION DATE: 22 June 2001
STUDY COMPLETION DATE: 17 Oct 2001
AMENDED STUDY DATE: 9 May 02

CONDUCTED BY:
STILLMEADOW, Inc.
10161 Harwin Drive, Suite 150
Houston, Texas 77036

LABORATORY STUDY NUMBER:

6419-01

VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 24

SUBMITTED TO:
Miller Chemical & Fertilizer Corporation
P.O. Box 333
Radio Road
Hanover, PA 17331

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA § 10(d) (1) (A), (B), or (C).

Company: Miller Chemical and Fertilizer Corporation

Company Agent:_____ Date: _____

Title_____ Signature _____

These data are the property of Miller Chemical and Fertilizer Corporation and as such, are considered to be confidential for all purposes other than compliance with FIFRA § 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality that may exist under any other statute or in any other country.

GLP COMPLIANCE STATEMENT

This study was designed and performed in STILLMEADOW, Inc.'s laboratory in compliance with the following:

· United States Environmental Protection Agency (USEPA) FIFRA; Good Laboratory Practice Standards 40 CFR 160 with exception of sections 160.105 (b)(e) and 160.31 (d), stability information was not provided; 160.105 (b) solubility not determined; and 40 CFR 160.113 (a) mixture analysis not performed.

· Organization for Economic Cooperation and Development's Principles of Good Laboratory Practice, Annex 2, C(97)186 with exception of section 6.2 (4), stability information was not provided, and section 6.2 (5), mixture analysis was not conducted.

· Japan Ministry of Agriculture, Forestry and Fisheries, Notification No. 59 Nohsan 3850, Director-General of Agricultural Production Bureau, 10 August 1984 with the exception of Article 5 (2) (9) and Article 21 (3), stability information was not provided, and Article 23 (1), mixture analysis was not conducted.

Neil A. Rodrigue, M.S.
Study Director
STILLMEADOW, Inc.

09 May 02
Date
Original Date: 17 Oct 01

Signature of Agent of Sponsor

Date

Agent Name
Sponsor: Miller Chemical & Fertilizer Corporation
P.O. Box 333
Radio Road
Hanover, PA 17331

Signature of Agent of Submitter

Date

Agent Name
Submitter: Mandava Associates

TABLE OF CONTENTS

QUALITY ASSURANCE STATEMENT

Study Title: Bluegill Sunfish (*Lepomis macrochirus*) Static 96-Hour Acute Toxicity Test
Test Substance: Miller 6064

The study has been inspected and the report and data have been audited in accordance with STILLMEADOW, Inc. Standard Operating Procedures (SOPs). The findings from the inspection and audit were reported to the Study Director and Management as follows:

Phase of Study Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
Observation	10 Aug 01	10 Aug 01	10 Aug 01
Report/Data Audit	10 Oct 01	11 Oct 01	11 Oct 01
Amended Report Audit	06 Mar 02	07 Mar 02	07 Mar 02

B. Lynn Murphy

B. Lynn Murphy, B.S.
Quality Assurance Unit
STILLMEADOW, Inc.

09 May 2002
Date

SUMMARY

This study was conducted to assess the toxicity of the test substance (Miller 6064) to *Lepomis macrochirus* in a 96-hour static, non-renewal test.

Test considerations were determined by preliminary range-finding tests. The test substance concentrations chosen (25, 43, 71, 118 and 197 mg/L) were administered to the test system, *Lepomis macrochirus*, in reconstituted water. For each test concentration, two replicates of ten organisms each were treated with the appropriate concentration of the test substance. A control group containing twenty organisms was not exposed to test substance. Dissolved oxygen, temperature, conductivity, and pH measurements were recorded at dosing and daily throughout the study. Observations of mortality were made at 24, 48, 72, and 96 hours after treatment. The test was terminated after 96 ± 2 hours of exposure.

Survival rates of 100, 100, 100, 30 and 0% were observed in fish treated with 25, 43, 71, 118, and 197 mg/L of the test substance (target concentrations), respectively. A 100% survival rate was observed in both control and solvent control groups. Based on this data, the median lethal concentration (LC_{50}) was determined to be 106.67 mg/L with a 95% confidence interval of 96.08 to 118.43 mg/L, and the NOEC was determined to be 71 mg/L.

INTRODUCTION

The objective of this study was to assess the toxicity of the test substance to *Lepomis macrochirus* in a 96-hour test. This study was conducted for Miller Chemical & Fertilizer Corporation according to the approved protocol, STILLMEADOW, Inc. SOPs, and Product Properties Test Guidelines, Series 850, Section 1075 of the United States Environmental Protection Agency Office of Prevention, Pesticides, and Toxic Substances. This study was initiated on 22 June 2001. The laboratory portion of the study was conducted between 19 Jul 01 to 23 Jul 01 and 25 Jul 01 to 28 Jul 01 for the range-finding tests, and 09 Aug 01 and 13 Aug 01 for the definitive test. The original protocol, raw data, and report are on file in the STILLMEADOW, Inc. archives.

TEST SUBSTANCE

Identification:	MILLER 6064
Date and Quantity Received:	19 Dec 00; 2 x 1 gal
Physical Description:	Amber liquid
Storage:	Room temperature
Purity and Composition:	Refer to Certificate of Analysis (Appendix D)
Stability:	Not provided by the Sponsor

Records pertaining to stability, characterization, and verification of test substance identity are the responsibility of the Sponsor. A copy of the Certificate of Analysis is included in the report.

TEST SYSTEM

Experimental Organism	
Species:	*Lepomis macrochirus*
Source:	Osage Cat Fisheries (Lake Ozark, Missouri) 03 Jul 01
Age:	Juvenile
Size:	Less than 3.0 g at dosing; the longest fish was not more than twice the length of the shortest.
Quantity:	Range-finding: 2 per test concentration Definitive: 20 per test concentration
Organism Husbandry	
Test Room:	Environmentally controlled chamber (Chamber C)
Test Chambers:	1 liter glass beaker (range-finding) and 2.5 gallon glass aquaria (definitive)
Test Medium:	Reconstituted water with total hardness between 40 and 180 mg $CaCO_3$ and with a pH between 6.0 and 8.0.
Loading:	Maximum loading of 0.8 g fish/liter.
Holding:	All fish were held in the laboratory at least 14 days before they were used for testing. The fish were held in water of the quality used in the test for at least seven days immediately before testing.
Environmental Controls	
Set to Maintain:	Temperature Range of 22±2°C 16-hours light / 8-hours dark cycle Dissolved oxygen concentration of at least 60 percent saturation
Food:	Fish were fed daily until 48 hours before the test was started. Fish were not fed during the test.

Animal husbandry and housing at STILLMEADOW, Inc. comply with Animal Welfare Act Regulations. No contaminants were expected to have been present in the feed or water which would have interfered with or affected the results of the study.

PROCEDURES

Range-finding Test

Two preliminary range-finding tests were conducted using five concentrations of the test substance (1, 5, 10, 50, and 100 mg/L). Since the test substance was insoluble in water, the test substance was administered using N,N-Dimethylformamide as a solvent at a rate of 1 mL N,N-Dimethylformamide per liter of solution volume. Following randomization, two organisms per each range finding were placed into each beaker containing the appropriate concentration of test substance. Two organisms per each range finding, which were not exposed to test substance, served as controls to demonstrate the condition of the test population. Additionally, two organisms per each range finding which were not exposed to test substance were exposed to the solvent (N,N-Dimethylformamide). This solvent control was used to demonstrate artifactual toxicity produced by the solvent. At 24, 48, 72, and 96 hours following dosing, each beaker was examined for mortality and the number of live fish was recorded.

PROCEDURES (cont.)

Definitive Test

Based on the results of the range-finding tests, test substance concentrations were chosen for definitive testing. Five target concentrations of the test substance were used (25, 43, 71, 118, and 197 mg/L). Since the test substance was insoluble in water, the test substance was administered using N,N-Dimethylformamide as a solvent in the same method used for the range-finder. Each test concentration consisted of two replicates of ten fish per replicate. Two replicates containing ten fish each were not exposed to test substance and served as controls to demonstrate the condition of the test population. Additionally, two replicates containing ten fish each which were not exposed to test substance were exposed to the solvent (N,N-Dimethylformamide). The solvent control was used to demonstrate artifactual toxicity produced by the solvent. At 24, 48, 72, and 96 hours following dosing, each of the aquaria was examined for mortality and the number of live fish was recorded. Fish were considered dead when there was no visible movement (e.g. gill movements) and if touching of the caudal peduncle produced no reaction. Dead fish were removed when observed. Visible abnormalities were also recorded (e.g. loss of reflex, erratic swimming, increased excitability, lethargy, and changes in appearance or physiology such as discoloration, excess mucous production, hyperventilation, opaque eyes, curved spine, or hemorrhaging, etc.).

Chemical and Physical Monitoring

The following measurements were recorded daily during definitive testing: dissolved oxygen, temperature, conductivity, and pH of control and treated containers.

RESULTS AND DISCUSSION

Test Validity

The test was considered valid if control mortality did not exceed 10 percent. Since control mortality was zero percent, the definitive test was considered valid.

Range-finding

A 0% survival rate was observed in fish treated at concentration of 100 mg/L. A 50% survival rate was observed in fish treated at concentrations of 0 and 5mg/L. A 75% survival rate was observed in fish treated with solvent only and with fish treated at concentrations of 1 and 10 mg/L. A 100% survival rate was observed in fish treated at concentrations of 50 mg/L. Mortality was observed in fish treated with 0, 1, 5, 10 and 100 mg/L of test substance.

Target Concentration (mg/L)	Range Finding Test	Number of Surviving Organisms				
		0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	A	2	2	1[a]	1	1
	B	2	1[b]	1	1	1
1	A	2	2	2	2	2
	B	2	2	2	2[c]	1[b]
5	A	2	2	2[d]	1[b]	1
	B	2	2	1[b]	1	1
10	A	2	2	2	2	2
	B	2	2	1[b]	1	1
50	A	2	2	2	2	2
	B	2	2	2	2	2
100	A	2	0[e]	0	0	0
	B	2	0	0[f]	0	0
Solvent Control	A	2	2	1[b]	1	1
	B	2	2	2	2	2

A – Range-finding test of 19 Jul 01 to 23 Jul 01

B – Range-finding test of 25 Jul 01 to 28 Jul 01

[a] - One fish floating on top, dead no movement

[b] – One fish dead lying on side at the bottom of the tank

[c] – One fish lying on side at bottom of tank still breathing, trying to swim

[d] – One fish dark in color staying at bottom of tank, has gill movement but appears weak

[e] – Fish white in color with curved spines

[f] - Both fish dead lying on side with curved spine

RESULTS AND DISCUSSION (cont.)

Definitive

Survival rates of 0 and 30% were observed in fish treated with 197and 118 mg/L of the test substance (target concentrations), respectively. A 100% survival rate was observed in fish treated with solvent only, and 0, 25, 43 and 71 mg/L of the test substance. Chemical and physical monitoring data (dissolved oxygen, temperature, conductivity, and pH) of control and treated containers for the definitive test are presented in Appendix A.

Target Concentration (mg/L)	Replicate	Number of Surviving Organisms				
		0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	A	10	10	10	10	10
	B	10	10	10	10	10
25	A	10	10	10	10	10
	B	10	10	10	10	10
43	A	10	†	†	†	10
	B	10	†	†	†	10
71	A	10	10[a]	10[a]	10	10
	B	10	10[a]	10[a]	10	10
118	A	10	6†[b]	6[c]	5[d]	5
	B	10	2†[e]	1[f]	1	1
197	A	10	†[g]	0[h]	0	0
	B	10	†[e]	0[h]	0	0
C2	A	10	10	10	10	10
	B	10	10	10	10	10

† – Too turbid to count
[a] – Fish swimming irregularly on side and vertically; hemorrhage around gills
[b] – Four dead animals on bottom; some fish swimming on side
[c] – Fish floating on top with gill movement
[d] – One fish dead floating on top of water
[e] – Eight animals dead; pale in color
[f] – One fish dead at bottom
[g] – Ten dead animals; pale in color; curved dorsal fin
[h] – All fish dead lying on side at the bottom of tank

RESULTS AND DISCUSSION (cont.)

Evaluation of Results

The median lethal concentration (LC_{50}) for the test substance, Miller 6064, was determined to be 106.67 mg/L with a 95% confidence interval of 96.08 to 118.43 mg/L using the Trimmed Spearman-Karber statistical method, and the NOEC was determined to be 71 mg/L.

CONCLUSION

The test substance, Miller 6064, was evaluated for toxicity to *Lepomis macrochirus* in a 96-hour static, non-renewal test. The median lethal concentration (LC_{50}) was determined to be 106.67 mg/L with a 95% confidence interval of 96.08 to 118.43 mg/L, and the NOEC was determined to be 71 mg/L

Neil A. Rodrigue

Neil A. Rodrigue, M.S.
Study Director
STILLMEADOW, Inc.

09 May 02

Date
Original Date: 17 Oct 01

STUDY PERSONNEL

Technical Staff
Mel Rivera, B.S.
Rob Stowe. B.S.
Abigail Campbell, B.S.
Brandy Goffinet

Technical Writer
Diana W. Cook, B.S.

Appendix A: Chemical and Physical Monitoring Data
Bluegill Sunfish (*Lepomis macrochirus*) Static 96-Hour Acute Toxicity Test
Test Substance: Miller 6064

Table 1. Temperature (°C)

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	21	21	21	21	21
25	21	21	21	21	21
43	21	21	21	21	21
71	21	21	21	21	21
118	21	21	21	21	21
197	21	21	21	-	-
Solvent Cntrl	21	21	21	21	21

Table 2. pH

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	7.9	7.2	7.4	7.4	7.5
25	7.9	7.2	7.4	7.5	7.5
43	7.9	7.2	7.4	7.5	7.5
71	7.9	7.2	7.5	7.5	7.6
118	8.0	7.2	7.5	7.5	7.5
197	7.9	7.2	7.5	-	-
Solvent Cntrl	7.9	7.2	7.4	7.4	7.5

Table 3. Dissolved Oxygen (mg/L)

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	7.9	5.8	6.0	6.0	4.6
25	8.0	6.2	6.2	6.0	4.7
43	8.0	6.0	6.2	5.6	5.0
71	7.9	6.2	6.2	5.6	5.0
118	7.8	6.2	6.4	5.6	4.8
197	7.8	6.8	6.4	-	-
Solvent Cntrl	7.8	6.0	6.2	5.8	5.2

Appendix A: Chemical and Physical Monitoring Data (cont.)
Bluegill Sunfish (*Lepomis macrochirus*) Static 96-Hour Acute Toxicity Test
Test Substance: Miller 6064

Table 4. Conductivity (µmhos/cm)

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	315	290	290	290	290
25	320	290	290	300	290
43	320	295	290	300	290
71	320	290	290	300	295
118	320	295	295	300	300
197	320	295	295	-	-
Solvent Cntrl	315	285	285	300	295

Appendix B: Statistics
Bluegill Sunfish (*Lepomis macrochirus*) Static 96-Hour Acute Toxicity Test
Test Substance: Miller 6064

Concentration (mg/L)	Number Exposed	Mortalities
0.00	20	0
25	20	0
43	20	0
71	20	0
118	20	14
197	20	20

LC_{50}:	106.67
95% Lower Confidence:	96.08
95% Upper Confidence	118.43

Appendix C: Protocol and Amendment

STILLMEADOW
INCORPORATED

PROTOCOL AMENDMENT #1
STILLMEADOW, Inc. Study Number 6419-01

Sponsor: Miller Chemical & Fertilizer Corporation
PO Box 333
Radio Road
Hanover PA 17331

Effective Date: 17 Oct 2001

Test Substance: Miller 6064

Study Title: BLUEGILL SUNFISH (*Lepomis macrochirus*) STATIC 96-HOUR ACUTE TOXICITY TEST

The following alteration is being made to the cover and Section A.7 of the protocol.

To Change:	Abigail Campbell, B.S.
To Read:	Neil Rodrigue, M.S.
Justification:	The study director is being changed because Abigail Campbell is no longer with the company.
Impact:	There will be no impact on the study.

This amendment has been reviewed and/or approved by the following:

Approved: Neil A. Rodrigue — 17OCT01 — Date
Neil Rodrigue, M.S.
Study Director
STILLMEADOW, Inc.

Approved: [signature] — 17Oct01 — Date
Mark S. Holbert
Vice President
STILLMEADOW, Inc.

Reviewed:: [signature] — 17Oct01 — Date
Vicki S. Crutchfield, R.Q.A.P.
Director, Quality Assurance Unit
STILLMEADOW, Inc.

12852 Park One Drive • Sugar Land, Texas 77478
281 240-8828 • Fax 281 240-8448
www.stillmeadow.com
10161 Harwin, Suite 150 • Houston, Texas 7703
713 272-7444 • Fax 713 272-7501

Appendix C: Protocol and Amendment (cont.)

STILLMEADOW
INCORPORATED

PROTOCOL FOR STUDY 6419-01

Study Title: BLUEGILL SUNFISH (*Lepomis macrochirus*) STATIC 96-HOUR ACUTE TOXICITY TEST

Test Substance: Miller 6064

Test Facility: STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, Texas 77479

Approved: _____ 23 June 01
Abigail Campbell, B.S.
Study Director
STILLMEADOW, Inc.
Date

Approved: _____ 5 June 2001
Elizabeth D. Sabol, B.A., B.S.Ed
Vice President
STILLMEADOW, Inc.
Date

Reviewed: _____ 5 June 2001
Vicki Crutchfield, R.Q.A.P.
Director, Quality Assurance Unit
STILLMEADOW, Inc.
Date

Sponsor: Miller Chemical & Fertilizer Corporation
PO Box 333
Radio Road
Hanover PA 17331

Sponsor Representative
Mandava Associates
1730 M Street, Suite 906
Washington, D.C. 20036-4510

Approved: _____ 15 JUNE 2001
N. Bhushan Mandava, Ph.D.
Date

Appendix C: Protocol and Amendment (cont.)

PROTOCOL FOR STUDY 6419-01

A. GENERAL

1. Study Title:	BLUEGILL SUNFISH (*Lepomis macrochirus*) STATIC 96-HOUR ACUTE TOXICITY TEST
2. Purpose:	To assess the toxicity of the test substance to bluegill sunfish (*Lepomis macrochirus*) in a static 96-hour test.
3. Regulatory Compliance:	This study will be conducted according to OPPTS 850.1075, which is intended to meet testing requirements of both FIFRA (7 U.S.C. 136. *et seq.*) and TSCA (15 U.S.C. 2601). This study will be conducted in compliance with Good Laboratory Practice Standards: 1. EPA FIFRA: 40 CFR 160 2. OECD: C(81)30 (Final) 3. Japanese MAFF All methods can be found in STILLMEADOW, Inc. Standard Operating Procedures (SOPs).
4. Quality Assurance:	The Protocol is reviewed by the Quality Assurance Unit (QAU). The study information will be entered into the Master Schedule. The study will be inspected at least once during its progress. Further inspections may be scheduled as needed to ensure the integrity of the study. Any deviations from SOPs, the Protocol, or Good Laboratory Practice Standards will be immediately reported to the Study Director and Management. The report will be audited and a statement prepared and signed which shall specify the dates inspections were made and findings reported to Management and to the Study Director.
5. Test Substance:	Miller 6064. Test substance identification should include the name, batch number, and purity. Information regarding safety, stability, storage conditions, and disposal should also be provided by the Sponsor. The Sponsor assumes responsibility for purity and stability determinations.
6. Proposed Schedule:	Testing will begin within approximately three weeks of receipt of the test substance and authorization to conduct the study. Proposed Start Date: 13 Jun 01 Proposed End Date: 11 Jul 01
7. Study Director:	Abigail Campbell, B.S.

Appendix C: Protocol and Amendment (cont.)

A. GENERAL (cont.)

8. Experimental Summary:

Definitive test concentrations will be determined by a preliminary range finder. The test substance concentrations chosen will be administered to the test system, bluegill sunfish (*Lepomis macrochirus)*, in reconstituted water. For each test concentration, 20 organisms will be treated with the appropriate concentration of the test substance. Two control groups which will not contain test substance will be used in this test. One group will have solvent added at the highest volume used for any test concentration preparation and will represent the solvent control. The other control group will remain untreated and will demonstrate the condition of the test population. Dissolved oxygen, temperature, conductivity, and pH will be measured and recorded in each treatment and the control at test initiation and daily throughout the study. Observations of mortality in each test chamber will be made at 24, 48, 72, and 96 hours. The test will be terminated after 96 ± 2 hours of exposure.

The test will be considered valid if control mortality does not exceed 10 percent.

9. Protocol Amendments:

Any alteration in the Protocol will be justified, approved by the Study Director, and recorded in writing.

10. Sponsor Audits:

The Sponsor may send an authorized representative to inspect the test system and/or data on the STILLMEADOW, Inc. premises during normal working hours.

Appendix C: Protocol and Amendment (cont.)

B. EXPERIMENTAL DESIGN

1. Organism

a. Species:	Bluegill sunfish (*Lepomis macrochirus*)
b. Justification of Species:	Specified in the OPPTS regulations.
c. Age/Size:	Juvenile fish, less than 3.0 g at test initiation. The longest fish will not be more than twice the length of the shortest.
d. Number:	The rangefinder will use 2 fish for each concentration and the controls. The definitive test will use 20 sunfish for each concentration and each control group (2 replicates each containing 10 fish).
e. Source:	*Lepomis macrochirus* will be obtained from Aquatic Research Organisms, Inc. (Hampton, New Hampshire) or another suitable supplier.
f. Identification:	Organisms will be labeled by study number, lot number, date of receipt, and number of organisms.

2. Animal Husbandry

a. Test Medium:	Reconstituted water with total hardness between 40 and 180 mg $CaCO_3$ and with a pH between 6.0 and 8.0.
b. Acclimation:	All fish will be held in the laboratory for at least 14 days before they are used for testing. They will be held in water of the quality to be used in the test for at least seven days immediately before testing. Pretest mortality must be less than 5% during acclimation or the organisms will be held for an additional seven days. If pretest mortality is greater than 10%, then the entire lot will be rejected and a new lot of fish will be obtained to begin acclimation.
b. Test Chamber:	Test containers will be 2½ gallon aquaria. Test containers will be loosely covered to reduce the loss of test solution or dilution water due to evaporation and to minimize the entry of dust or other particles into the solutions.
c. Temperature:	Test temperature will be 22±2°C.
d. Photoperiod:	16 hours light, 8 hours dark
e. Dissolved Oxygen Concentrations:	At least 60 percent air saturation value.
f. Food:	Fish will be fed daily until 48 hours prior to test initiation. Fish will not be fed during test.
g. Loading:	Maximum loading of 0.8 g fish/liter.

Appendix C: Protocol and Amendment (cont.)

B. EXPERIMENTAL DESIGN (cont.)

3. Pre-test Preparation

a. Test Substance Receipt:	Test substance will be supplied by the Sponsor in appropriately sized glass containers sealed and delivered to STILLMEADOW, Inc. Samples will be stored according to the Sponsor's instructions until prepared for testing.
b. Test Substance Preparation:	The test substance is insoluble in water and will be administered using an appropriate solvent (DMF, ethanol, methanol, etc.) as weight/volume concentrations. A solvent control will be included in the test design. The test substance dilutions will be prepared on the day of treatment.
c. Route of Administration:	The test substance will be administered to the test system at test initiation by introduction to the test containers containing the test system.
d. Reason for Route of Administration:	Specified by the cited guidelines for evaluation of the toxicity potential of a test substance.
e. Preparation of Test System:	The organisms will be randomized into aquaria containing the appropriate concentration of test substance. Each test concentration will consist of 20 fish. Each aquaria will house of a maximum of 10 fish.
f. Control Groups:	Twenty fish will not have test substance added and will be considered the control. This control will be used to demonstrate the condition of the test population. An additional 20 fish will not have test substance added but will contain the solvent at the highest volume used for any test concentration preparation. The solvent control will be used to demonstrate artifactual toxicity produced by the solvent.

4. Test Substance Administration

a. Dosing Concentrations:	A range finder will be conducted with at least five concentrations of the test substance to obtain an approximate LC_{50} value for the test substance. The test concentrations will be at least 50% greater than the lowest test concentrations (not to exceed 120%). Five test concentrations chosen from the range-finding data and the controls will be prepared on the day of test initiation.
b. Initial Measurements:	Dissolved oxygen, temperature, conductivity, and pH of the control and treated containers will be measured and recorded at test initiation.

Appendix C: Protocol and Amendment (cont.)

B. EXPERIMENTAL DESIGN (cont.)

5. Observations

a. Biological Monitoring: Containers will be inspected at 24, 48, 72, and 96 hours for mortality. Fish are considered dead if there is no visible movement (e.g. gill movements) and if touching of the caudal peduncle produces no reaction. Dead fish will be removed when observed, and mortalities will be recorded. Visible abnormalities will be recorded (e.g. loss of reflex, erratic swimming, increased excitability, lethargy, and changes in appearance or physiology such as discoloration, excess mucous production, hyperventilation, opaque eyes, curved spine, or hemorrhaging, etc.)

b. Chemical and Physical Monitoring: At a minimum, the following measurements will be made daily: dissolved oxygen, temperature, conductivity, and pH of the controls and treated containers.

6. Test Duration: The test will be terminated after 96 ± 2 hours.

7. Quality Criterion: The test will be considered valid if the control mortality does not exceed 10 percent.

8. Evaluation of Results: The survival in the test concentrations will be statistically compared to survival in the control to determine the highest concentration of test substance that demonstrates no significant reduction in survival. This concentration will be the No Observed Effect Level (NOEL) for survival. The NOEL will be determined by using a commercially available statistical program (Toxstat®).

The median lethal concentration (LC_{50}) will be estimated using a linear regression model. Several models are available for LC_{50} determination: Probit, Trimmed Spearman-Karber, and Binomial. The most appropriate model will be selected for estimating the LC_{50} if a dose response is exhibited in the study.

9. Test Substance Accountability: A comprehensive inventory of test substance received and used will be kept. The test substance container(s) will be weighed when received at this facility, and a record of all test substance use will be maintained. Test substance and test substance dosing solutions will be stored in the original containers or in the equivalent thereof, or in glass containers with Teflon-lined caps.

Appendix C: Protocol and Amendment (cont.)

B. EXPERIMENTAL DESIGN (cont.)

10. Disposal of Unused Test Substance:

Unused test substance will be disposed of at the Sponsor's expense after the termination of the study. STILLMEADOW, Inc. will retain a reserve sample.

11. Safety Precautions:

General safety precautions required by laboratory SOPs will be followed. The Sponsor will supply basic toxicity data on the test substance to be used. However, since the toxicity of test substances is often not well characterized, STILLMEADOW, Inc. will be conservative in setting safety procedures. The Sponsor or Sponsor's Representative shall be notified of any exposures requiring a physician's examination or care.

C. DATA MANAGEMENT

1. Records:

The following records will be maintained during the study and transferred to the STILLMEADOW, Inc. archives upon study termination.

a. Protocol and Protocol Amendments (if any).
b. Final report and amendments (if any).
c. Study correspondence.
d. Test culture data.
e. Test substance receipt, identification as supplied by Sponsor, preparation, administration, and disposition.
f. Range finder data and results.
g. Initial and daily measurements for dissolved oxygen, temperature, and pH of the control and treated containers.
h. Cumulative mortality at each concentration at each observation time.
i. Determination of the validity of the study.
j. Other pertinent data.

2. Data Storage:

All raw data and a reserve sample of the test substance will be retained at STILLMEADOW, Inc. for a period of at least five years.

Appendix C: Protocol and Amendment (cont.)

C. DATA MANAGEMENT (cont.)

3. Data Reporting:

The final report will include all data as described in the Good Laboratory Practice Standards, including:

a. Statement from the Quality Assurance Unit.
b. Signature of the Study Director.
c. A GLP Compliance Statement signed by the Study Director.
d. Names of scientific personnel involved in the study.
e. Dates of study initiation and termination.
f. Identification, description, preparation, and storage of the test substance.
g. All pertinent culture information, preparation of test medium, test conditions, dosing information, and observation methods.
h. Initial and daily data for dissolved oxygen, temperature, and pH of the control and treated containers.
i. Cumulative mortality at each concentration at each observation time.
j. Graph of the concentration-mortality curve at the end of the test.
k. Statistical procedures used for determining the LC_{50} and NOEL values.
l. Determination of the validity of the test based on the control data.
m. Abnormalities observed in test and control animals.
n. Any protocol deviations or occurrences which may have influenced the final results of the test.
o. Evaluation of results.
p. A reference to this Protocol.

4. Report Submission:

A report will be submitted after termination of the laboratory portion of the study.

Appendix D: Certificate of Analysis

MILLER CHEMICAL & FERTILIZER CORPORATION
P O. BOX 333, 120 RADIO ROAD
HANOVER, PENNSYLVANIA 17331 U.S.A.
TELEPHONE: 717-632-8921
FAX NO.: 717-632-1561

1/18/01

CERTIFICATE OF ANALYSIS

Product Name: MILLER 6064
Quantity Produced: 7.4 kilograms
Batch Number: 150386
Date of Formulation: 12/13/00

Appearance: Yellow to Amber Liquid
Odor: Terpenic
Moisture: Negligible (non-aqueous)
Specific Gravity: 0.92 - 0.94 @ 20c
Viscosity @ 20c: 100 to 500 cps
pH(1% solution): 6.0 to 8.0
Solubility in Water: Insoluble (Emulsifies)
Flash Point: over 200 f
Purity: 100%

Signature:

Name: Andrew G. Smith
Title: Compliance Officer

Appendix E: Amendment

Miller 6064

Bluegill Sunfish (*Lepomis macrochirus*) Static 96-Hour Acute Toxicity Test
(OPPTS 850. 1075)

Study Number 6419-01

Sponsor: **Miller Chemical & Fertilizer Corporation**

Final Report Amendment

This amendment makes the following changes in the final report:

To change: Page 6, Test Substance; Purity and Composition: Certificate of Analysis not provided by sponsor
To: Purity and Composition: Refer to Certificate of Analysis (Appendix D)

Reason: The Certificate of Analysis with composition information was not included in the original report.

To Add: Pages 6 and 11, "and the NOEC was determined to be 71 mg/L."

Reason: The NOEC was not included in the original report.

To Change: Page 6, Summary
From: Survival rates of 100, 100, 100, 100, 30 and 0% were observed in fish treated with 25, 43, 71, 118 and 197 mg/L of the test substance (target concentrations), respectively.

To: Survival rates of 100, 100, 100, 30 and 0% were observed in fish treated with 25, 43, 71, 118 and 197 mg/L of the test substance (target concentrations), respectively.

Reason: Typographical error.

To add: Appendix C, D and E to the Table of Contents and the report.

Reason: The protocol and Certificate of Analysis were not included in the original report.

Amendment Approval:

Neil A. Rodrigue

Neil A. Rodrigue, M.S.
Study Director
STILLMEADOW, Inc.

09 May 02
Date

ATTACHMENT 41

Rainbow Trout (*Oncorhynchus mykiss*)
Static 96-Hour Acute Toxicity Study
on Miller 6064

STILLMEADOW
INCORPORATED

VOLUME __ OF __ OF SUBMISSION

Miller 6064

FINAL REPORT

RAINBOW TROUT (*Oncorhynchus mykiss*) STATIC 96-HOUR ACUTE TOXICITY TEST

AUTHOR:

Neil A. Rodrigue, M.S.

STUDY INITIATION DATE: 22 Jun 2001
STUDY COMPLETION DATE: 9 May 2002

CONDUCTED BY:
STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, Texas 77048

LABORATORY STUDY NUMBER:

6420-01

VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 26

SUBMITTED TO:
Miller Chemical and Fertilizer Corporation
P. O. Box 333
Radio Road
Hanover, PA 17331

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA § 10(d) (1) (A), (B), or (C).

Company: Miller Chemical and Fertilizer Corporation

Company Agent_____ Date_____

Title_____ Signature_____

These data are the property of Miller Chemical and Fertilizer Corporation and as such, are considered to be confidential for all purposes other than compliance with FIFRA § 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality that may exist under any other statute or in any other country.

GLP COMPLIANCE STATEMENT

This study was designed and performed in STILLMEADOW, Inc.'s laboratory in compliance with the following:

· United States Environmental Protection Agency (USEPA) FIFRA; Good Laboratory Practice Standards 40 CFR 160 with exception of sections 160.105 (b) (e) and 160.31 (d), stability information was not provided; 160.105 (b) solubility not determined; and 160.113 (a) mixture was not performed.

· Organization for Economic Cooperation and Development's Principles of Good Laboratory Practice, Annex 2, C(97)186 with the exception of section 6.2 (4), stability information was not provided and section 6.2 (5), mixture analysis was not performed.

· Japan Ministry of Agriculture, Forestry and Fisheries, Notification No. 59 Nohsan 3850, Director-General of Agricultural Production Bureau, 10 August 1984 with the exception of Article 5 (2) (9) and Article 21 (3), stability information was not provided and Article 23 (1), mixture analysis was not performed.

Neil A. Rodrigue

Neil A. Rodrigue, M.S.
Study Director
STILLMEADOW, Inc.

09 May 02
Date
Original Date: 17 Oct 01

Signature of Agent of Sponsor

Date

Agent Name
Sponsor: Miller Chemical & Fertilizer Corporation
P.O. Box 333
Radio Road
Hanover, PA 17331

Signature of Agent of Submitter

Date

Agent Name
Submitter: Mandava Associates

TABLE OF CONTENTS

QUALITY ASSURANCE STATEMENT

Study Title: Rainbow Trout (*Oncorhynchus mykiss*) Static 96-Hour Acute Toxicity Test
Test Substance: Miller 6064

The study has been inspected and the report and data have been audited in accordance with STILLMEADOW, Inc. Standard Operating Procedures (SOPs). The findings from the inspection and audit were reported to the Study Director and Management as follows:

Phase of Study Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
Randomization/Dosing	12 Jul 01	13 Jul 01	13 Jul 01
Report/Data Audit	16 Nov 01	16 Nov 01	16 Nov 01
Final Report Audit	25 Mar 02	25 Mar 02	25 Mar 02

B. Lynn Murphy

B. Lynn Murphy, B.S.
Quality Assurance Unit
STILLMEADOW, Inc.

09 May 2002
Date

SUMMARY

This study was conducted to assess the toxicity of the test substance Miller 6064 to *Oncorhynchus mykiss* in a 96-hour static, non-renewal test.

Test considerations were determined by a preliminary range-finding test. The test substance concentrations chosen by the range-finding test (5, 8, 13, 21 and 35 mg/L) were administered to the test system, *Oncorhynchus mykiss*, in laboratory fresh water. There were two control groups without the test substance, one to determine the condition of the test population, and another, a solvent control, to demonstrate artifactual toxicity produced by the solvent N,N-Dimethylformamide. For each test concentration, two replicates of ten organisms each were treated with the appropriate concentration of the test substance. Dissolved oxygen, temperature, conductivity, and pH measurements were recorded at dosing and daily throughout definitive portion of the study. Observations of mortality were made at 24, 48, 72 and 96 hours after treatment. The test was terminated after 96 ± 2 hours of exposure.

Since there was mortality only at the 35-mg/L concentration for the first definitive test, a second definitive test was conducted in the same manner with concentrations of 13, 21, 35, and 58 mg/L. An even higher concentration was not used due to the potential toxicity of the solvent. A 100% survival rate was observed in fish treated with 0, 13, 21 and 35 mg/L of the test substance and the solvent control. A 55% survival rate was observed in fish treated with 58 mg/L of the test substance.

It was determined that the solvent did not have a high enough toxicity level to interfere with using higher concentrations of the test substance, so a third definitive level was conducted at concentrations of 20, 40, 60, 80 and 100 mg/L, as well as another control and solvent control. There was 100% survival in both controls as well as the 20 mg/L concentration, and a 35% survival rate in the 40 mg/L level. All fish died at the 60, 80 and 100 mg/L concentrations. The LC_{50} level, based on the three definitive tests conducted, is 46.90 mg/L with 95% confidence limits of 38.21 – 58.88 mg/L. The NOEC level is 35 mg/L.

INTRODUCTION

The objective of this study was to assess the toxicity of the test substance, Miller 6064, to *Oncorhynchus mykiss* in a 96-hour test. This study was conducted for Miller Chemical and Fertilizer Corporation according to the approved protocol, STILLMEADOW, Inc. SOPs, and OPPTS 850.1075. This study was initiated on 22 Jun 01. The laboratory portions of the study were conducted between 06 Jul 01 and 25 Feb 02. The original protocol, raw data, and report are on file in the STILLMEADOW, Inc. archives. A reserve sample of the test substance will be retained at STILLMEADOW, Inc. for a period of at least five years.

TEST SUBSTANCE AND SOLVENT

Identification:	Miller 6064
Date and Quantity Received:	19 Dec 00; 2 X 1 gal.
Physical Description:	Amber liquid
Storage:	Room temperature
Purity and Composition:	Certificate of Analysis not provided by Sponsor
Stability:	Not provided by the Sponsor
Solvent:	N,N-Dimethylformamide, Fisher Lot No. 001380, Exp. 19 Jul 05

Records pertaining to stability, characterization, and verification of test substance identity are the responsibility of the Sponsor.

TEST SYSTEM

Experimental Organism

Species:	Rainbow Trout, *Oncorhynchus mykiss*
Source and Receive Date:	Lost River Trout Farm (Mackay, Idaho), 12 Jun, 23 Aug 01 and 28 Jan 02
Age:	Juvenile (DOB 28 Mar 01, 21 Jul 01 and 26 Nov 02)
Size:	Less than 3.0 g at test initiation; the longest fish was not more than twice the length of the shortest.
Quantity:	Range-finding: 2 per test concentration Definitives: 20 per test concentration

Organism Husbandry

Test Room:	Environmentally controlled chamber (Chambers B, D and E)
Test Chambers:	2 ½-gallon glass aquaria
Test Medium:	Reconstituted water with total hardness between 40 and 180 mg/L $CaCO_3$ and with a pH between 6.0 and 8.0.
Loading:	Maximum loading of 0.8 g fish/liter.
Holding:	All fish were held in the laboratory at least 14 days before they were used for testing. The fish were held in water of the quality used in the test for at least seven days immediately before testing.

Environmental Controls

Set to Maintain:	Temperature Range of 12 ±2°C 16-hours light/8-hours dark cycle Dissolved oxygen concentration of at least 60 percent saturation
Food:	Fish were fed daily until 48 hours before the test was started. Fish were not fed during the test.

Animal husbandry and housing at STILLMEADOW, Inc. comply with Animal Welfare Act Regulations. No contaminants were expected to have been present in the feed or water which would have interfered with or affected the results of the study.

PROCEDURES

Range-finding Test

A range-finding test was conducted using five concentrations (1, 5, 10, 50, and 100 mg/L) of the test substance Miller 6064. Organisms were randomly placed individually into each beaker containing the appropriate concentration of test substance. Each beaker contained two organisms. Two organisms that were not exposed to test substance served as controls to demonstrate the condition of the test population. Two organisms were exposed to the solvent N,N-dimethylformamide only in order to demonstrate any artifactual toxicity produced. At 24, 48, 72, and 96 hours following dosing, each beaker was examined for mortality and the number of live fish was recorded. Final parameters were recorded for all test concentrations either at study termination or when there were no survivors in a replicate.

Definitive Test

Based on the results of the range-finding test, concentrations of Miller 6064 chosen for definitive testing were 5, 8, 13, 21, and 35 mg/L. Each test concentration consisted of two replicates of ten fish per replicate. Two replicates containing ten fish each were not exposed to test substance and served as controls to demonstrate the condition of the test population and two replicates containing ten fish each were exposed to the solvent only. Small groups of fish were randomly placed in the test vessels until each aquarium contained the appropriate number of organisms. At 24, 48, 72, and 96 hours following dosing, each aquarium was examined for mortality and the number of live fish was recorded. Fish were considered dead when there was no visible movement (e.g. gill movements) and if touching of the caudal peduncle produced no reaction. Dead fish were removed when observed. Visible abnormalities were also recorded (e.g. loss of reflex, erratic swimming, increased excitability, lethargy, and changes in appearance or physiology such as discoloration, excess mucous production, hyperventilation, opaque eyes, curved spine, or hemorrhaging, etc.). Since there was only a 5% mortality rate at the highest concentration, a second definitive test was set in the same manner using concentrations of 13, 21, 35 and 58 mg/L. Once it was determined that the solvent was not toxic at the higher concentrations necessary for higher concentrations of the test substance, a third definitive test was conducted at concentrations of 20, 40, 60, 80 and 100 mg/L.

Chemical and Physical Monitoring

The following measurements of control and treated containers were recorded daily during definitive testing: dissolved oxygen, temperature, conductivity, and pH.

RESULTS AND DISCUSSION

Test Validity

The test was considered valid if control mortality did not exceed 10 percent. Since control and solvent control mortality were zero percent, the range-finding test was considered valid. Control data were compared with the mortality endpoints of the test concentrations.

RESULTS AND DISCUSSION (cont.)

Range-finding

A 100% survival rate was observed in fish treated at concentrations of 0, 1 and 10 mg/L. 100% mortality was observed in all fish treated with 50 and 100 mg/L of test substance.

Concentration (mg/L)	Number of Surviving Organisms				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	2	2	2	2	2
1	2	2	2	2	2
5	2	2	2	1[d]	1
10	2	2	2	2	2
50	2	2[a]	0[c]	-	-
100	2	0[b]	-	-	-
C2	2	2	2	2	2

C2 – Solvent control
[a] - Fish laying on the bottom of the tank in curved position. Gills still moving, appear dead but jerk when stimulated.
[b] - Fish all dead. No movement, with curved spines.
[c] - All fish dead, white in color with curved spines.
[d] - Dead fish has very extended gills and mouth.

Definitive

In the first definitive test conducted, a 100% survival rate was observed in fish treated with 0, 5, 8, 13 and 21 mg/L of the test substance and in the solvent control. A 95% survival rate was observed in fish treated at the 35mg/L level. Since there was only a 5% mortality rate at the highest concentration, a second definitive test was set in the same manner using concentrations of 13, 21, 35, 58 and 97 mg/L. The 97 mg/L concentration subsequently had to be dropped from the test since it was feared that the amount of solvent needed to dissolve the test substance would exceed the toxicity level for the fish. In this second definitive test, a 100% survival rate was seen in both controls and in the 13, 21 and 35 mg/L levels. A 55% survival rate was seen at the 58-mg/L level. Once it was determined that the higher concentrations of solvent could be used, a third definitive test was run at higher concentrations. The concentrations used were 20, 40, 60, 80 and 100 mg/L. There were 100% survival rates observed in both controls as well as the 20-mg/L concentration. The 40-mg/L level had only a 35% survival rate, and the 60, 80 and 100 mg/L levels had 100% mortality. Results of the definitive tests follow.

Chemical and physical monitoring data (dissolved oxygen, temperature, conductivity, and pH) of control and treated containers for the three definitive tests are presented in Appendix A.

RESULTS AND DISCUSSION (cont.)

First Definitive Test (12 to 16 Jul 01)

Target Concentration (mg/L)	Replicate	Number of Surviving Organisms				
		0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	A	10	10	10	10	10
	B	10	10	10	10	10
5	A	10	10	10	10	10
	B	10	10	10	10	10
8	A	10	10	10	10	10
	B	10	10	10	10	10
13	A	10	10	10	10	10
	B	10	10	10	10	10
21	A	10	10	10	10	10
	B	10	10	10	10	10
35	A	10	10[a]	10	10[d]	9[e]
	B	10	10[b]	10[c]	10[c]	10[c]
C2	A	10	10	10	10	10
	B	10	10	10	10	10

C2 – Solvent control
[a] - All fish dark in color, swimming on bottom of tank. Three fish laying on side with curved spine and gill movement.
[b] - All fish dark in color laying on bottom of tank with curved spine and gill movement.
[c] - Four fish laying on side with curved spine and gill movement.
[d] - One fish laying on side with curved spine and gill movement.
[e] - One fish dead with curved spine.

RESULTS AND DISCUSSION (cont.)

Second Definitive Test (04 to 08 Oct 01)

Target Concentration (mg/L)	Replicate	Number of Surviving Organisms				
		0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	A	10	10	10	10	10
	B	10	10	10	10	10
13	A	10	10	10	10	10
	B	10	10	10	10	10
21	A	10	10	10	10	10
	B	10	10	10	10	10
35	A	10	10	10	10	10
	B	10	10	10	10	10
58	A	10	10[a]	10[a]	7[b]	5[d]
	B	10	10[a]	10[a]	6[c]	6[a]
C2	A	10	10	10	10	10
	B	10	10	10	10	10

C2 – Solvent control
[a] - All fish laying on side at botom of tank with gill movement.
[b] - Three fish dead laying on side at bottom, no gill movement.
[c] - Four fish dead laying on side at bottom.
[d] - Two fish dead laying on side with curved spine, white in color.

Third Definitive Test (21 to 25 Feb 02)

Target Concentration (mg/L)	Replicate	Number of Surviving Organisms				
		0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	A	10	10	10	10	10
	B	10	10	10	10	10
20	A	10	10	10	10	10
	B	10	10	10	10	10
40	A	10	4[a]	4	4	4
	B	10	3[a]	3	3	3
60	A	10	2[a]	0[a]	0	0
	B	10	2[a]	0[a]	0	0
80	A	10	0[a]	0	0	0
	B	10	0[a]	0	0	0
100	A	10	0[a]	0	0	0
	B	10	0[a]	0	0	0
C2	A	10	10	10	10	10

C2 – Solvent control. There was insufficient solvent to run two replicates of the solvent control.
[a] - Fish dead, laying on bottom.

RESULTS AND DISCUSSION (cont.)

Evaluation of Results

The LC_{50} level for the test substance, Miller 6064, based on the three definitive tests conducted, is 46.90 mg/L with 95% confidence limits of 38.21 – 58.88 mg/L. The NOEC level is 35 mg/L.

CONCLUSION

The test substance, Miller 6064, was evaluated for toxicity to *Oncorhynchus mykiss* in a 96-hour static, non-renewal test. The LC_{50} level, based on the three definitive tests conducted, is 46.90 mg/L with 95% confidence limits of 38.21 – 58.88 mg/L. The NOEC level is 35 mg/L.

Neil A. Rodrigue

09 May 02

Neil A. Rodrigue, M.S.
Study Director
STILLMEADOW, Inc.

Date

STUDY PERSONNEL

Technical Staff

Mel S. Rivera, B.S.
Brandy Goffinet
Abigail Campbell, B.S.
Hernan Hernandez
Richard Sankar, B.S.
Jennifer Thompson

Technical Writer

Lynne Magee, B.S., R.Q.A.P.

Appendix A: Chemical and Physical Monitoring Data (First Definitive Test – 12 to 16 Jul 01)

Table 1. Temperature (°C)

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	14	12[a]	12[a]	12[a]	12[a]
5	14	12[a]	12[a]	12[a]	12[a]
8	14	12[a]	12[a]	12[a]	12[a]
13	14	12[a]	12[a]	12[a]	12[a]
21	14	12[a]	12[a]	12[a]	12[a]
35	14	12[a]	12[a]	12[a]	12[a]
C2	14	12[a]	12[a]	12[a]	12[a]

[a] – Temperature taken from chamber thermometer

Table 2. pH

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	7.7	7.5	7.3	7.3	7.4
5	7.8	7.5	7.3	7.3	7.3
8	7.9	7.4	7.3	7.3	7.3
13	7.9	7.4	7.3	7.4	7.3
21	7.9	7.4	7.3	7.4	7.3
35	7.9	7.4	7.2	7.4	7.2
C2	7.8	7.4	7.3	7.4	7.3

Table 3. Dissolved Oxygen (mg/L)

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	10.2	8.2	8.0	8.0	8.0
5	9.8	8.2	8.0	8.0	7.8
8	10.0	7.6	8.0	8.0	7.8
13	10.0	7.6	8.0	7.8	7.8
21	10.2	7.0	7.4	7.8	7.0
35	10.2	6.6	7.0	5.8	6.4
C2	10.0	8.2	8.0	8.1	7.7

Table 4. Conductivity (µmhos/cm)

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	250	245	250	250	250
5	265	250	250	250	250
8	270	250	245	250	250
13	265	250	245	245	250
21	265	245	245	245	245
35	260	245	230	245	245
C2	260	245	245	245	245

Appendix A: Chemical and Physical Monitoring Data (Second Definitive Test – 04 to 08 Oct 01)

Table 1. Temperature (°C)

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	13	11[a]	11[a]	11[a]	11[a]
13	13	11[a]	11[a]	11[a]	11[a]
21	13	11[a]	11[a]	11[a]	11[a]
35	13	11[a]	11[a]	11[a]	11[a]
58	13	11[a]	11[a]	11[a]	11[a]
C2	13	11[a]	11[a]	11[a]	11[a]

[a] – Temperature taken from chamber thermometer

Table 2. pH

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	7.6	7.4	7.8	7.8	7.6
13	7.5	7.4	7.8	7.7	7.6
21	7.6	7.4	7.8	7.7	7.6
35	7.6	7.4	7.7	7.7	7.6
58	7.6	7.4	7.7	7.7	7.5
C2	7.5	7.4	7.6	7.7	7.6

Table 3. Dissolved Oxygen (mg/L)

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	8.4	6.4	7.2	6.6	6.0
13	8.6	6.7	7.4	6.4	5.4
21	8.6	6.4	7.4	6.4	5.8
35	8.6	6.5	7.2	5.8	4.4
58	8.6	6.5	7.2	3.9	4.6
C2	8.6	6.6	7.2	6.2	5.4

Table 4. Conductivity (µmhos/cm)

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	260	230	230	240	240
13	260	235	230	235	240
21	260	235	230	230	235
35	260	230	230	230	235
58	255	230	235	235	230
C2	260	230	230	230	235

Appendix A: Chemical and Physical Monitoring Data (Third Definitive Test – 21 to 25 Feb 02)

Table 1. Temperature (°C)

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	12	13[a]	13[a]	13[a]	13[a]
20	12	13[a]	13[a]	13[a]	13[a]
40	12	13[a]	13[a]	13[a]	13[a]
60	12	13[a]	13[a]	13[a]	13[a]
80	12	13[a]	13[a]	13[a]	13[a]
100	12	13[a]	13[a]	13[a]	13[a]
C2	13	13[a]	13[a]	13[a]	13[a]

[a] – Temperature taken from chamber thermometer

Table 2. pH

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	8.0	7.7	7.5	7.2	7.7
20	8.2	7.7	7.5	7.2	7.7
40	8.2	7.7	7.5	7.2	7.7
60	8.2	7.7	7.6	7.2	-
80	8.2	7.7	-	-	-
100	8.2	7.7	-	-	-
C2	8.1	7.7	7.5	7.2	7.6

Table 3. Dissolved Oxygen (mg/L)

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	9.8	9.6	9.4	9.0	8.8
20	10.0	9.4	9.6	9.2	8.8
40	10.0	9.4	9.6	9.2	8.8
60	10.2	10.2	9.6	-	-
80	10.3	10.0	-	-	-
100	10.2	10.0	-	-	-
C2	10.2	9.6	9.5	9.0	9.0

Table 4. Conductivity (µmhos/cm)

Target Concentration (mg/L)	Definitive				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0	210	210	210	210	210
20	205	210	210	210	210
40	205	205	210	210	210
60	205	205	210	-	-
80	205	210	-	-	-
100	205	210	-	-	-
C2	215	210	210	210	210

Appendix B: Statistics

96 hour Static, Non-Renewal Acute Definitive Toxicity Test-96 Hr Survival

Start Date:	2/21/02 17:16	Test ID:	6420-01	Sample ID:	Miller 6064
End Date:	2/25/02 16:42	Lab ID:	Miller 6064	Sample Type:	Product
Sample Date:		Protocol:	OPPTS 850.1075	Test Species:	OM-Oncorhynchus mykiss
Comments:	Data from 3 Definitive tests				

Conc-mg/L	1	2	3	4	5	6
Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
t Control (C2)	1.0000	1.0000	1.0000	1.0000	1.0000	
5	1.0000	1.0000				
8	1.0000	1.0000				
13	1.0000	1.0000	1.0000	1.0000		
20	1.0000	1.0000				
21	1.0000	1.0000	1.0000	1.0000		
35	0.9000	1.0000	1.0000	1.0000		
40	0.4000	0.3000				
58	0.5000	0.6000				
60	0.0000	0.0000				
80	0.0000	0.0000				
100	0.0000	0.0000				

			Transform: Arcsin Square Root					1-Tailed			Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	6				0	60
t Control (C2)	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	5					
5	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.861	0.0870	0	20
8	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.861	0.0870	0	20
13	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	0.000	2.861	0.0688	0	40
20	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.861	0.0870	0	20
21	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	0.000	2.861	0.0688	0	40
35	0.9750	0.9750	1.3713	1.2490	1.4120	5.942	4	1.695	2.861	0.0688	1	40
*40	0.3500	0.3500	0.6322	0.5796	0.6847	11.753	2	25.648	2.861	0.0870	13	20
*58	0.5500	0.5500	0.8357	0.7854	0.8861	8.518	2	18.953	2.861	0.0870	9	20
*60	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	41.217	2.861	0.0870	20	20
*80	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	41.217	2.861	0.0870	20	20
*100	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	41.217	2.861	0.0870	20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.64297	0.908	-1.8591	7.74529
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 1.00)	0	2.26216		

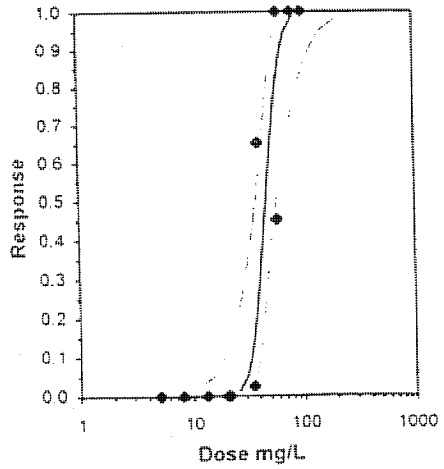
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	35	40	37.4166		0.03419	0.03507	0.73935	0.00139	4.9E-24	11, 22

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	8.8605	2.38084	3.47465	14.2463	0	37.5254	16.919	2.1E-05	1.67115	0.11286	5
Intercept	-9.8072	3.96924	-18.786	-0.8281							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	25.6209	9.94312	33.2567
EC05	3.355	30.5849	15.3809	37.7044
EC10	3.718	33.6132	19.3003	40.5385
EC15	3.964	35.824	22.4031	42.7438
EC20	4.158	37.6843	25.1296	44.7442
EC25	4.326	39.3571	27.6311	46.7043
EC40	4.747	43.909	34.3001	53.2385
EC50	5.000	46.8972	38.2191	58.8759
EC60	5.253	50.0887	41.8204	66.3018
EC75	5.674	55.8817	47.0753	83.3463
EC80	5.842	58.3623	48.9853	91.9275
EC85	6.036	61.393	51.1426	103.388
EC90	6.282	65.4309	53.8014	120.284
EC95	6.645	71.9094	57.724	151.254
EC99	7.326	85.8419	65.3165	234.428

Significant heterogeneity detected (p = 2.12E-05)



Appendix C: Protocol and Amendment

STILLMEADOW
INCORPORATED

PROTOCOL AMENDMENT #1
STILLMEADOW, Inc. Study Number 6420-01

Sponsor: Miller Chemical & Fertilizer Corporation
PO Box 333
Radio Road
Hanover PA 17331

Effective Date: 22 Oct 2001

Test Substance: Miller 6064

Study Title: RAINBOW TROUT (*Oncorhynchus mykiss*) STATIC 96-HOUR ACUTE TOXICITY TEST

The following alteration is being made to the cover and Section A.7 of the protocol.

To Change:	Abigail Campbell, B.S.
To Read:	Neil Rodrigue, M.S.
Justification:	The study director is being changed because Abigail Campbell is no longer with the company.
Impact:	There will be no impact on the study.

This amendment has been reviewed and/or approved by the following:

Approved: Neil A. Rodrigue — 08 Nov 01
Neil Rodrigue, M.S.
Study Director
STILLMEADOW, Inc.
Date

Approved: — 6 Nov 01
Mark S. Holbert
Vice President
STILLMEADOW, Inc.
Date

Reviewed:: — 6 Nov 2001
Vicki S. Crutchfield, R.Q.A.P.
Director, Quality Assurance Unit
STILLMEADOW, Inc.
Date

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Appendix C: Protocol and Amendment (cont.)

STILLMEADOW
INCORPORATED

PROTOCOL FOR STUDY 6420-01

Study Title: RAINBOW TROUT (*Oncorhynchus mykiss*) STATIC 96-HOUR ACUTE TOXICITY TEST

Test Substance: Miller 6064

Test Facility: STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, Texas 77479

Approved: Abigail Campbell — 22 Jun 01
Abigail Campbell, B.S.
Study Director
STILLMEADOW, Inc.
Date

Approved: Elizabeth L. Sabol — 5 June 2001
Elizabeth L. Sabol, B.A., B.S.Ed
Vice President
STILLMEADOW, Inc.
Date

Reviewed: Vicki Crutchfield — 5 June 2001
Vicki Crutchfield, R.Q.A.P.
Director, Quality Assurance Unit
STILLMEADOW, Inc.
Date

Sponsor: Miller Chemical & Fertilizer Corporation
PO Box 333
Radio Road
Hanover PA 17331

Sponsor Representative
Mandava Associates
1730 M Street, Suite 906
Washington, D.C. 20036-4510

Approved: N. Bhushan Mandava — 15 June 2001
N. Bhushan Mandava, Ph.D.
Date

Appendix C: Protocol and Amendment (cont.)

PROTOCOL FOR STUDY 6420-01

A. GENERAL

1. Study Title:	RAINBOW TROUT (*Oncorhynchus mykiss*) STATIC 96-HOUR ACUTE TOXICITY TEST
2. Purpose:	To assess the toxicity of the test substance to rainbow trout (*Oncorhynchus mykiss*) in a static 96-hour test.
3. Regulatory Compliance:	This study will be conducted according to OPPTS §50.1075, which is intended to meet testing requirements of both FIFRA (7 U.S.C. 136, *et seq.*) and TSCA (15 U.S.C. 2601). This study will be conducted in compliance with Good Laboratory Practice Standards: 1. EPA FIFRA: 40 CFR 160 2. OECD: C(81)30 (Final) 3. Japanese MAFF All methods can be found in STILLMEADOW, Inc. Standard Operating Procedures (SOPs).
4. Quality Assurance:	The Protocol is reviewed by the Quality Assurance Unit (QAU). The study information will be entered into the Master Schedule. The study will be inspected at least once during its progress. Further inspections may be scheduled as needed to ensure the integrity of the study. Any deviations from SOPs, the Protocol, or Good Laboratory Practice Standards will be immediately reported to the Study Director and Management. The report will be audited and a statement prepared and signed which shall specify the dates inspections were made and findings reported to Management and to the Study Director.
5. Test Substance:	Miller 6064. Test substance identification should include the name, batch number, and purity. Information regarding safety, stability, storage conditions, and disposal should also be provided by the Sponsor
6. Proposed Schedule:	Testing will begin within approximately three weeks of receipt of the test substance and authorization to conduct the study. Proposed Start Date: 13 Jun 01 Proposed End Date: 11 Jul 01
7. Study Director:	Abigail Campbell, B.S.

Appendix C: Protocol and Amendment (cont.)

A. GENERAL (cont.)

8. Experimental Summary:	Definitive test concentrations will be determined by a preliminary range finder. The test substance concentrations chosen will be administered to the test system, rainbow trout (*Oncorhynchus mykiss)*, in reconstituted water. For each test concentration, 20 organisms will be treated with the appropriate concentration of the test substance. Two control groups, which will not contain test substance, will be used in this test. One group will have solvent added at the highest volume used for any test concentration preparation and will represent the solvent control. The other control group will remain untreated and will demonstrate the condition of the test population. Dissolved oxygen, temperature, conductivity, and pH will be measured and recorded in each treatment and the control at test initiation and daily throughout the study. Observations of mortality in each test chamber will be made at 24, 48, 72, and 96 hours. The test will be terminated after 96 ± 2 hours of exposure. The test will be considered valid if control mortality does not exceed 10 percent.
9. Protocol Amendments:	Any alteration in the Protocol will be justified, approved by the Study Director, and recorded in writing.
10. Sponsor Audits:	The Sponsor may send an authorized representative to inspect the test system and/or data on the STILLMEADOW, Inc. premises during normal working hours.

Appendix C: Protocol and Amendment (cont.)

B. EXPERIMENTAL DESIGN

1. Organism

a. Species:	Rainbow trout (*Oncorhynchus mykiss*)
b. Justification of Species:	Specified in the OPPTS regulations.
c. Age/Size:	Juvenile fish, less than 3.0 g at test initiation. The longest fish will not be more than twice the length of the shortest.
d. Number:	The range finder will use 2 fish for each concentration and the controls. The definitive test will use 20 fish for each concentration and each control group (2 replicates each containing 10 fish).
e. Source:	*Oncorhynchus mykiss* will be obtained from Aquatic Research Organisms, Inc. (Hampton, New Hampshire) or another suitable supplier.
f. Identification:	Organisms will be labeled by study number, lot number, date of receipt, and number of organisms.

2. Animal Husbandry

a. Test Medium:	Reconstituted water with total hardness between 40 and 180 mg $CaCO_3$ and with a pH between 6.0 and 8.0.
b. Acclimation:	All fish will be held in the laboratory for at least 14 days before they are used for testing. They will be held in water of the quality to be used in the test for at least seven days immediately before testing. Pretest mortality must be less than 5% during acclimation or the organisms will be held for an additional seven days. If pretest mortality is greater than 10%, then the entire lot will be rejected and a new lot of fish will be obtained to begin acclimation.
b. Test Chamber:	Test containers will be 2½ gallon aquaria. Test containers will be loosely covered to reduce the loss of test solution or dilution water due to evaporation and to minimize the entry of dust or other particles into the solutions.
c. Temperature:	Test temperature will be 12±2°C.
d. Photoperiod:	16 hours light, 8 hours dark
e. Dissolved Oxygen Concentrations:	At least 60 percent air saturation value.
f. Food:	Fish will be fed daily until 48 hours prior to test initiation. Fish will not be fed during test.
g. Loading:	Maximum loading of 0.8 g fish/liter.

Appendix C: Protocol and Amendment (cont.)

B. EXPERIMENTAL DESIGN (cont.)

3. Pre-test Preparation

a. Test Substance Receipt: Test substance will be supplied by the Sponsor in appropriately sized glass containers, sealed and delivered to STILLMEADOW, Inc. Samples will be stored according to the Sponsor's instructions until prepared for testing.

b. Test Substance Preparation: The test substance is insoluble in water and will be administered using an appropriate solvent (DMF, ethanol, methanol, etc.) as weight/volume concentrations. A solvent control will be included in the test design. The test substance dilutions will be prepared on the day of treatment.

c. Route of Administration: The test substance will be administered to the test system at test initiation by introduction to the test containers containing the test system.

d. Reason for Route of Administration: Specified by the cited guidelines for evaluation of the toxicity potential of a test substance.

e. Preparation of Test System: The organisms will be randomized into aquaria containing the appropriate concentration of test substance. Each test concentration will consist of 20 fish. Each aquarium will house of a maximum of 10 fish.

f. Control Groups: Twenty fish will not have test substance added and will be considered the control. This control will be used to demonstrate the condition of the test population. An additional 20 fish will not have test substance added but will contain the solvent at the highest volume used for any test concentration preparation. The solvent control will be used to demonstrate artifactual toxicity produced by the solvent.

4. Test Substance Administration

a. Dosing Concentrations: A range finder will be conducted with at least five concentrations of the test substance to obtain an approximate LC_{50} value for the test substance. The test concentrations will be at least 50% greater than the lowest test concentrations (not to exceed 120%).

Five test concentrations chosen from the range-finding data and the controls will be prepared on the day of test initiation.

b. Initial Measurements: Dissolved oxygen, temperature, conductivity, and pH of the control and treated containers will be measured and recorded at test initiation.

Appendix C: Protocol and Amendment (cont.)

B. EXPERIMENTAL DESIGN (cont.)

5. Observations

a. Biological Monitoring: Containers will be inspected at 24, 48, 72, and 96 hours for mortality. Fish are considered dead if there is no visible movement (e.g. gill movements) and if touching of the caudal peduncle produces no reaction. Dead fish will be removed when observed, and mortalities will be recorded. Visible abnormalities will be recorded (e.g. loss of reflex, erratic swimming, increased excitability, lethargy, and changes in appearance or physiology such as discoloration, excess mucous production, hyperventilation, opaque eyes, curved spine, or hemorrhaging, etc.)

b. Chemical and Physical Monitoring: At a minimum, the following measurements will be made daily: dissolved oxygen, temperature, conductivity, and pH of the controls and treated containers.

6. Test Duration: The test will be terminated after 96 ± 2 hours.

7. Quality Criterion: The test will be considered valid if the control mortality does not exceed 10 percent.

8. Evaluation of Results: The survival in the test concentrations will be statistically compared to survival in the control to determine the highest concentration of test substance that demonstrates no significant reduction in survival. This concentration will be the No Observed Effect Level (NOEL) for survival. The NOEL will be determined by using a commercially available statistical program (Toxstat®).

The median lethal concentration (LC_{50}) will be estimated using a linear regression model. Several models are available for LC_{50} determination: Probit, Trimmed Spearman-Karber, and Binomial. The most appropriate model will be selected for estimating the LC_{50} if a dose response is exhibited in the study.

9. Test Substance Accountability: A comprehensive inventory of test substance received and used will be kept. The test substance container(s) will be weighed when received at this facility, and a record of all test substance use will be maintained. Test substance and test substance dosing solutions will be stored in the original containers, or in the equivalent thereof, or in glass containers with Teflon-lined caps.

Appendix C: Protocol and Amendment (cont.)

B. EXPERIMENTAL DESIGN (cont.)

10. Disposal of Unused Test Substance:

Unused test substance will be disposed of at the Sponsor's expense after the termination of the study. STILLMEADOW, Inc. will retain a reserve sample.

11. Safety Precautions:

General safety precautions required by laboratory SOPs will be followed. The Sponsor will supply basic toxicity data on the test substance to be used. However, since the toxicity of test substances is often not well characterized, STILLMEADOW, Inc. will be conservative in setting safety procedures. The Sponsor or Sponsor's Representative shall be notified of any exposures requiring a physician's examination or care.

C. DATA MANAGEMENT

1. Records:

The following records will be maintained during the study and transferred to the STILLMEADOW, Inc. archives upon study termination.

a. Protocol and Protocol Amendments (if any).
b. Final report and amendments (if any).
c. Study correspondence.
d. Test culture data.
e. Test substance receipt, identification as supplied by Sponsor, preparation, administration, and disposition.
f. Range finder data and results.
g. Initial and daily measurements for dissolved oxygen, temperature, and pH of the control and treated containers.
h. Cumulative mortality at each concentration at each observation time.
i. Determination of the validity of the study.
j. Other pertinent data.

2. Data Storage:

All raw data and a reserve sample of the test substance will be retained at STILLMEADOW, Inc. for a period of at least five years.

Appendix C: Protocol and Amendment (cont.)

C. DATA MANAGEMENT (cont.)

3. Data Reporting:

The final report will include all data as described in the Good Laboratory Practice Standards, including:

a. Statement from the Quality Assurance Unit.
b. Signature of the Study Director.
c. A GLP Compliance Statement signed by the Study Director.
d. Names of scientific personnel involved in the study.
e. Dates of study initiation and termination.
f. Identification, description, preparation, and storage of the test substance.
g. All pertinent culture information, preparation of test medium, test conditions, dosing information, and observation methods.
h. Initial and daily data for dissolved oxygen, temperature, and pH of the control and treated containers.
i. Cumulative mortality at each concentration at each observation time.
j. Graph of the concentration-mortality curve at the end of the test.
k. Statistical procedures used for determining the LC_{50} and NOEL values.
l. Determination of the validity of the test based on the control data.
m. Abnormalities observed in test and control animals.
n. Any protocol deviations or occurrences which may have influenced the final results of the test.
o. Evaluation of results.
p. A reference to this Protocol.

4. Report Submission:

A report will be submitted after termination of the laboratory portion of the study.

Appendix D: Certificate of Analysis

MILLER CHEMICAL & FERTILIZER CORPORATION

P O. BOX 333, 120 RADIO ROAD
HANOVER, PENNSYLVANIA 17331 U.S.A.
TELEPHONE 717-632-8921
FAX NO.: 717-632-4561

1/18/01

CERTIFICATE OF ANALYSIS

Product Name: MILLER 6064
Quantity Produced: 7.4 kilograms
Batch Number: 150386
Date of Formulation: 12/13/00

Appearance: Yellow to Amber Liquid
Odor: Terpenic
Moisture: Negligble (non-aqueous)
Specific Gravity: 0.92 - 0.94 @ 20c
Viscosity @ 20c: 100 to 500 cps
pH(1% solution): 6.0 to 8.0
Solubility in Water: Insoluble (Emulsifies)
Flash Point: over 200 f
Purity: 100%

Signature:

Name: Andrew G. Smith
Title: Compliance Officer

ATTACHMENT 42

Daphnia magna
Static 48-Hour Acute Toxicity Test
on Miller 6064

STILLMEADOW
INCORPORATED

VOLUME __ OF __ OF SUBMISSION

Miller 6064

FINAL REPORT

***Daphnia magna* STATIC 48-Hour ACUTE TOXICITY TEST**

OPPTS No. 850.1010

AUTHOR:

Neil A. Rodrigue, M.S.

STUDY INITIATION DATE: 29 June 2001
STUDY COMPLETION DATE: 9 May 2002

CONDUCTED BY:
STILLMEADOW, Inc.
10161 Harwin Drive, Suite 150
Houston, Texas 77036

LABORATORY STUDY NUMBER:

6421-01

VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 23

SUBMITTED TO:
Miller Chemical & Fertilizer Corporation
P.O. Box 333
Radio Road
Hanover, PA 17331

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA § 10(d) (1) (A), (B), or (C).

Company: Miller Chemical and Fertilizer Corporation

Company Agent:_____ Date: _____

Title_____ Signature _____

These data are the property of Miller Chemical and Fertilizer Corporation and as such, are considered to be confidential for all purposes other than compliance with FIFRA § 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality that may exist under any other statute or in any other country.

GLP COMPLIANCE STATEMENT

This study was designed and performed in STILLMEADOW, Inc.'s laboratory in compliance with the following:

· United States Environmental Protection Agency (USEPA) FIFRA; Good Laboratory Practice Standards 40 CFR 160 with exception of sections 160.105 (b) (e) and 160.31 (d), stability information was not provided; 160.105 (b) solubility not determined; and 160.113 (a) mixture was not performed.

· Organization for Economic Cooperation and Development's Principles of Good Laboratory Practice, Annex 2, C(97)186 with the exception of section 6.2 (4), stability information was not provided and section 6.2 (5), mixture analysis was not performed.

· Japan Ministry of Agriculture, Forestry and Fisheries, Notification No. 59 Nohsan 3850, Director-General of Agricultural Production Bureau, 10 August 1984 with the exception of Article 5 (2) (9) and Article 21 (3), stability information was not provided and Article 23 (1), mixture analysis was not performed.

Neil A. Rodrigue — 09 May 02

Neil A. Rodrigue, M.S.
Study Director
STILLMEADOW, Inc.

Date

Signature of Agent of Sponsor

Date

Agent Name
Sponsor: Miller Chemical & Fertilizer Corporation
P.O. Box 333
Radio Road
Hanover, PA 17331

Signature of Agent of Submitter

Date

Agent Name
Submitter: Mandava Associates

TABLE OF CONTENTS

QUALITY ASSURANCE STATEMENT

Study Title: *Daphnia magna* Static 48-Hour Acute Toxicity Test
Test Substance: Miller 6064

The study has been inspected and the report and data have been audited in accordance with STILLMEADOW, Inc. Standard Operating Procedures (SOPs). The findings from the inspection and audit were reported to the Study Director and Management as follows:

Phase of Study Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
Randomization and Dosing	21 Aug 01	22 Aug 01	22 Aug 01
Counts	12 Oct 01	15 Oct 01	15 Oct 01
Report/Data Audit	14 Dec 01	15 Dec 01	15 Dec 01
Final Report/Data Audit	06 Mar 02	07 Mar 02	07 Mar 02

B. Lynn Murphy
Quality Assurance Unit
STILLMEADOW, Inc.

09 May 2002
Date

SUMMARY

This study was conducted to assess the toxicity of the test substance (Miller 6064) to *Daphnia magna* in a 48-hour static, non-renewal test.

Test considerations were determined by a preliminary range-finding test. The test substance concentrations chosen (0, 0.5, 0.9, 1.5, 2.5 and 5 mg/L) were administered to the test system, *Daphnia magna*, in reconstituted water. For each target test concentration, two replicates of ten organisms each were treated with the appropriate concentration of the test substance. Two control containers each contained 10 daphnids in reconstituted water and no test substance. Because the test substance was insoluble in water, N,N-Dimethylformamide was used as a solvent. Two control containers each contained 10 daphnids in the solvent as solvent controls. Dissolved oxygen, temperature, conductivity and pH measurements were recorded at dosing and termination. Observations for immobilization in each test chamber were made daily. The test was terminated after 48 ± 1 hours of exposure.

100% survival rates were seen in the daphnids treated with 0 mg/L of Miller 6064 and in the solvent control, and mortality was observed in daphnids treated with 0.5, 0.9, 1.5, 2.5 and 5 mg/L of the test substance.

Because of the high mortality rate, it was determined to run another definitive test. The test substance concentrations chosen (0, 0.1, 0.3, 0.5, 0.9 and 1.5mg/L) were administered to the test system using identical criteria as the first definitive test. The median lethal concentration (LC_{50}) was determined to be 0.54 mg/L with 95% confidence limits of 0.39 to 0.78 mg/L and a NOEC of less that 0.1 mg/L.

INTRODUCTION

The objective of this study was to assess the toxicity of the test substance to *Daphnia magna* in a 48-hour test. This study was conducted for Miller Chemical & Fertilizer Corporation according to the approved protocol, STILLMEADOW, Inc. SOPs, and Product Properties Test Guidelines, Series 850, Section 1010 of the United States Environmental Protection Agency Office of Prevention, Pesticides, and Toxic Substances. This study was initiated on 29 Jun 01. The laboratory portion of the study was conducted between 19 Jul 01 to 21 Jul 01 for the range-finding test, 21 Aug 01 to 23 Aug 01 for the first definitive test, and 11 Oct 01 to 13 Oct 01 for the second definitive test. The original protocol, raw data, and report are on file in the STILLMEADOW, Inc. archives. A reserve sample of the test substance will be retained at STILLMEADOW, Inc. for a period of at least five years.

TEST SUBSTANCE

Identification:	MILLER 6064
Date and Quantity Received:	19 Dec 00; 2 X 1 gal
Physical Description:	Amber liquid
Storage:	Room temperature
Purity and Composition:	Certificate of Analysis not provided by Sponsor
Stability:	Not provided by the Sponsor

Records pertaining to stability, characterization, and verification of test substance identity are the responsibility of the Sponsor.

TEST SYSTEM

Experimental Organism

Species:	*Daphnia magna*
Source:	STILLMEADOW, Inc. culture laboratory
Age at dosing:	Less than 24 hours old at dosing
Quantity:	Range-finding: 5 per test concentration Definitive: 20 per target test concentration

Organism Husbandry

Test Room:	Environmentally controlled chambers (Chamber D for the range-finding test, and Chamber C for the definitive tests)
Test Chambers:	250 mL glass beaker (range-finding and definitive)
Test Medium:	Reconstituted water
Holding:	The daphnids were held in water of the quality used in the tests for at least 48 hours immediately before testing.

Environmental Controls

Set to Maintain:	Temperature Range of 20±2°C 16-hours light / 8-hours dark cycle Dissolved oxygen concentration of at least 60 percent saturation at dosing
Food:	Daphnids were not fed during the tests

Animal husbandry and housing at STILLMEADOW, Inc. comply with Animal Welfare Act Regulations. No contaminants were expected to have been present in the feed or water which would have interfered with or affected the results of the study.

PROCEDURES

Range-finding Test

A preliminary range-finding test was conducted using five concentrations of the test substance (1, 5, 10, 50, and 100 mg/L). Following randomization, five organisms were placed into each beaker containing the appropriate concentration of test substance. Five organisms, which were not exposed to test substance, served as controls to demonstrate the condition of the test population. Additionally, five organisms, which were not exposed to test substance were exposed to the solvent (N,N-Dimethylformamide). This solvent control was used to demonstrate artifactual toxicity produced by the solvent. At 24 and 48 hours following dosing, each beaker was examined for mortality and the number of live daphnids was recorded.

PROCEDURES (cont.)

Definitive Test

Based on the results of the range-finding test, test substance concentrations were chosen for definitive testing. Five target concentrations of the test substance were used (0.5, 0.9, 1.5, 2.5 and 5 mg/L). Each target test concentration consisted of two replicates of ten daphnids per replicate. Two replicates containing ten daphnids each were not exposed to test substance and served as controls to demonstrate the condition of the test population. Additionally, two replicates of ten organisms, which were not exposed to test substance were exposed to the solvent (N,N-Dimethylformamide). This solvent control was used to demonstrate artifactual toxicity produced by the solvent. At 24 and 48 hours following dosing, each beaker was examined for mortality and the number of live daphnids was recorded.

Because of high mortality rates, it was determined to run another definitive test. Five target concentrations of the test substance were used (0.1, 0.3, 0.5, 0.9 and 1.5 mg/L). Identical criteria were used for the second test.

Chemical and Physical Monitoring

The following measurements were recorded during definitive testing: dissolved oxygen, temperature, conductivity, and pH of control and treated containers.

RESULTS AND DISCUSSION

Test Validity

The test was considered valid if control mortality did not exceed 10 percent. Since control mortality was zero percent, the range-finding and definitive tests were considered valid.

Range-finding

A 100% survival rate was observed in daphnids treated at a concentration of 0 mg/L of the test substance and the solvent control. Mortality was observed in all daphnids treated with 1, 5, 10, 50 and100 mg/L of test substance.

Concentration (mg/L)	Number of Surviving Organisms		
	0 Hours	24 Hours	48 Hours
0	5	5	5
1	5	5[a]	3
5	5	5[b]	1
10	5	5[c]	0
50	5	5	1
100	5	5	0
C2	5	5	5

[a] – Daphnids floating on surface of water, still alive with gill movement.
[b] – Daphnids floating on surface of water, still alive with gill movement. Two stuck together.
[c] – Daphnids collected on surface of water, alive with gill movement.

RESULTS AND DISCUSSION (cont.)

Definitive

In the definitive study of 21 Aug 01 to 23 Aug 01, 100% survival rates were seen in the daphnids treated with 0 mg/L of Miller 6064 and the solvent control. Mortality was observed in daphnids treated with 0.5, 0.9, 1.5, 2.5 and 5 mg/L of the test substance. In the definitive study of 11 Oct 01 to 13 Oct 01, 100% survival was observed in daphnids treated with 0 mg/L of the test substance and in the solvent control. Mortality was observed in daphnids treated with 0.1, 0.3, 0.5, 0.9 and 1.5 mg/L of the test substance. Chemical and physical monitoring data (dissolved oxygen, temperature, conductivity, and pH) of control and treated containers for the definitive tests are presented in Appendix A.

Definitive Test of 21 Aug 01 to 23 Aug 01

Target Concentration (mg/L)	Replicate	Number of Surviving Organisms		
		0 Hours	24 Hours	48 Hours
0	A	10	10	10
	B	10	10	10
0.5	A	10	10	4
	B	10	9	1
0.9	A	10	10	4
	B	10	10	3
1.5	A	10	10	2
	B	10	10	4
2.5	A	10	10	2
	B	10	10	3
5	A	10	10	1
	B	10	10	3
C2	A	10	10	10
	B	10	10	10

RESULTS AND DISCUSSION (cont.)

Definitive Test of 11 Oct 01 to 13 Oct 01

Target Concentration (mg/L)	Replicate	Number of Surviving Organisms		
		0 Hours	24 Hours	48 Hours
0	A	10	10	10
	B	10	10	10
0.1	A	10	9	9
	B	10	10	8
0.3	A	10	10[a]	8
	B	10	10[a]	9
0.5	A	10	10[a]	5
	B	10	10[a]	3
0.9	A	10	9[ab]	2
	B	10	9[ab]	4
1.5	A	10	10[a]	3
	B	10	10[ab]	1
C2	A	10	10	10
	B	10	10	10

[a] – Surviving animals floating on surface.

[b] – Two pair of animals stuck together but alive.

Evaluation of Results

Using the Trimmed Spearman-Karber Statistical Method, the median lethal concentration (LC_{50}) of Miller 6064 was determined to be 0.54 mg/L with 95% confidence limits of 0.39 0.78 mg/L, and a NOEC of less than 0.1 mg.l

CONCLUSION

The test substance, Miller 6064, was evaluated for toxicity to *Daphnia magna* in a 48-hour static, non-renewal test. The median lethal concentration (LC_{50}) of Miller 6064 was determined to 0.54 mg/L with 95% confidence limits of 0.39 to 0.78 mg/L and a NOEC of less than 0.1 mg/L

Neil A. Rodrigue

Neil A. Rodrigue, M.S.
Study Director
STILLMEADOW, Inc.

09 May 02
Date

STUDY PERSONNEL

Technical Staff

Mel S. Rivera, B.S.
Richard Sankar, B.S.
Rob Stowe, B.S.
Abigail Campbell, B.S.
Brandy Goffinet

Technical Writer

Diana W. Cook, B.S.

Appendix A: Chemical and Physical Monitoring Data
Definitive Test of 21 Aug 01 to 23 Aug 01

Table 1. Temperature (°C)

Target Concentration (mg/L)	0 Hours	48 Hours
0	21	21
0.5	21	21
0.9	21	21
1.5	21	21
2.5	21	21
5	21	21
C2	21	21

Table 2. pH

Target Concentration (mg/L)	0 Hours	48 Hours
0	8.1	7.8
0.5	8.2	7.8
0.9	8.2	7.8
1.5	8.2	7.8
2.5	8.2	7.9
5	8.2	7.9
C2	8.1	7.9

Table 3. Dissolved Oxygen (mg/L)

Target Concentration (mg/L)	0 Hours	48 Hours
0	6.8	8.4
0.5	6.6	8.4
0.9	6.6	8.4
1.5	6.6	8.4
2.5	6.4	8.4
5	6.4	8.4
C2	6.8	8.4

Table 4. Conductivity (µmhos/cm)

Target Concentration (mg/L)	0 Hours	48 Hours
0	290	265
0.5	290	270
0.9	290	270
1.5	290	270
2.5	290	270
5	290	270
C2	290	260

Appendix A: Chemical and Physical Monitoring Data (cont.)
Definitive Test of 11 Oct 01 to 13 Oct 01

Table 1. Temperature (°C)

Target Concentration (mg/L)	0 Hours	48 Hours
0	21	21
0.1	21	21
0.3	21	21
0.5	21	21
0.9	21	21
1.5	21	21
C2	21	21

Table 2. pH

Target Concentration (mg/L)	0 Hours	48 Hours
0	8.0	8.4
0.1	8.0	8.3
0.3	8.1	8.3
0.5	8.1	8.3
0.9	8.1	8.3
1.5	8.1	8.3
C2	*	*

* - pH not recorded

Table 3. Dissolved Oxygen (mg/L)

Target Concentration (mg/L)	0 Hours	48 Hours
0	7.6	7.4
0.1	7.8	7.4
0.3	7.9	7.5
0.5	7.9	7.4
0.9	7.9	7.4
1.5	7.9	7.4
C2	8.0	7.6

Table 4. Conductivity (µmhos/cm)

Target Concentration (mg/L)	0 Hours	48 Hours
0	295	290
0.1	300	290
0.3	300	290
0.5	300	290
0.9	300	290
1.5	300	290
C2	290	295

Appendix B: Trimmed Spearman-Karber Method Statistics

Concentration (mg/L)	Number Exposed	Mortalities
0	40	0
0.10	20	3
0.30	20	3
0.50	20	12
0.54	20	15
0.90	40	27
1.50	40	30
2.50	20	15
5.00	20	16

Spearman-Karber Estimates:

LC_{50}:	0.54
95% Lower Confidence:	0.39
95% Upper Confidence:	0.78

Fisher's Exact Test: NOEC: <0.1

Appendix C: Protocol and Amendment

STILLMEADOW
INCORPORATED

PROTOCOL AMENDMENT #1
STILLMEADOW, Inc. Study Number 6421-01

Sponsor: Miller Chemical & Fertilizer Corporation
PO Box 333
Radio Road
Hanover PA 17331

Effective Date: 22 Oct 2001

Test Substance: Miller 6064

Study Title: *Daphnia magna* STATIC 48-HOUR ACUTE TOXICITY TEST

The following alteration is being made to the cover and Section A.7 of the protocol.

To Change:	Abigail Campbell, B.S.
To Read:	Neil Rodrigue, M.S.
Justification:	The study director is being changed because Abigail Campbell is no longer with the company.
Impact:	There will be no impact on the study.

This amendment has been reviewed and/or approved by the following:

Approved: Neil A. Rodrigue — 08 Nov 01
Neil Rodrigue, M.S.
Study Director
STILLMEADOW, Inc.
Date

Approved: [signature] — 6 Nov 01
Mark S. Holbert
Vice President
STILLMEADOW, Inc.
Date

Reviewed:: [signature] — 6 Nov 2001
Vicki S. Crutchfield, R.Q.A.P.
Director, Quality Assurance Unit
STILLMEADOW, Inc.
Date

12852 Park One Drive • Sugar Land, Texas 77478
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www.stillmeadow.com
10161 Harwin, Suite 150 • Houston, Texas 7703
713 272-7444 • Fax 713 272-7501

STILLMEADOW
INCORPORATED

PROTOCOL FOR STUDY 6421-01

Study Title: *Daphnia magna* STATIC 48-HOUR ACUTE TOXICITY TEST

Test Substance: Miller 6064

Test Facility: STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, Texas 77479

Approved: _____ 29 June 01
Abigail Campbell, B.S.
Study Director
STILLMEADOW, Inc.
Date

Approved: _____ 5 June 2001
Elizabeth J. Sabol, B.A., B.S.Ed
Vice President
STILLMEADOW, Inc.
Date

Reviewed: _____ 5 June 2001
Vicki Crutchfield, R.Q.A.P.
Director, Quality Assurance Unit
STILLMEADOW, Inc.
Date

Sponsor: Miller Chemical & Fertilizer Corporation
PO Box 333
Radio Road
Hanover PA 17331

Sponsor Representative
Mandava Associates
1730 M Street, Suite 906
Washington, D.C. 20036-4510

Approved: _____ 27 June 01
N. Bhushan Mandava, Ph.D.
Date

Appendix C: Protocol and Amendment

PROTOCOL FOR STUDY 6421-01

A. GENERAL

1. Study Title: *Daphnia magna* STATIC 48-HOUR ACUTE TOXICITY TEST

2. Purpose: To assess the toxicity of the test substance to the marine invertebrate *Daphnia magna* through a 48-hour test.

3. Regulatory Compliance: This study will be conducted according to OPPTS 850.1010, which is intended to meet testing requirements of both FIFRA (7 U.S.C. 136, *et seq.*) and TSCA (15 U.S.C. 2601).

This study will be conducted in compliance with Good Laboratory Practice Standards:
1. EPA FIFRA: 40 CFR 160
2. OECD: C(81)30 (Final)
3. Japanese MAFF

All methods can be found in STILLMEADOW, Inc. Standard Operating Procedures (SOPs).

4. Quality Assurance: The Protocol is reviewed by the Quality Assurance Unit (QAU). The study information will be entered into the Master Schedule. The study will be inspected at least once during its progress. Further inspections may be scheduled as needed to ensure the integrity of the study. Any deviations from SOPs, the Protocol, or Good Laboratory Practice Standards will be immediately reported to the Study Director and Management. The report will be audited and a statement prepared and signed which shall specify the dates inspections were made and findings reported to Management and to the Study Director.

5. Test Substance: Miller 6064. Test substance identification should include the name, batch number, and purity. Information regarding safety, stability, storage conditions, and disposal should also be provided by the Sponsor. The Sponsor assumes responsibility for purity and stability determinations.

6. Proposed Schedule: Testing will begin within approximately three weeks of receipt of the test substance and authorization to conduct the study.

Proposed Start Date: 13 Jun 01
Proposed End Date: 11 Jul 01

7. Study Director: Abigail Campbell, B.S.

A. GENERAL (cont.)

8. Experimental Summary: Test considerations will be determined by a preliminary range finding test which will give an approximate value for the 24 and 48 hr EC_{50} for the test substance. The test substance concentrations chosen will be administered to the test system, *Daphnia magna*, in reconstituted water. All definitive test concentrations will contain 2 replicates with 10 daphnids each. Two control containers will contain 10 test organisms in reconstituted water and no test substance. Dissolved oxygen, temperature, and pH will be measured and recorded in each treatment and the control at test initiation and termination. Observations for immobilization in each test chamber will be made daily. The test will be terminated after 48±1 hours of exposure.

The test will be considered valid if the control immobilization does not exceed 10 percent at 48 hours.

9. Protocol Amendments: Any alteration in the Protocol will be justified, approved by the Study Director, and recorded in writing.

10. Sponsor Audits: The Sponsor may send an authorized representative to inspect the test system and/or data on the STILLMEADOW, Inc. premises during normal working hours.

Appendix C: Protocol and Amendment

B. EXPERIMENTAL DESIGN

1. Organism

a.	Species:	*Daphnia magna*
b.	Justification of Species:	One of the daphnid species specified in the OPPTS regulations.
c.	Age:	Less than 24 hours old at test initiation.
d.	Number:	The range-finding test will use 5 daphnids for each concentration and the control. The definitive test will use 20 daphnids for each concentration and the control (2 replicates each containing 10 daphnids).
e.	Source:	*Daphnia magna* will be obtained from the STILLMEADOW, Inc. culture laboratory. Cultures which should not be used for testing include: cultures containing ephippia, if adults do not produce young before day 12, if more than 20 percent of the culture stock die during the two days preceding the test, or if adults in the culture do not produce an average of at least three young adult per day over a 7-day period prior to test.
f.	Acclimation:	Brood daphnids will be maintained in 100 percent dilution water at the test temperature for at least 48 hours prior to the start of the test.
g.	Identification:	Organisms will be labeled by study number, lot number, date, and number of organisms.

2. Animal Husbandry

a.	Test Medium:	Reconstituted water.
b.	Test Room:	Testing will be conducted in a light/temperature-controlled cabinet.
c.	Temperature:	Test temperature will be 20 ± 2°C.
d.	Photoperiod:	16-hours light and 8-hours dark.
e.	Test Chambers:	Test containers will be 250 mL beakers. Test containers will be loosely covered to reduce the loss of test solution or dilution water due to evaporation and to minimize the entry of dust or other particles into the solutions.
f.	Dissolved Oxygen Concentrations:	Test containers will not be aerated, but dissolved oxygen level for the dilution water at test initiation will 60-105% saturation.
g.	Food:	A variety of foods (e.g. unicellular green algae) is adequate for daphnid culture. However, the organisms will not be fed during the test.

3. Contaminants: No contaminants are expected during the study that are known to be capable of interfering with the purpose or conduct of the study.

Appendix C: Protocol and Amendment

B. EXPERIMENTAL DESIGN (cont.)

4. Pretest Preparation

a. Test Substance Receipt: Test substances will be supplied by the Sponsor in appropriately sized glass containers sealed and delivered to STILLMEADOW, Inc. Samples will be stored according to the Sponsor's instructions until prepared for testing.

b. Range-finding: A preliminary range-finding test will be conducted with several concentrations of the test substance to obtain an approximate value for the 24 and 48 hour EC_{50} for the test substance. Concentrations for the definitive test will be chosen from this data.

c. Test Substance Preparation: The test substance is insoluble in water and will be administered using an appropriate solvent (DMF, ethanol, methanol, etc.) as weight/volume concentrations. A solvent control will be included in the test design. The test substance dilutions will be prepared on the day of treatment.

d. Route of Administration: The test substance will be administered to the test system at test initiation by introduction to the test containers.

e. Reason for Route of Administration: Specified by the cited guidelines for evaluation of the toxicity potential of a test substance.

f. Preparation of Test System: The organisms will be randomized into cups containing holding water. Each cup will contain a maximum of five daphnids. Each test concentration will consist of two replicates of ten daphnids per replicate.

g. Control Group: Two replicates containing ten daphnids will not have test substance added and will be considered the control. This control will be used to demonstrate the condition of the test population.

5. Test Substance Administration

a. Dosing Concentrations: A preliminary range-finding test will be conducted with at least five concentrations of the test substance to obtain an approximate EC_{50} value for the test substance. The concentrations selected in a geometric series will be between a factor of 1.5 and 2.0.

Five test concentrations chosen from the range-finding data will be prepared on the day of test initiation.

b. Initial Measurements: Dissolved oxygen, temperature, and pH of the control and treated containers will be measured and recorded at test initiation.

Appendix C: Protocol and Amendment

B. EXPERIMENTAL DESIGN (cont.)

6. Observations:

a. Biological Monitoring: Containers will be inspected at 24 and 48 hours for immobility. In addition to immobility, any abnormal behavior or appearance will be recorded.

b. Chemical and Physical Monitoring: At a minimum, the following measurements will be made at test termination: dissolved oxygen, temperature, and pH of the control and treated containers.

7. Test Duration: The test will be terminated at the end of 48 ± 1 hours.

8. Evaluation of Results: The test will be considered valid if the control immobilization does not exceed 10 percent at 48 hours.

The 24 and 48 hour EC_{50} values and their respective 95 percent confidence limits will be determined using a linear regression model. Several models are available for EC_{50} determination: Probit, Trimmed Spearman-Karber, and Binomial. The most appropriate model will be selected for the determination if a dose response is exhibited in the study.

9. Test Substance Accountability: A comprehensive inventory of test substance received and used will be kept. The test substance container(s) will be weighed when received at this facility, and a record of all test substance use will be maintained. Test substance and test substance dosing solutions will be stored in the original containers, or in the equivalent thereof, or in glass containers with Teflon-lined caps.

10. Disposal of Unused Test Substance: Unused test substance will be disposed of at the Sponsor's expense after the termination of the study. STILLMEADOW, Inc. will retain a reserve sample.

11. Safety Precautions: General safety precautions required by laboratory SOPs will be followed. The Sponsor will supply basic toxicity data on the test substance to be used. However, since the toxicity of test substances is often not well characterized, this laboratory will be conservative in setting safety procedures. The Sponsor or Sponsor's Representative shall be notified of any exposures requiring a physician's examination or care.

C. DATA MANAGEMENT

1. Records:

The following records will be maintained during the study and transferred to the STILLMEADOW, Inc. archives upon study termination.

a. Protocol and Protocol Amendments (if any).
b. Final report and amendments (if any).
c. Study correspondence.
d. Test culture data.
e. Test substance receipt, identification as supplied by Sponsor, preparation, administration, and disposition.
f. Range-finding data and results.
g. Initial and terminal measurements for dissolved oxygen, temperature, and pH of the control and treated containers.
h. Daily counts for each container.
i. Other pertinent data.

2. Data Storage:

All raw data and a reserve sample of the test substance will be retained at STILLMEADOW, Inc. for a period of at least five years..

3. Data Reporting:

The final report will include all data as described in the Good Laboratory Practice Standards, including:

a. Statement from the Quality Assurance Unit.
b. Signature of the Study Director.
c. A GLP Compliance Statement signed by the Study Director.
d. Names of scientific personnel involved in the study.
e. Dates of study initiation and termination.
f. Identification, description, preparation, and storage of the test substance.
g. All pertinent culture information, preparation of test medium, test conditions, dosing information, and observation methods.
h. Initial and terminal data for dissolved oxygen, temperature, and pH of the control and treated containers.
i. Daily counts for each container and mean number of immobile organisms per treatment.
j. Determination of the validity of the test based on the control data.
k. The 24 and 48 hour EC_{50} values and their respective 95 percent confidence limits.
l. Evaluation of results.
m. A reference to this Protocol.

4. Report Submission:

A report will be submitted after termination of the laboratory portion of the study.

Appendix D: Certificate of Analysis

MILLER CHEMICAL & FERTILIZER CORPORATION
P.O. BOX 333, 120 RADIO ROAD
HANOVER, PENNSYLVANIA 17331 U.S.A.
TELEPHONE: 717-633-8821
FAX NO.: 717-632-4561

1/18/01

CERTIFICATE OF ANALYSIS

Product Name: MILLER 6064
Quantity Produced: 7.4 kilograms
Batch Number: 150386
Date of Formulation: 12/13/00

Appearance: Yellow to Amber Liquid
Odor: Terpenic
Moisture: Negligible (non-aqueous)
Specific Gravity: 0.92 - 0.94 @ 20c
Viscosity @ 20c: 100 to 500 cps
pH(1% solution): 6.0 to 8.0
Solubility in Water: Insoluble (Emulsifies)
Flash Point: over 200 f
Purity: 100%

Signature:

Name: Andrew G. Smith
Title: Compliance Officer