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January 22, 2004

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National Organic Standards Board
C/o Robert Pooler, Agricultural marketing Specialist
USDA/AMS/TM/NOP
Room 2510 –So.
Ag. Stop 0268
P.O. Box 96456
Washington, D.C. 20090-6456

SUBJECT: Petition to Allow Use of Sucrose Octanoate Esters As a Synthetic Substance in Organic Livestock Production

Dear Mr. Pooler:

I am pleased to submit the enclosed two copies of the subject petition and I look forward to responding to any questions you may have.

Very truly yours

AVA CHEMICAL VENTURES, L.L.C.

Anthony Barrington
Managing Member

CHEMICAL VENTURES, L.L.C.
65 Aviation Avenue
Portsmouth, NH 03801

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Room. 4008

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Washington, D.C. [redacted] 20250

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PETITION TO ALLOW USE OF SUCROSE OCTANOATE ESTERS AS A SYNTHETIC SUBSTANCE IN ORGANIC LIVESTOCK PRODUCTION

SUBMITTED

TO

NATIONAL ORGANIC STANDARDS BOARD

BY

AVA CHEMICAL VENTURES, L.L.C.

RECEIVED JAN 3 0 2004

JANUARY, 2004

1. COMMON NAME

The subject of this petition is the biochemical insecticide/miticide active ingredient, Sucrose Octanoate Esters. This substance belongs to the organic chemical family of "sucrose fatty acid esters."

2. MANUFACTURER'S NAME

Sucrose Octanoate Esters is manufactured under contract by the following company:

Applied Power Concepts, Inc.
411 East Julianna Street
Anaheim, CA 92801
ATTN: William A. Farone, Ph.D., President
714-502-2446, Extn. 110

3. INTENDED USE

Sucrose Octanoate Esters is a contact-type insecticide/miticide that is registered as a bio-pesticide for foliar spray on greenhouse, nursery and field crops; for *Sciarid* fly control in mushroom growing media; and for *Varroa* mite control on honey bees.

The *Varroa* mite control on honey bees use is the subject of this petition.

4. LIVESTOCK LIST & APPLICATION METHOD

Please see **Attachment 1** which includes the EPA-approved label for *Varroa* mite control on honey bees use. Other EPA documents relating to the pesticide registration of Sucrose Octanoate Esters are also included in **Attachment 1.**

5. SOURCE AND MANUFACTURING PROCESS

Sucrose Octanoate Esters is a synthesis of sugar and food-grade octanoic acid derived from tropical vegetable oils. The substance is designed to mimic the pest control properties of naturally-occurring sugar ester isolates of *Nicotiana gossei* Domin and other *Nicotiana* species that have been demonstrated to have insecticidal activity. In addition to the tobacco plant, sugar esters have been found in wild tomato and wild potato species and in the petunia plant. (Please see **Attachment 2** which contains copies of relevant scientific papers).

Naturally-occurring sugar ester bio-pesticides are present at low concentrations in their host plants – the highest-yielding plant *Nicotiana trigonophylla* has less than 3 grams per kilogram of plant material. The low concentration plus the cost of extraction means that naturally-occurring sugar esters are not an economically viable or environmentally sound source of this bio-pesticide active ingredient. It is estimated that the cost of sugar esters extracted from the tobacco plant would be several thousand dollars per kilogram of active ingredient; it would mean the cultivation of very large acreage to obtain commercially useful quantities; and there would be a significant waste stream.

As demonstrated in the scientific papers contained in **Attachment 2,** early trials with synthetic sugar esters showed them to be effective insecticides against certain pest types.

The Sucrose Octanoate Esters active ingredient (AI) that is the subject of this petition is manufactured from sugar and from food-grade C-8 fatty acids derived from tropical vegetable oils. Short-chain fatty acids, including C-8, are found in palm kernel oil and in coconut oil at concentrations of 3.0-4.5% and 5.8%, respectively.

A manufacturing process for Sucrose Octanoate Esters is described in U.S. Patent # 5,756,716, titled "Sugar-Ester Manufacturing Process." The patent describes processes for manufacturing sugar esters that have no liquid waste streams and minimal air emissions. Solvents used in the manufacturing process are recovered and re-used in subsequent batches. (Please see **Attachment 3** for a copy of the patent).

Steps in Manufacturing Process

Ethyl Octanoate Ester Production

1. SDA-3A ethanol, food-grade octanoic acid and 90.0% sulfuric acid are added to a reactor and mixed.

2. Heat is applied and the mixture is reacted until the free fatty acid is less than 0.5%

3. Potassium carbonate is added to neutralize the sulfuric acid. Potassium sulfate and carbon dioxide are produced.

4. The product is filtered to remove the potassium sulfate.

5. The product is returned to the reactor where the ethanol is removed by vacuum distillation until it is a small fraction of the ethyl octanoate ester. The recovered ethanol is dried prior to re-use in subsequent batches.

Sucrose Octanoate Esters Production

1. Dimethyl sulfoxide (DMSO) and the ethyl octanoate ester are mixed in the reactor.

2. Potassium carbonate is added to the reactor.

3. The temperature and vacuum are set to reflux the DMSO and remove any ethanol produced in the reaction.

4. After completion of the transesterification reaction to produce the sucrose octanoate esters, the mixture is subject to vacuum evaporation to recover most of the DMSO for re-use in later batches.

Sucrose Octanoate Esters Purification

1. The product is pumped from the reactor to a mixing tank, water is added and the mixture is stirred.

2. Butanol is added to the mixture and stirred.

3. The mixer is turned off and two layers are allowed to separate.

4. The bottom layer, consisting of water, un-reacted sucrose, DMSO and potassium sulfate, is decanted off.

5. Water is again added to the sucrose octanoate/butanol mixture and stirred. After two layers form, the water layer is again decanted.

6. A third water was/decant cycle is performed to meet the residual sucrose specification and the residual DMSO specification (max. of 4500ppm).

7. The sucrose octanoate/butanol mixture is returned to the reactor and butanol is removed by vacuum distillation until the residual butanol specification is met. The recovered butanol is used in subsequent batches.

8. The following analyses are performed on the finished Manufacturing Use Product (MUP) to confirm compliance with the specifications shown in the EPA-approved **Confidential Statement of Formula (CSF)** in **Attachment 4 – Confidential Business Information (CBI).**

   Potassium Octanoate, Potassium Sulfate and Potassium Carbonate
   LC for Sucrose Analysis
   GC for Ethyl Octanoate, DMSO, Butanol
   Moisture Analysis
   Ash Analysis

LC for Active Ingredient

The analytic methods used to perform the above are in **Attachment 4 - CBI.**

Discussion of Raw Materials and Manufactured Product

Raw Materials

The following raw materials are listed in order of their addition to the manufacturing process:

SDA-3A Anhydrous Ethanol (CAS # 64-17-5)

Octanoic Acid (CAS # 124-07-02)

Sulfuric Acid (CAS # 7664-93-9)

Potassium Carbonate (CAS # 584-08-7)

Dimethyl Sulfoxide (CAS # 61-71-0)

Sucrose (CAS # 57-50-1)

Water (CAS # 7732-18-5)

Butanol (CAS # 71-36-3)

All of the above, with the exception of dimethyl sulfoxide (DMSO), appear on the EPA **List 4- Inerts of Minimal Concern.**

Manufacturing Use Product (MUP)

Sucrose Octanoate Esters is manufactured to the specification contained in the CSF, a copy of which is contained in **Attachment 4-CBI.** The CSF lists the following components of the MUP:

Sucrose Octanoate Esters (CAS # 42922-74-7 (monooctanoate), CAS # 58064-47-4 (dioctanoate). This is the active ingredient (AI) and is the predominant component of the MUP. Per the CSF, the minimum AI content in the MUP is 80%. In practice, it is significantly higher.

Sucrose fatty acid esters, including sucrose octanoate esters, conforming to the specification in 21CFR§172.858 are approved by the Food and Drug Administration (FDA) as emulsifiers for direct addition to certain foods and as preservative coatings for certain fruits. The European Union, The World Health Organization, Japan and other countries have issued similar approvals.

Croda, Inc., an importer of food-grade sucrose fatty acid esters manufactured in Japan, maintains an FDA drug master file (DMF) for applications involving the use of sucrose fatty acid esters as excipients in pharmaceutical formulations.

Ethyl Caprylate (Cas # 106-32-1). This is an ester of ethanol and caprylic acid. Sulfuric acid is added as a catalyst and is neutralized with potassium carbonate.

Un-reacted ethanol is removed until it constitutes a small percentage of the ethyl aprylate ester.

The ethyl caprylate is substantially reacted with the sucrose to form the Sucrose Octanoate Esters. The CSF allows for the MUP to contain a small percentage of ethyl caprylate as a manufacturing impurity.

Sucrose. The CSF allows the MUP to contain a small percentage of sucrose as a manufacturing impurity.

Butanol. This solvent is introduced in the product purification phase of the manufacturing process. Most of it is distilled off for use in subsequent batches. THE CSF allows for 2-16% by weight of butanol to remain in the MUP, where it helps to control viscosity. Typically, batches of MUP contain 8-12% butanol.

Potassium Octanoate. (CAS # 764-71-6). Following completion of the ethyl octanoate ester reaction, a small amount of free fatty acid may remain. When the reaction is neutralized with potassium carbonate, the free fatty acid is converted to potassium octanoate and is substantially filtered out. The CSF allows the MUP to contain a small percentage of potassium octanoate as a manufacturing impurity.

Potassium octanoate is chemically similar to the active ingredient in the insecticidal soaps that are approved for pest control in organic agriculture, the only difference being the carbon chain-length of the fatty acid.

Dimethyl Sulfoxide (DMSO). This is a polar solvent which is used in the transesterification reaction because of its ability to make the reaction go to a high level of completion, thereby reducing manufacturing costs and waste streams. The only known alternative is Dimethylformamide (DMF).

The DMSO used in the Sucrose Octanoate Esters transesterification reaction is substantially removed by vacuum distillation for use in subsequent batches. The

portion remaining is substantially removed during the purification phase of the process. The CSF allows for residual DMSO to remain in the MUP as a manufacturing impurity at a maximum level of 4500ppm.

Potassium Sulfate/Potassium Carbonate (CAS # 7778-80-5; 584-08-7). Some potassium sulfate may remain in the ethyl caprylate after filtration and may remain in the sucrose octanoate after it has been purified. Some potassium carbonate added to the transesterification may be emulsified with the AI. The CSF allows a small percentage of potassium sulfate/potassium carbonate to remain in the MUP as a manufacturing impurity.

End-Use Product (EUP)

EUP is formulated by adding water to the MUP in sufficient quantity to achieve an AI concentration of 40%, per the EPA-approved labels.

6. PREVIOUS REVIEWS

Sucrose Octanoate Esters have not previously been reviewed for use in organic livestock production.

7. EPA, FDA AND STATE REGULATORY AUTHORITY REGISTRATIONS

EPA: Sucrose Octanoate Esters is registered by EPA as a biopesticide AI for insecticidal use as a foliar spray on greenhouse, ornamental and field crops; for *Sciarid* fly control in mushroom growing media; and for *Varroa* mite control on honey bees. The AI has been granted a waiver from the requirement for a tolerance for all food crops. (Copies of the EPA-approved labels and documents relating to the waiver of tolerance are contained in **Attachment 1**).

Of the starting raw materials, all but DMSO appear on the EPA **List 4 – Inerts of Minimal Concern.**

Of the manufacturing impurities, ethyl caprylate, sucrose , butanol, potassium sulfate and potassium carbonate appear on the **EPA List 4 –Inerts of Minimal Concern**. The manufacturing impurities not so listed are DMSO and Potassium Octanoate.

The MUP registration number is 70950-1 and the EUP number is 70950-2.

FDA: 21CFR §172.859 grants sucrose fatty acid esters conforming to the specification stated therein approval for use as emulsifiers in certain foods and as coatings for use on certain fruits.

21CFR§184.1025 lists caprylic (octanoic) acid as a substance added directly to human food affirmed as generally recognized as safe (GRAS).

The FDA document titled, "**Guidance for Industry, Q3C Impurities: Residual Solvents,"** (December, 1997) recommends acceptable amounts for residual solvents in pharmaceuticals for the safety of the patient. Solvents are divided into the following classes:

***Class 1 solvents*: Solvents to be avoided –**

Known human carcinogens, strongly suspected human carcinogens and environmental hazards.

***Class 2 solvents:* Solvents to be limited –**

Nongenotoxic animal carcinogens or possible causative agents of other irreversible toxicity such as neurotoxicity or teratogenicity.

***Class 3 solvents:* Solvents with low toxic potential --**

Solvents with low toxic potential to man; no health-based exposure limit is needed. Class 3 solvents have PDE's[1] of 50 milligrams(mg) or more per day. A daily dose of 10 grams of a product containing 50 mg of solvent corresponds to a solvent concentration in the product of 5000 ppm.

All three solvents used in the Sucrose Octanoate Esters manufacturing process – ethanol, butanol and DMSO – appear on the FDA list of Class 3 solvents. Methanol (the alternative to ethanol in the manufacture of Ethyl Caprylate) appears on the Class 2 list, as does Dimethylformamide (DMF), the alternative to DMSO in the manufacture of Sucrose Octanoate Esters).

Ethanol: The maximum concentration of ethanol in the ethyl caprylate is 1% (10,000ppm) and the maximum concentration of ethyl caprylate in the MUP is 1%, so that the maximum concentration of ethanol in the MP is 100ppm. That is reduced to a maximum of about 50ppm when the MUP is formulated into EUP and to a maximum of about 0.3ppm when the diluted EUP is applied to the bees.

Butanol: The maximum concentration of butanol in the MUP is 16% (160,000ppm). That is reduced to approximately 80,000ppm in the EP and to about 500ppm when the diluted EUP is applied to the bees.

[1] **Permitted daily exposure**

In practice, batches of Sucrose Octanoate Esters MUP typically have 8-10% residual butanol, so the concentration delivered to the bees will be 250 – 300 ppm.

DMSO: The maximum concentration of DMSO in the MP is 4500ppm. That is diluted to approximately 2250ppm when the MP is formulated into EUP and to about 14ppm when the diluted EP is applied to the bees.

Attachment 5 contains a copy of the FDA document cited above and its companion document, **"Q3C – Tables and List,** (December, 1997).

Bulletin # 106, "Dimethyl Sulfoxide (DMSO) Health Effects Information", Gaylord Chemical Corp., Rev. 11/09/98, documents an extensive range of studies of the human health and environmental effects of DMSO, including its natural occurrence in food:

> "Naturally-occurring DMSO has been identified in alfalfa, asparagus, barley, beans, beets, cabbage, corn, cucumbers, oats, onions, swiss chard, tomatoes, apples, raspberries, spearmint, beer, milk, coffee and tea."

A copy of Bulletin # 106 is contained in **Attachment 5.**

California Department of Pesticide Regulation: Conditional registration has been granted for the Manufacturing Use Product (70950-1-AA) and the Foliar Spray Sub-Label (70950-2-AA) and the *Varroa* Mite on Honey Bee Sub-label. The registrations are subject to the following condition:

> " AVA Chemical Ventures, L.L.C. shall submit five batch analysis data for commercially produced batches of the manufacturing use product, (EPA Reg. No. 70950-1), to the Department , not later than November 1, 2003."

An extension to the above-stated deadline has been requested, as manufacture of the necessary five commercial batches had not been completed by the November 1, 2003 deadline.

Other State Registrations: As of January, 2004 registration petitions for the use of Sucrose Octanoate Esters for *Varroa* mite control on honey bees had been filed in all 50 states.

8. CAS NUMBERS & LABELS

CAS Registry Number: 42922-74-7

CA Index Name: α-D-Glucopyranoside, β-D-fructofuranosyl, monooctanoate

CAS Registry Number: 58064-47-4

CA Index Name: α-D-Glucopyranoside, β-D-fructofuranosyl, dioctanoate

Copies of the labels approved by EPA and CDPR are contained in **Attachment 1.**

9. PHYSICAL PROPERTIES AND CHEMICAL MODE OF ACTION

Physical Properties: Sucrose Octanoate Esters is a non-ionic surfactant with the physical and chemical properties shown in **Attachment 4 –CBI.**

Chemical Mode of Action: Sucrose Octanoate Esters is a contact-type insecticide/miticide that is rapidly toxic to soft-bodied arthropods including mites, aphids, whitefly and psyllids. The specific mode of action is physical and is either suffocation or de-waxing of the insect's cuticle which causes it to dessicate. (Please see **Attachment 2** for additional information.)

(a). Chemical Interactions with Other Substances: Sucrose Octanoate Esters is a non-ionic surfactant. Field trials have demonstrated that it has synergistic pest control effects when used in conjunction with other active ingredients and adjuvents. No negative chemical interactions have been observed.

(b). Toxicity and Environmental Persistence: Sucrose fatty acid esters do not persist in the environment and biodegrade within approximately five days at approximately 20-27°C, in both aerobic and anaerobic conditions.[2] Sucrose fatty acid esters are not soluble in water.

Based on the information submitted in connection with the registration, including the document cited in the prior paragraph, EPA concluded that Sucrose Octanoate Esters has extremely low toxicity and is not likely to cause toxic effects to birds, fish, and aquatic invertebrates when the product is used according to the label directions. Waivers were granted from studies for Avian Acute Oral Toxicity, Avian Dietary, Freshwater Fish and Freshwater Invertebrate.

(c). Environmental Impacts from Use and Manufacture: As noted under (b) above, sucrose esters biodegrade rapidly following application.

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[2] **Wyman, Cooper H., Ph.D., *"Biodegradation of Synthetic Detergents"*, Progress in Industrial Microbiology, 1971. pp.219-271. (Copy in Attachment 6).**

The Sucrose Octanoate Esters MUP is manufactured by a patented process from which air emissions are minimal and from which all solid and liquid output streams are recycled, re-used or sold as products or by-products. (Please see **Attachment 3** for a copy of the patent).

(d). Effects on Human Health: Sucrose fatty acid esters were approved as emulsifiers for direct addition to certain foods and post harvest protective coatings by the FDA in 1983. (**21 CFR § 172.858).** The food additive use was expanded in 1995 to include use as, " emulsifiers, stabilizers, and texturizers in chewing gum, confections, and frostings: texturizers in surimi-based fabricated seafood products; and emulsifiers in coffee and tea beverages with added dairy ingredients and/or dairy production analogs." **(Federal Register, August 29, 1995, 60 FR 44755).** The European Union, Japan and the World Health Organization (WHO) have also approved sucrose fatty acid esters for direct addition to food.

Acute oral and dietary toxicity of sucrose fatty acid esters was evaluated by the Food and Agricultural Organization (FAO) of the WHO in the following publications: (I) ***Toxicological Evaluation Certain Food Additives*** [20th Report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series No. 599, FAO Food and Nutrition Series No. 1 (1976); (ii) **Same title as above** [24th Report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series No. 653 (1980); and (iii) ***Toxicological Evaluation of some Food Colours, Emulsifiers, Stabilizers, AntiCaking Agents, and Certain Other Substances*** (Undated and unnumbered FAO/WHO report).

The data contained in the reports cited above demonstrate that sucrose fatty acid esters had extremely low oral toxicity in laboratory studies, even at concentrations substantially higher than are found in human food. Extremely high concentrations of sucrose fatty acid esters were needed to produce toxic symptoms in laboratory studies.

Long- and short-term dietary studies (100 days to 2.5 years) evaluated in the aforementioned FAO/WHO reports demonstrated that dietary consumption of individual or mixed sucrose fatty acid esters at levels up to 3% in the diet caused no substantial toxicological effects in rats, mice or dogs. Some mortalities were observed when 5% sucrose fatty acid esters were present in the diet, and 100% mortalities were observed when the level of sucrose fatty acid esters was ≥10% in the diet. The LD_{50} values were > 20,000 mg/kg. of body weight. The level at which no toxicological effects are observed in the diet is 1%, a daily diet equivalent of 500mg/kg of body weight.

An acceptable daily intake (ADI) of sucrose fatty acid esters for humans was estimated to be up to 16mg/kg of body weight. [**Joint FAO/WHO Expert Committee on Food Additives** ((1992)].

In studies with rats and humans, it was demonstrated that sucrose fatty acid esters were rapidly hydrolyzed and absorbed by the body [***Toxicological Evaluation Certain Food Additives***, 20th Report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series No. 653 (1980).

Two Primary Dermal Irritation Studies conforming to EPA guidelines were performed with Sucrose Octanoate Esters, one with the MUP and the other with the EUP. Both showed a low incidence of irritation. All symptoms cleared by 24 hours in both studies. Both the MUP and the EUP are classified in Toxicity Category IV with respect to skin irritation.

Two Primary Eye Irritation Studies conforming to EPA guidelines were performed, one with the MUP and the second with the EUP.

Following ocular instillation of 0.1 mL of undiluted MUP (approximately 85% active ingredient) into the eyes of rabbits, moderate to severe eye irritation and mild corneal opacity was observed in the treated eyes of all rabbits at 24 hours and persisted in one rabbit to 21 days post dosing. Mild iritis was exhibited in three rabbits at 24 hours and persisted in one rabbit to 72 hours. The MUP is classified in Toxicity Category I for eye irritation.

Following ocular instillation of 0.1mL of undiluted EUP (40% active ingredient) into the eyes of rabbits, moderate to severe eye irritation was observed in the treated eyes of all six rabbits at 72 hours post dosing, was mild at 7 days and cleared by 14 days. Mild corneal opacity was observed in all six rabbits at 24 hours and persisted to 7 days in one rabbit then cleared by 14 days post dosing. Mild iritis persisted in four rabbits to 72 hours, then cleared. The EUP is classified in Toxicity Category II for eye irritation.

(e). Effects on Soil Organisms, Crops or Livestock. Sucrose Octanoate Esters has been tested on a range of crops, including almond, apple, pear, citrus, cotton, grape, peach, lettuce, tomato, mint, cabbage, melon and several ornamental crops, including rose and poinsettia. No phytotoxicity has been reported on any crop.

Sucrose Octanoate Esters has been tested for its effect on honey bees (*Apis mellifera* L, order Hymenoptera) and the following beneficial insects, all of which are important predators of homopteran pests:

Lady Beetles	(*Harmonia sp.*),
"	(*Curinus coeruleus* Mulsant)
"	(*Cycloneda sanguinea* L.)
"	(*Harmonia axyridis* Pallas)
"	(*Olla v-nigrum* Mulsant)

Green Lacewing	(*Chrysopidae rufilabris*)
Red Scale Parasoid	(*Aphytis melinus* De Bach)
Insidious Flower Bug	(*Orius insidiosus* (Say)

A Honey Bees Acute Contact Toxicity Study was conducted under an EPA protocol. Five geometrically spaced dosages of Sucrose Octanoate Esters EUP were administered to groups of 30 adult worker honey bees. At initiation of the study each bee received a single dose of the chemical. The bees were observed after 4, 24 and 48 hours for toxicological response and the number of bees that were alive and the number that were dead recorded at 24 and 48 hours. An LD_{50} with 95% confidence limits could not be calculated because there was little or no mortality at any of the rates used.

The study concluded: "AVACHEM Sucrose Octanoate 40% is not toxic to honey bees." (A copy of the study in included in **Attachment 7).**

Protocol for Sucrose Octanoate Ester Effects on the Target Insect Pest, Pear Psylla, and the Beneficial Insect, Lady Beetle (G. Puterka, USDA-ARS*, 1996)* included a field study that evaluated the effects of Sucrose Octanoate Esters on lady beetle (*Harmonia sp.*). Sucrose Octanoate Esters, M-Pede (insecticidal soap) and Agri-Mek (a conventional insecticide) were applied to five year-old pear trees infested with pear psylla and having a population of lady beetles. At two days post treatment, no dead lady beetles were found below the trees treated with Sucrose Octanoate Esters, M-Pede or the untreated controls, whereas below trees treated with Agri-Mek a mean of 3.6 dead lady beetles was observed.

Safety of a Novel Insecticide, Sucrose Octanoate, to Beneficial Insects, J.P. Michaud and C.L. Mckenzie, University of Florida Citrus Research and Education Center (Undated), examined the toxicity of Sucrose Octanoate Esters to beneficial insects representing four insect orders of importance in biological control in citrus. First instar larvae of the lady beetles *Cycloneda sanguina L., Curinus coeruleus* Mulsant, *Harmonia axyridis* Pallas and *Olla v-nigrum* Mulsant and the lacewing *Chrysoperla rufilabris* Burmeister (Neuroptera: Chrysopidea) all survived topical sprays of Sucrose Octanoate Esters at 8,000ppm without significant mortality, a concentration corresponding to twice the recommended rate to kill target pests. Similarly, adults of the red scale parasitoid, *Aphytis melinus* De Bach (Hymenoptera: Aphelinidae) and 2nd instar nymphs of the predatory bug *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) both survived 24 hour exposures to 8,000ppm residues of Sucrose Octanoate Esters without significant mortality.

10. Safety Information

An MSDS for Sucrose Octanoate Esters is included in **Attachment 8.**

A search of the National Institute of Environmental Health Studies database did not produce any reports on Sucrose Octanoate Esters, or other sucrose fatty acid esters.

11. Research Information

Other than the documents cited herein, petitioner is not aware of comprehensive substance research reviews and research bibliographies. Petitioner is not aware of any reviews or bibliographies which present contrasting positions to those presented herein.

12. Petition Justification Statement

This is a petition for the inclusion of a synthetic substance, Sucrose Octanoate Esters, on the National List. As a synthesis of sucrose and food-grade fatty acids derived from tropical vegetable oils manufactured by a "zero discharge" process, Sucrose Octanoate Esters is a commercially-viable version of naturally-occurring sugar esters found in several plant species. It is manufactured by a process that has significantly less environmental impact than a process to cultivate plants containing sucrose esters and to extract them for use as natural pesticides.

With the exception of one solvent (DMSO) used in the manufacturing process, all of the raw materials in the MUP and the EUP appear on the **EPA List 4 – Inerts of Minimal Concern**. In the dilute form applied to bees, the maximum concentration of DMSO is approximately 14ppm, which is similar to the concentration found in a range of fruits and vegetables.

With the exception of potassium octanoate, the impurities formed during the manufacturing process appear on List 4 also. Potassium octanoate is a "potassium salt of fatty acids" (also known as "insecticidal soaps") which are registered as pesticide active ingredients and are approved for used in organic agriculture.

Sucrose Octanoate Esters is one of the sucrose fatty acid esters that are FDA-approved for direct addition to foods and as fruit coatings.

As a biochemical insecticide/miticide, Sucrose Octanoate Esters is selective as to the insect species it kills and it does not harm major classes of beneficial insects. It is therefore suitable for use in a crop management system that includes cultural practices and integrated pest management (IPM) techniques.

Sucrose Octanoate Esters is not toxic to honey bees, but it does kill *Varroa* mites, a widespread predator of honey bees that causes significant bee mortality and reduced productivity among bee colonies. It would augment the very limited number of registered treatments for *Varroa* currently available to beekeepers, none of which are approved for use in organic production. One of the registered substances available for *Varroa* mite control is an organo-phosphate that in 2003 was available to beekeepers under Section 18 emergency registrations in approximately 38 states.

In summary: Sucrose Octanoate Esters is a bio-pesticide manufactured from natural ingredients by a "zero discharge" process that kills insects selectively by a non-toxic mode of action and biodegrades rapidly following use. It provides beekeepers with a safe, environmentally friendly alternative means of controlling *Varroa* mites, a major pest.

13. Commercial Confidential Information Statement:

Confidential information has been assembled in **Attachment 4 –CBI.** The documents included are the EPA-approved Physical and Chemical Properties, the CSF for Sucrose Octanoate Esters MUP, including Certified Limits for each Component, and the analytic methods used to measure compliance with the Certified Limits. This information is commercially valuable; is used in the applicant's business to manufacture Sucrose Octanoate Esters; and is maintained in secrecy by the applicant and its manufacturing contractor.

HONEY BEE TREATMENT

AVACHEM SUCROSE OCTANOATE [40.0%]

Biochemical Miticide For *Varroa* Mite Control on Honey Bees

ACTIVE INGREDIENT

Sucrose Octanoate Esters (α-D-Glucopyranosyl - ß-D-fructofuranosyl - octanoate), mono-, di-, and triesters of sucrose octanoate 40.0%

OTHER INGREDIENT 60.0%

TOTAL 100.0%

EPA Reg. No. 70950-2-2205 EPA Est. No. 075197-CA-001

U.S. Patent #'s 5,756,716; 6,419,941

STOP - READ THE LABEL BEFORE USE

KEEP OUT OF REACH OF CHILDREN

WARNING - AVISO

Si usted no entiende la etiqueta, busque a alguien para que se la explique a usted en detalle.
(If you do not understand the label, find someone to explain it to you in detail.)

FIRST AID		
If in eyes:	•	Hold eye open and rinse slowly and gently with water for 15-20 minutes.
	•	Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye.
	•	Call a poison control center or doctor for treatment advice.
If swallowed:	•	Call poison control center or doctor immediately for treatment advice.
	•	Have person sip a glass of water if able to swallow.
	•	Do not induce vomiting unless told to do so by the poison control center or doctor.
	•	Do not give anything by mouth to an unconscious person.
HOT LINE NUMBER: 888-229-7414		
Have the product container or label with you when calling a poison control center or doctor, or going for treatment. You may also contact AVA Chemical Ventures, L.L.C. at 603-766-0288 for emergency medical treatment information.		

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Net Contents: 5 Gallons

PRECAUTIONARY STATEMENTS

Hazard to Humans and Domestic Animals:

WARNING: Causes substantial but temporary eye injury. Do not get in eyes or on clothing. Wear protective eyewear (goggles or face shield). Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash clothing before reuse.

Environmental Hazards:

Do not apply directly to water, or to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when cleaning equipment or disposing of equipment wash waters.

DIRECTIONS FOR USE

It is a violation of Federal Law to use this product in a manner inconsistent with its labeling. Do not apply this product in a way that will contact workers or other persons, either directly or through drift. Only protected handlers may be in the area during application. For any requirements specific to your State or Tribe, consult the agency responsible for pesticide regulation.

Do not allow spray to drift from the application site and contact people, structures people occupy at any time and the associated property, parks and recreational areas, non-target crops, aquatic and wetland areas, woodlands, pastures, rangelands or animals. Apply only when wind speed is not more than 10 mph. For sprays, apply largest size droplets possible.

GENERAL INFORMATION

AVACHEM SUCROSE OCTANOATE is a biochemical miticide for use against *Varroa* mites (*Varroa destructor*) on adult honey bees. It is primarily a contact miticide with limited residual activity.

- Do not apply this product through any type of feeding/watering system.
- Apply as soon as infestation is detected.
- Use in sufficient water to achieve adequate coverage of the adult honey bee population.
- Repeat applications at intervals of 7 - 10 days, up to three times per infestation, to control mites emerging from brood cells.
- Thorough spray coverage of adult honey bees on frames is essential for good control of the pest. Remove frames with adhering bees and spray both sides.

MIXING AND APPLICATION

Shake or stir before use.

AVACHEM SUCROSE OCTANOATE dissolves readily in water. To achieve and to maintain the suspension, add the appropriate quantity to water with agitation and maintain gentle agitation during application.

Rate: Use a 0.625% v/v solution of this product.

Apply 1.5 fl. Oz. (45 ml) of total mix volume on each full depth frame of bees. [Typical 18 frame two story colony will receive 27 fl. Oz. (810 ml) of total mix volume.]

Rate Table

Total Mix Volume	% v/v Solution	Amount English (Metric)
2 gal. (7.57 liters)	0.625%	3 TBS (50 ml.)
10 gal. (37.85 liters)	0.625%	1 Cup (235 ml.)

Timing of Application

Initiate applications as soon as *Varroa* mites are observed. Repeat applications at intervals of 7 - 10 days, up to three times per infestation, to control mites emerging from brood cells.

Test Application

This product has been tested on European-derived honey bees managed in standard Langstroth style

beehives in temperate areas of North America. However, it is not possible to evaluate all management systems and climatic conditions. Test AVACHEM Sucrose Octanoate for possible detrimental effects on honey bees by treating a few beehives at the label use rate prior to large scale use.

Application

- This product may be applied with a garden type hand held or backpack sprayer. Do not apply this product through any type of feeding/ watering system.
- This product must come into contact with the *Varroa* mites to be effective. Complete wetting of the adult honey bee population on frames is essential for maximum control.
- Do not apply when honey bees are in winter cluster or at temperatures below 55°F to avoid chilling the bee population.

STORAGE AND DISPOSAL

Do not contaminate water, food, or feed by storage or disposal.

Pesticide Storage: Store in a cool, dry location.

Pesticide Disposal: Wastes resulting from the use of this product may be disposed of on site or at an approved waste disposal facility.

Container Disposal: Triple rinse (or equivalent). Then offer for recycling or reconditioning, or puncture and dispose of in a sanitary landfill, or by incineration, or, if allowed by state and local authorities, by burning. If burned, stay out of smoke.

WARRANTY STATEMENT, DISCLAIMER

Dadant & Sons, Inc. (Dadant) seeks to present reliable information concerning the composition, properties and use of the product, however: (1) All advice concerning selection and use of this product is provided at no charge and with no warranty. (2) No warranty is made hereby. The product described herein is warranted to conform to Dadant specifications, therefore, only at the time of sale. THIS WARRANTY IS EXCLUSIVE AND IN LIEU OF ANY AND ALL OTHER WARRANTIES, EXPRESS OR IMPLIED, ARISING BY LAW OR CUSTOM, INCLUDING BUT NOT BY WAY OF LIMITATION, THE IMPLIED WARRANTY OF MERCHANTABILITY AND THE IMPLIED WARRANTY OF FITNESS FOR A PARTICULAR PURPOSE. Remedy for any breach of warranty is limited to replacement of the defective product. (3) Dadant assumes no responsibility for any patent liability arising from the use of the product in a process, manner or formula not designed by Dadant. Nothing in the listed information shall be construed as an inducement or recommendation to use any process or to produce or use the product in conflict with existing or future patents.

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

SEP 25 2002

Anthony Barrington
AVA Chemical Ventures, L.L.C.
80 Rochester Avenue, Suite 214
Portsmouth, NH 03801

Subject: Pesticide Petition Approval (No. 8E4926)
Approval of an Exemption from the Requirement of a Tolerance
For Sucrose Octanoate Esters

Dear Mr. Barrington:

The exemption from the requirement of a tolerance for residues of the biochemical insecticide/miticide sucrose octanoate esters, in or on all food commodities, has been established under section 408(d) of the Food, Drug and Cosmetic Act as amended by the Food Quality Protection Act (FQPA) of August 3, 1996. The scientific data show the tolerance exemption is in the public interest and will protect the public health.

The approval is listed under 40 CFR section 180.1222 Sucrose octanoate esters; exemption from the requirement of a tolerance. The tolerance exemption has been established for residues of the biochemical insecticide/miticide, sucrose octanoate esters, in or on all food commodities. Please find enclosed a copy of the final rule (for your records), which will become effective upon its pending publication in the *Federal Register*. You will be alerted to the publication date and citation as soon as they become available.

Should you have any questions or concerns regarding this letter, please contact me, or telephone Denise Greenway on (703) 308-8263.

Sincerely,

Janet L. Andersen, Ph.D.
Director
Biopesticides and Pollution
Prevention Division (7511C)

Enclosure

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division (7511C)
1200 Pennsylvania Avenue NW
Washington, DC 20460

EPA Reg. Number:	Date of Issuance:
70950-1	9-16-02

Term of Issuance: Unconditional

NOTICE OF PESTICIDE:

X Registration

___ Reregistration

(under FIFRA, as amended)

Name of Pesticide Product:

Avachem Sucrose Octanoate
Manufacturing Use Product

Name and Address of Registrant (include ZIP Code):

AVA Chemical Ventures, L.L.C.
80 Rochester Avenue, Suite 214
Portsmouth, NH 03801

Note: Changes in labeling differing in substance from that accepted in connection with this registration must be submitted to and accepted by the Biopesticides and Pollution Prevention Division prior to use of the label in commerce. In any correspondence on this product always refer to the above EPA registration number.

On the basis of information furnished by the registrant, the above named pesticide is hereby registered/reregistered under the Federal Insecticide, Fungicide and Rodenticide Act.

Registration is in no way to be construed as an endorsement or recommendation of this product by the Agency. In order to protect health and the environment, the Administrator, on his motion, may at any time suspend or cancel the registration of a pesticide in accordance with the Act. The acceptance of any name in connection with the registration of a product under this Act is not to be construed as giving the registrant a right to exclusive use of the name or to its use if it has been covered by others.

This registration does not eliminate the need for continual reassessment of the pesticide. If EPA determines at any time, that additional data are required to maintain in effect an existing registration, the Agency will require submission of such data under section 3(c)(2)(B) of FIFRA.

This product is registered in accordance with FIFRA section 3(c)(5) and is subject to the following terms and conditions:

1. Make the following modification to your label before your release your product for shipment:

   a. Revise the EPA Reg. No. to "70950-1."

Signature of Approving Official:	Date:
(See second page for signature)	9-16-02

EPA Form 8570-6

2. Submit two (2) copies of the revised final printed labeling before you release the product for shipment. Refer to the A-79 enclosure for a further description of final printed labeling.

A stamped copy of the label is enclosed for your records.

Sincerely,

Janet L. Andersen, Ph.D., Director
Biopesticides and Pollution
Prevention Division (7511C)

Enclosure

AVACHEM SUCROSE OCTANOATE MANUFACTURING USE PRODUCT

BIOCHEMICAL INSECTICIDE/MITICIDE FOR FORMULATING USE ONLY

Active Ingredient:

Sucrose Octanoate Esters (α-D-Glucopyranosyl, ß-D-fructofuranosyl - octanoate), mono, di-, and triesters of sucrose octanoate 85.43%

Other Ingredients 14.57%

TOTAL 100.00%

EPA Reg. No. 70950-R
U.S. Patent # 5,756,716

EPA Est. No._____

KEEP OUT OF REACH OF CHILDREN

DANGER

FIRST AID		
If in eyes:	•	Hold eye open and rinse slowly and gently with water for 15-20 minutes.
	•	Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye.
	•	Call a poison control center or doctor for treatment advice.
If swallowed:	•	Call poison control center or doctor immediately for treatment advice.
	•	Have person sip a glass of water if able to swallow.
	•	Do not induce vomiting unless told to do so by the poison control center or doctor.
	•	Do not give anything by mouth to an unconscious person.
HOT LINE NUMBER: 888-229-7414		
Have the product container or label with you when calling a poison control center or doctor, or going for treatment. You may also contact AVA Chemical Ventures, L.L.C. at 603-431-4242 for emergency medical treatment information.		
NOTE TO PHYSICIAN: Probable mucosal damage may contraindicate the use of gastric lavage.		

ACCEPTED
SEP 16 2002
Under the Federal Insecticide, Fungicide, and Rodenticide Act as amended, for the pesticide registered under EPA Reg. No. 70950-1

Manufactured for:
AVA Chemical Ventures, L.L.C.
80 Rochester Avenue, Suite 214
Portsmouth, NH 03801

Net Contents: 1140 lbs.

Revised 09/16/02 (MP-1)

Type Size Requirements for 3 Front Panel Headings

Size of front panel square inches	Minimum type size for "RESTRICTED USE PESTICIDE" (if required) and Signal Word in capital letters	Minimum type for "Storage and Disposal" heading & "Keep Out of Reach of Children" warning
5 and under	6 point	6 point
above 5 to 10	10 point	6 point
above 10 to 15	12 point	8 point
above 15 to 30	14 point	10 point
over 30	18 point	12 point

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division (7511C)
1200 Pennsylvania Avenue NW
Washington, DC 20460

EPA Reg. Number:	Date of Issuance:
70950-2	9-16-02

Term of Issuance: Unconditional

Name of Pesticide Product:
Avachem Sucrose Octanoate [40.0%]

NOTICE OF PESTICIDE:

X Registration
___ Reregistration
(under FIFRA, as amended)

Name and Address of Registrant (include ZIP Code):

AVA Chemical Ventures, L.L.C.
80 Rochester Avenue, Suite 214
Portsmouth, NH 03801

Note: Changes in labeling differing in substance from that accepted in connection with this registration must be submitted to and accepted by the Biopesticides and Pollution Prevention Division prior to use of the label in commerce. In any correspondence on this product always refer to the above EPA registration number.

On the basis of information furnished by the registrant, the above named pesticide is hereby registered/reregistered under the Federal Insecticide, Fungicide and Rodenticide Act.

Registration is in no way to be construed as an endorsement or recommendation of this product by the Agency. In order to protect health and the environment, the Administrator, on his motion, may at any time suspend or cancel the registration of a pesticide in accordance with the Act. The acceptance of any name in connection with the registration of a product under this Act is not to be construed as giving the registrant a right to exclusive use of the name or to its use if it has been covered by others.

This registration does not eliminate the need for continual reassessment of the pesticide. If EPA determines at any time, that additional data are required to maintain in effect an existing registration, the Agency will require submission of such data under section 3(c)(2)(B) of FIFRA.

This product is registered in accordance with FIFRA section 3(c)(5) and is subject to the following terms and conditions:

1. Make the following modification to your label before your release your product for shipment:

   a. Revise the EPA Reg. No. to "70950-2."

Signature of Approving Official:	Date:
(See second page for signature)	9-16-02

EPA Form 8570-6

PRECAUTIONARY STATEMENTS

HAZARD TO HUMANS AND DOMESTIC ANIMALS

DANGER: CORROSIVE. Causes irreversible eye damage. Do not get in eyes or on clothing. Wear protective eyewear (goggles or face shield). Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash clothing before reuse.

ENVIRONMENTAL HAZARDS

Do not discharge effluent containing this product into lakes, streams, ponds, estuaries, oceans or other waters unless in accordance with the requirements of a National Pollutant Discharge Elimination System (NPDES) permit and the permitting authority has been notified in writing prior to discharge. Do not discharge effluent containing this product to sewer systems without previously notifying the local sewage treatment plant authority. For guidance contact your State Water Board or Regional Office of the EPA.

DIRECTIONS FOR USE

It is a violation of Federal Law to use this product in a manner inconsistent with its labeling.

Only for formulation into end-use insecticide/ miticide products for use on: All food commodities, ornamental landscape trees and shrubs, Christmas trees, roses, flowers and bedding plants; for sciarid fly control in mushroom growing media; and for *Varroa* mite control on adult honey bees.

Each formulator is responsible for obtaining EPA registration for his end use products.

STORAGE AND DISPOSAL

DO NOT CONTAMINATE WATER, FOOD OR FEED BY STORAGE OR DISPOSAL.

PESTICIDE STORAGE: Store in a cool, dry location.

PESTICIDE DISPOSAL: Pesticide wastes are acutely hazardous. Improper disposal of excess pesticide, spray mixture, or rinsate is a violation of Federal Law. If these wastes cannot be disposed of by use according to label instructions, contact your State Pesticide or Environmental Control Agency or the Hazardous Waste Representative at the nearest EPA Regional Office for guidance.

CONTAINER DISPOSAL: Triple rinse (or equivalent). Then offer for recycling or reconditioning, or puncture and dispose of in a sanitary landfill, or by incineration, or, if allowed by state and local authorities, by burning. If burned, stay out of smoke.

WARRANTY STATEMENT, DISCLAIMER

AVA Chemical Ventures, L.L.C. (AVA Chemical) seeks to present reliable information concerning the composition, properties and use of the product, however: (1) All advice concerning selection and use of this product is provided at no charge and with no warranty. (2) No warranty is made hereby. The product described herein is warranted to conform to AVA Chemical's specifications, therefore, only at the time of sale. THIS WARRANTY IS EXCLUSIVE AND IN LIEU OF ANY AND ALL OTHER WARRANTIES, EXPRESS OR IMPLIED, ARISING BY LAW OR CUSTOM, INCLUDING BUT NOT BY WAY OF LIMITATION, THE IMPLIED WARRANTY OF MERCHANTABILITY AND THE IMPLIED WARRANTY OF FITNESS FOR A PARTICULAR PURPOSE. All sales are subject to AVA Chemical's standard terms and conditions, which are reproduced on the reverse side of each invoice. Remedy for any breach of warranty is limited to replacement of the defective product. (3) AVA Chemical assumes no responsibility for any patent liability arising from the use of the product in a process, manner or formula not designed by AVA Chemical. Nothing in the listed information shall be construed as an inducement or recommendation to use any process or to produce or use the product in conflict with existing or future patents.

Revised 5/82

A-79 ENCLOSURE

Final printed labeling is defined as that labeling which will accompany the pesticide product to market, and includes not only the container label, but also all accompanying technical information, brochures, etc.

Final printed labeling for the Agency's files should be of a size that can be stored conveniently in 8 1/2 x 11 inch files. Labels may be mounted or photoreduced to meet the size requirements provided the printing is legible and is of microfilm reproduction quality. Should photo reduction make any of the text illegible, the text must be typed out on an accompanying sheet of paper.

PASTE-ON LABELING: This should be submitted as is, unless it requires photo reduction.

SCREEN PRINTED LABELING: These labels should be printed by taping paper on the container as it goes through the printing process. The actual container should not be submitted.

EMBOSSED LABELING: These labels should be photocopied.

UNUSUAL SIZE LABELING: Large bags or boxes must be photoreduced, either the entire label on one reduction or in sections so that each section is 8 1/2 x 11 inches.

2. Submit two (2) copies of the revised final printed labeling before you release the product for shipment. Refer to the A-79 enclosure for a further description of final printed labeling.

A stamped copy of the label is enclosed for your records.

Sincerely,

Janet L. Andersen, Ph.D., Director
Biopesticides and Pollution
Prevention Division (7511C)

Enclosure

MASTER LABEL

AVACHEM SUCROSE OCTANOATE [40.0%]

BIOCHEMICAL INSECTICIDE/MITICIDE

ACTIVE INGREDIENT

Sucrose Octanoate Esters (α-D-Glucopyranosyl - ß-D-fructofuranosyl - octanoate), mono-, di-, and triesters of sucrose octanoate 40.0%

OTHER INGREDIENT 60.0%

TOTAL 100.0%

EPA Reg. No. 70950-E

EPA Est. No._____

U.S. Patent #'s 5,756,716; 6,419,941

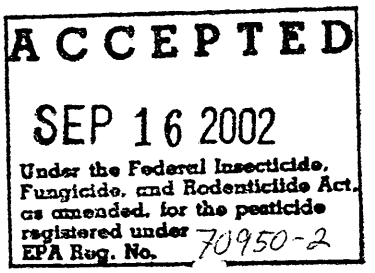
KEEP OUT OF REACH OF CHILDREN

WARNING

Manufactured for:

AVA Chemical Ventures, L.L.C.
80 Rochester Avenue, Suite 214
Portsmouth, NH 03801

Net Contents: 1 Gallon, 5 Gallons



Revised 09/16/02

Type Size Requirements for 3 Front Panel Headings

Size of front panel square inches	Minimum type size for "RESTRICTED USE PESTICIDE" (if required) and Signal Word in capital letters	Minimum type for "Storage and Disposal" heading & "Keep Out of Reach of Children" warning
5 and under	6 point	6 point
above 5 to 10	10 point	6 point
above 10 to 15	12 point	8 point
above 15 to 30	14 point	10 point
over 30	18 point	12 point

I. General Information

A. Does this Action Apply to Me?

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

Categories	NAICS Codes	Examples of potentially affected entities
Industry	111	Crop production
	112	Animal production
	311	Food manufacturing
	32532	Pesticide manufacturing

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 the table could also be affected. The North American Industrial Classification System (NAICS) codes have been provided to assist you and others in determining whether or not this action might apply to certain entities. If you have 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 Additional Information, Including Copies of this Document and Other Related Documents?

1. Electronically. You may obtain electronic copies of this document, and certain other related documents that might be available electronically, from the EPA Internet home page at <A HREF="
http://www.epa.gov/">http://www.epa.gov/</A>.
To access this document, on the home page select ``Laws and Regulations,'' ``Regulations and Proposed Rules,'' and then look up the entry for this document under the ``Federal Register--Environmental Documents.'' You can also go directly to the Federal Register listings at <A HREF="http://www.epa.gov/fedrgstr/">http://www.epa.gov/fedrgstr/</A>. A frequently updated electronic
version of 40 CFR part 180 is available at <A HREF="
http://www.access.gpo.gov/nara/cfr/cfrhtml_00/Title_40/40cfr180_00.html
">http://www.access.gpo.gov/
nara/cfr/cfrhtml_00/Title_40/40cfr180_00.html</A>, a beta site currently under development. To access the OPPTS Harmonized Guidelines referenced in this document, go directly to the guidelines at <A HREF="
http://www.epa.gov/opptsfrs/home/guidelin.htm">http://
www.epa.gov/opptsfrs/home/guidelin.htm</A>.

2. In person. The Agency has established an official record for this action under docket ID number OPP-2002-0016. The official record consists of the documents specifically referenced in this action, and other information related to this action, including any information claimed as Confidential Business Information (CBI). This official

Brian Steinwand
09/25/02 12:36 PM

To: OPP BPPD
cc:
Subject: Sucrose Octanoate Esters; Exemption from the Requirement

[Federal Register: September 25, 2002 (Volume 67, Number 186)]
[Rules and Regulations]
[Page 60146-60152]
>From the Federal Register Online via GPO Access [wais.access.gpo.gov]
[DOCID:fr25se02-9]

ENVIRONMENTAL PROTECTION AGENCY
40 CFR Part 180
[OPP-2002-0016; FRL-7199-1

Sucrose Octanoate Esters; Exemption from the Requirement of a Tolerance

AGENCY: Environmental Protection Agency (EPA).
ACTION: Final rule.

SUMMARY: This regulation establishes an exemption from the requirement of a tolerance for residues of certain sucrose octanoate esters on all food commodities when applied/used in accordance with good agricultural practices. AVA Chemical Ventures, L.L.C. submitted a petition to EPA under the Federal Food, Drug, and Cosmetic Act (FFDCA), as amended by the Food Quality Protection Act (FQPA) of 1996, requesting an exemption from the requirement of a tolerance. This regulation eliminates the need to establish a maximum permissible level for residues of sucrose octanoate esters.

DATES: This regulation is effective September 25, 2002. Objections and requests for hearings, identified by docket identification (ID) number OPP-2002-0016, must be received on or before November 25, 2002.

ADDRESSES: Written objections and hearing requests may be submitted by mail, electronically, or in person. Please follow the detailed instructions for each method as provided in Unit IX. of the SUPPLEMENTARY INFORMATION. To ensure proper receipt by EPA, your objections and hearing requests must identify docket ID number OPP-2002-0016 in the subject line on the first page of your response.

FOR FURTHER INFORMATION CONTACT: By mail: Denise Greenway, c/o Product Manager (PM) 90, Biopesticides and Pollution Prevention Division (7511C), Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington,

[[Page 60147]]

DC 20460-0001; telephone number: (703) 308-8263; e-mail address: <A HREF="mailto:greenway.denise@epa.gov">greenway.denise@epa.gov</A>.

SUPPLEMENTARY INFORMATION:

pesticide residues may not be moved in interstate commerce without an appropriate tolerance or an exemption from the requirement of a tolerance.

Section 408(c)(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(c)(2)(A)(ii) 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) 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....'' FFDCA section 408(b)(2)(D) specifies other, general factors EPA must consider in establishing an exemption, including the consideration of the cumulative effects of a particular pesticide's residues and ``other substances that have a common mechanism of toxicity.'' FFDCA section 408(c)(3) prohibits an exemption unless

[[Page 60148]]

there is either a practical method for detecting and measuring levels of pesticide chemical residue in or on food or EPA determines that there is no need for such a method and states the reason for such determination.

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.

IV. Toxicological Profile

Consistent with section 408(b)(2)(D) of 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.

Sucrose octanoate esters are made from a caprylic fatty acid ester derived from an edible oil or fat, and sucrose. Sucrose is the primary product of photosynthesis (Reference 1) and therefore, common in food crops eaten regularly by humans and animals. Sucrose, also known as table sugar, has an exceedingly long history of human dietary exposure (Reference 1). The octanoate esters are made from octanoic acid (caprylic acid), a common fatty acid in plants, which is produced in small quantities in the human body and is marketed as a human dietary supplement (Reference 1). Sucrose octanoate esters derived from edible vegetable oils, edible tallow or hydrogenated edible tallow have been FDA-approved since 1983 (21 CFR 172.859) when used (as an additive for

record includes the documents that are physically located in the docket, as well as the documents that are referenced in those documents. The public version of the official record does not include any information claimed as CBI. The public version of the official record, which includes printed, paper versions of any electronic comments submitted during an applicable comment period is available for inspection in the Public Information and Records Integrity Branch (PIRIB), Rm. 119, Crystal Mall <greek-i>2, 1921 Jefferson Davis Hwy., Arlington, VA, from 8:30 a.m. to 4 p.m., Monday through Friday, excluding legal holidays. The PIRIB telephone number is (703) 305-5805.

II. Background and Statutory Findings

In the Federal Register of September 9, 1999 (64 FR 49010) (FRL-6095-9), EPA issued a notice pursuant to section 408 of FFDCA, 21 U.S.C. 346a(d)(3), as amended by FQPA (Public Law 104-170), announcing the filing of a pesticide tolerance petition (PP 8E4926) by AVA Chemical Ventures, L.L.C., 65 Aviation Avenue (now at 80 Rochester Avenue, Suite 214), Portsmouth, NH 03801. This notice included a summary of the petition prepared by the petitioner AVA Chemical Ventures, L.L.C. There were no comments received in response to the notice of filing.

The petition requested that 40 CFR part 180 be amended by establishing an exemption from the requirement of a tolerance for residues of sucrose fatty acid esters. EPA has determined that the designation ``sucrose fatty acid esters'' is too broad, in that it could include other compounds not intended by the registrant, and for which the Agency has not reviewed relevant data. The data and information submitted by the registrant in support of the petition cover an exemption from the requirement of a tolerance for sucrose octanoate esters, which have been identified as the specific type of sucrose fatty acid esters that act as the active ingredient (a.i.) in the petitioner's pending products. EPA's general policy is to establish a tolerance or exemption from the requirement of a tolerance for the actual a.i. contained in the registrant's products. Because the a.i. for which the registrant actually is petitioning is technically defined as sucrose octanoate esters [([alpha]-D-glucopyranosyl-[beta]-D-fructofuranosyl-octanoate), mono-, di-, and triesters of sucrose octanoate], all discussions in this rule and in the resulting tolerance exemption expression refer only to ``sucrose octanoate esters [([alpha]-D-glucopyranosyl-[beta]-D-fructofuranosyl-octanoate), mono-, di-, and triesters of sucrose octanoate].'' Hereinafter, EPA uses the terms ``sucrose octanoate esters'' and ``SOEs'' to mean sucrose octanoate esters [([alpha]-D-glucopyranosyl-[beta]-D-fructofuranosyl-octanoate), mono-, di-, and triesters of sucrose octanoate].

III. Statutory Authority

This exemption from the requirement of a tolerance is being issued under the authority of section 408(c) of FFDCA (21 U.S.C. 346a(c)). Under FFDCA section 408, EPA regulates pesticide chemical residues by establishing tolerances limiting the amounts of residues that may be present in or on food, or by establishing exemptions from the requirement of a tolerance for such residues. Food includes articles used for food or drink by humans or other animals. A food containing

of rabbits, very slight erythema was exhibited by 6 rabbits at 0.5 hour post-treatment and 5 rabbits exhibited very slight to slight edema. All symptoms cleared by 24 hours. Classification: Acceptable; Toxicity Category IV for the end-use product.

Data waivers were requested for the following studies. Although no acute toxicity studies were conducted by the registrant, acceptable information/data was submitted from the open technical literature to support the data waiver requests.

3. Acute oral toxicity waiver (OPPTS 870.1100, 152-10) MRID 444158-03, and Amendment number 1: Acute oral and dietary toxicity data, previously evaluated in three publications by the Food and Agriculture Organization (FAO) of the United Nations World Health Organization (WHO), were submitted in support of this data waiver request (References 2, and 3). The data contained in these reports demonstrated that SOEs had extremely low oral toxicity (in laboratory studies), even at concentrations substantially higher than are found in human food. Extremely high concentrations were needed to produce toxic symptoms in laboratory studies (LD₅₀ <20,000 milligrams/kilogram (mg/kg)). Long-term and short-term dietary studies (100 days to 2.5 years), evaluated in the aforementioned FAO/WHO reports, demonstrated that dietary consumption at levels of up to 3% in the diets of rats, mice and dogs caused no substantial toxicological effects. An acceptable daily intake (ADI) of SOEs for humans was estimated to be up to 16 mg/kg body weight/day, which is equivalent to 1.28 kg of SOEs per day for a 176 lb person. In studies with rats and humans, it was demonstrated that SOEs were rapidly hydrolyzed and absorbed by the body. In addition, the National Toxicology Program lists the octanoic acid oral LD₅₀ for rats as 10,080 mg/kg (Reference 1). The information/data described above supports waivers from the data requirements for acute oral toxicity studies. The Agency concludes that SOEs have extremely low toxicity. Classification: Acceptable; Toxicity Category IV for the manufacturing-use product and end-use product.

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4. Acute dermal toxicity waiver (OPPTS 870.1200, 152-11) MRID 444158-03 and Amendment number 1: A data waiver was granted for this guideline study based on the strength of the supporting information/data submitted by the registrant. In addition, publically available sources list the octanoic acid dermal LD₅₀ for rabbits as > 5,000 mg/kg (Reference 1). Classification: Acceptable; Toxicity Category IV for the manufacturing-use product and end-use product.

5. Acute inhalation toxicity waiver (OPPTS 870.1300, 152-12) MRID 444158-03 and Amendment number 1: A data waiver was granted for this guideline study based on the strength of the supporting information/data submitted by the registrant (References 2, 3, and 5). Classification: Acceptable; Toxicity Category IV for the manufacturing-use product and end-use product.

6. Hypersensitivity study waiver (OPPTS 870.2600, 152-15) MRID 444158-04: No hypersensitivity incidents (152.16) have been reported for laboratory workers regularly exposed to SOEs for up to 6 years. In addition, the registrant is obliged under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) section 6(a)(2) to notify the Agency in the event of such incidents. Classification: Acceptable.

direct addition to food) as emulsifiers in certain processed foods and as post-harvest protective coatings for certain fruits. FDA expanded in 1995 the range of foods in which SOEs are permitted, to include use in emulsifiers, stabilizers, and texturizers in chewing gum, confections, and frostings; texturizers in surimi-based fabricated seafood products; and emulsifiers in coffee and tea beverages with added dairy ingredients and/or dairy product analogs (60 FR 44755). The applicant collected and summarized the toxicological data associated with the cited FDA food-use approvals and submitted this information in support of their tolerance exemption request (Reference 2). The Agency reviewed both the summaries and the underlying data.

Toxicity information/data submitted in support of this tolerance exemption are referenced below. New studies were contracted by the petitioner only for primary eye irritation and primary dermal irritation. Data waivers were requested and granted for all other toxicity data requirements. Publically available information/data were submitted, in lieu of studies, as part of the scientific justification necessary to support the data waiver requests (References 2, 3, and 4). In addition, the Agency has found additional relevant data from additional public sources including the National Toxicology Program which have been of value to the Agency's review of this petition (Reference 1). The submitted information/data, in combination, were found equivalent to what would normally be provided by guideline studies, and therefore would likely have been adequate to meet each toxicology requirement had they been submitted as such pursuant to 40 CFR 152.90(b)(4). More detailed analyses of these data and information can be found in specific Agency reviews of the studies and technical literature (References 1, 5, and 6).

1. Primary eye irritation (OPPTS 870.2400, 152-13) MRIDs 446101-05 and 446101-06: Following ocular instillation of 0.1 mL of undiluted manufacturing-use product into the eyes of rabbits, moderate to severe eye irritation and mild corneal opacity was observed in the treated eyes of all rabbits at 24 hours post-dosing, and persisted in 1 rabbit to 21 days post-dosing. Mild iritis was exhibited in 3 rabbits at 24 hours post-dosing, and persisted in 1 rabbit to 72 hours. Classification: Acceptable; Toxicity Category I for the manufacturing-use product. In a second primary eye irritation study, following ocular instillation of 0.1 mL of undiluted end-use product into the eyes of rabbits, moderate to severe eye irritation was observed in the treated eyes of all 6 rabbits at 72 hours post-dosing, was mild at 7 days, and cleared by 14 days. Mild corneal opacity was observed in all 6 rabbits at 24 hours, and persisted to 7 days in 1 rabbit, then cleared by 14 days post-dosing. Mild iritis persisted in 4 rabbits to 72 hours, then cleared. Classification: Acceptable; Toxicity Category II for the end-use product.

2. Primary dermal irritation (OPPTS 870.2500, 152-14) MRIDs 446101-03 and 446101-04: Following dermal application of 0.5 mL of undiluted manufacturing-use product to the skin of rabbits, 5 rabbits exhibited very slight erythema and one exhibited well-defined erythema at 1 hour post-treatment. Very slight erythema persisted on 4 rabbits to 24 hours, then cleared. No edema was observed on any rabbit. Classification: Acceptable; Toxicity Category IV for the manufacturing-use product. In a second primary dermal irritation study, following dermal application of 0.5 mL of undiluted end-use product to the skin

and post-harvest protective fruit coatings since 1983. Even if there is a significant increase in exposure to SOEs due to their use as a pesticide, the acute toxicity information and data available from the National Toxicology Program and submitted by the registrant demonstrating extremely low mammalian toxicity (Toxicity Category IV) indicate that risk associated with acute exposures by the oral, dermal and inhalation routes would be low to non-existent.

2. Drinking water exposure. No drinking water exposure is expected, as SOEs are not soluble in water, do not persist in the environment, and are biodegradable within approximately five days at approximately 20-27[deg]C, in both aerobic and anaerobic conditions (Reference 5). Because SOEs have extremely low toxicity, have been approved for food use by FDA, and are present as direct food additives in many foods, should exposure through drinking water occur, no risk is anticipated.

B. Other Non-Occupational Exposure

The potential for non-dietary exposure to SOEs residues for the general population, including infants and children, is unlikely because potential use sites are commercial, agricultural, and large-scale horticultural. Sucrose octanoate esters' constituent sugars and fatty acids are normal parts of the human diet. Sucrose octanoate esters' toxicity has been determined to be extremely low (except via the ocular exposure route). Therefore, while there exists a great likelihood of prior exposure for most, if not all, individuals to both SOEs and SOEs' components, any increased exposure due to the proposed products would be negligible because the product would very likely be degraded to sugars and fatty acids and/or consumed by microorganisms before the general public would come in contact with treated plants or food products from treated plants.

VI. Cumulative Effects

The Agency has considered the cumulative effects of SOEs and other substances in relation to a common mechanism of toxicity. These considerations include the possible cumulative effects of such residues on infants and children. There is no indication of mammalian toxicity from the submitted information/data (except by the ocular route of exposure) for SOEs. Therefore, no adverse cumulative effects are expected.

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

1. U.S. population. There is a reasonable certainty that no harm will result from aggregate exposure to residues of SOEs to the U.S. population. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. The Agency has arrived at this conclusion based on the extremely low levels of mammalian toxicity associated with

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SOEs (except for risk from ocular exposure, which will be mitigated via precautionary label language). Sucrose octanoate esters have extremely low toxicity (LD₅₀ >20,000 mg/kg in laboratory studies of oral exposure), and it is unlikely that any toxic effects will result

7. Genotoxicity and Mutagenicity waiver (OPPTS 870.5300, 870.5195; 152-17, and 152-19) MRID 444158-03 and Amendment number 1: No guideline studies were submitted, but it was determined that none are required because acceptable information/data were submitted from the open technical literature to scientifically justify a waiver of the data requirements for genotoxicity and mutagenicity. This information/data demonstrate that SOEs are not genotoxic and/or mutagenic, nor is the a.i. structurally and/or chemically similar to known mutagens or known classes of mutagens (References. 2, 3, and 5). In addition, a study reported by the National Toxicology Program shows octanoic acid to be negative for genotoxicity/mutagenicity (Reference 1). Classification: Acceptable.

8. Immune response and other Subdivision M toxicity data waivers (OPPTS 880.3800 through 870.4200, 152-18 through 152-29) MRID 444158-03 and Amendment number 1: Due to the low toxicity of SOEs (as demonstrated in the cited open technical literature (References 2, 3, 4, 5, and 6), the Agency granted waivers from all other Subdivision M toxicity data requirements, including the 90-day feeding and teratogenicity studies. In addition, octanoic acid is considered a nonteratogenic compound even at the very high dose rate of 18.75 millimoles/kg (Reference 1).

V. Aggregate Exposures

In examining aggregate exposure, FFDCA section 408 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).

A. Dietary Exposure

1. Food. An ADI of SOEs for humans was estimated by FAO/WHO to be up to 16 mg/kg body weight/day, which is equivalent to 1.28 kg of SOEs per day for a 176 lb person (References. 2, 3, and 5). There are no reasonably foreseeable circumstances in which the residue levels of SOEs would ever approach this amount. Sucrose octanoate esters break down into their natural constituents (sugar and fatty acids) shortly after application. The foliar application rate for the product would be at a volume to volume rate of 0.32% to 0.40% (Reference 7) for the a.i.. Likewise the a.i. use rate when applied to honey bees would be 0.25%, and would range from 0.5% to 1.0% when treating mushroom growing media. In studies with rats and humans, it was demonstrated that SOEs were rapidly hydrolyzed and absorbed by the body (Reference 5). Because SOEs are the mono-, di- and tri-esters of sucrose with fatty acids and are derived from sucrose (sugar) and edible tallow or edible vegetable oils, there is a great likelihood of exposure in the normal human diet to SOEs and SOEs' components for most, if not all individuals, including infants and children. Sucrose octanoate esters are a sucrose fatty acid ester, and sucrose fatty acid esters are a normal part of the human diet. Thus SOEs may be considered a normal part of the human diet. To date, there have been no reports of any hypersensitivity incidents or reports of any known adverse reactions in humans resulting from exposure to SOEs, which have been FDA-approved food emulsifiers

B. Analytical Method(s)

The Agency is establishing an exemption from the requirement of a tolerance without any numerical limitation for the reasons stated above, including SOEs' low mammalian toxicity (except by ocular exposure). For the same reasons, the Agency has concluded that an analytical method is not required for enforcement purposes for SOEs.

C. Codex Maximum Residue Level

There are no Codex Maximum Residue Levels (MRLs) established for residues of SOEs.

IX. Conclusions

Based on the toxicology information/data submitted and publically available, there is a reasonable certainty that no harm will result from aggregate exposure of residues of SOEs to the U.S. population, including infants and children, under reasonably foreseeable circumstances, when the biochemical pesticide is used in accordance with good agricultural practices. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. The Agency has arrived at this conclusion based on the information/data submitted and publically available data demonstrating no toxicity, except from ocular exposure. Potential risk from ocular exposure will be effectively addressed under FIFRA by mitigating precautionary label language. As a result, EPA establishes an exemption from the tolerance requirements pursuant to FFDCA 408(c) and (d) for residues of SOEs in or on all food commodities.

X. Objections and Hearing Requests

Under section 408(g) of the FFDCA, as amended by the 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 the FQPA of 1996, EPA will continue to use those procedures, with appropriate adjustments, until the necessary modifications can be made. The new section 408(g) 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), as was provided in the old FFDCA sections 408 and 409. 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-2002-0016 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 November 25, 2002.

from exposure to SOEs via the oral, dermal or inhalation pathways when the products are used according to proposed label directions (Reference 5). The amount of SOEs applied to food crops is many orders of magnitude lower than the concentrations of SOEs needed to cause toxicological effects. Because the worst case scenario exposure is far below the level of any dietary toxicity known for SOEs or their components and degradates, EPA has determined that residues will not pose a dietary risk under reasonably foreseeable circumstances and that the setting of a tolerance exemption is appropriate.

2. Infants and children. FFDCA section 408 provides that EPA shall apply an additional ten-fold margin of exposure (safety) for infants and children in the case of threshold effects to account for pre-natal and post-natal toxicity and the completeness of the data base unless EPA determines that a different margin of exposure (safety) will be safe for infants and children. Margins of exposure (safety) are often referred to as uncertainty (safety) factors. In this instance, based on all the available information, the Agency concludes that SOEs are practically non-toxic to mammals, including infants and children (except via ocular exposure). Thus, there are no threshold effects of concern, and so the provision requiring an additional margin of safety does not apply. Further, the provisions of consumption patterns, special susceptibility, and cumulative effects do not apply. As a result, EPA has not used a margin of exposure (safety) approach to assess the safety of SOEs.

VIII. Other Considerations

A. Endocrine Disruptors

EPA is required under the FFDCA as amended by FQPA to develop a screening program to determine whether certain substances (including all pesticide active and other ingredients) ``may have an effect in humans that is similar to an effect produced by a naturally-occurring estrogen, or other such endocrine effects as the Administrator may designate.'' Following the recommendations of its Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), EPA determined that there is no scientific basis for including, as part of the program, the androgen and thyroid hormone systems in addition to the estrogen hormone system. EPA also adopted EDSTAC's recommendation that the program include evaluations of potential effects in wildlife. For pesticide chemicals, EPA will use FIFRA and, to the extent that effects in wildlife may help determine whether a substance may have an effect in humans, FFDCA authority to require wildlife evaluations. As the science develops and resources allow, screening of additional hormone systems may be added to the Endocrine Disruptor Screening Program (EDSP). When the appropriate screening and/or testing protocols being considered under the Agency's EDSP have been developed, SOEs may be subjected to additional screening and/or testing to better characterize effects related to endocrine disruption.

Based on available data, no endocrine system-related effects have been identified with consumption of SOEs. It is an FDA-approved direct food additive comprised of sugars and fatty acids, having an ADI of 16 mg/kg body weight/day. To date, there is no evidence to suggest that SOEs affect the immune system, function in a manner similar to any known hormone, or that they act as an endocrine disruptor.

0001. In person or by courier, bring a copy to the location of the PIRIB described in Unit I.B.2. You may also send an electronic copy of your request via e-mail to: <A HREF="mailto:opp-docket@epa.gov ">opp-docket@epa.gov</A>. 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).

XI. References

1. USEPA. Brief summary of toxicity information to support registration/tolerance exemptions for sucrose octanoate. R. S. Jones to D. Greenway; August 8, 2002.

2. Barrington, T., and C. L. Hartman. Sucrose fatty acid esters-Safety data in support of petition proposing a temporary (sic) exemption from the requirement of a tolerance for use in all food commodities (MRID 444158-03); October 2, 1997.

3. Barrington, T. and W. L. Biehn. Sucrose fatty acid esters-safety data in support of petition proposing an exemption from the requirement of a tolerance for use in all food commodities, Amendment number 1 to MRID 444158-03; July 13, 1998.

4. Barrington, A. Waiver request; July 12, 2002.

5. USEPA. Science review in support of registration of sucrose octanoate esters. R.S. Jones to D. Greenway; February 14, 2000.

6. USEPA. Sucrose octanoate esters; A request for concurrence on a decision to waive the requirement for 90-day feeding (152-20) and teratogenicity (152-23) studies, based on the Registrant's correspondence of July 12, 2002. D. Greenway to R. S. Jones; August 7, 2002.

7. Barrington, A. Sucrose octanoate esters - per-acre application rates; July 12, 2002.

XII. Regulatory Assessment Requirements

This final rule establishes an exemption from the tolerance requirement under FFDCA section 408(d) 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

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

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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 (1900C), Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington, DC 20460-0001. You may also deliver your written request to the Office of the Hearing Clerk in Rm. 104, Crystal Mall <greek-i> 2, 1921 Jefferson Davis Hwy., Arlington, VA. 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 (703) 603-0061.

2. Tolerance fee payment. If you file an objection or request a hearing, you must also pay the fee prescribed by 40 CFR 180.33(i) or request a waiver of that fee pursuant to 40 CFR 180.33(m). You must mail the fee to: EPA Headquarters Accounting Operations Branch, Office of Pesticide Programs, P.O. Box 360277M, Pittsburgh, PA 15251. Please identify the fee submission by labeling it ``Tolerance Petition Fees.''

EPA is authorized to waive any fee requirement ``when in the judgement of the Administrator such a waiver or refund is equitable and not contrary to the purpose of this subsection.'' For additional information regarding the waiver of these fees, you may contact James Tompkins by phone at (703) 305-5697, by e-mail at <A HREF="mailto:tompkins.jim@epa.gov">tompkins.jim@epa.gov</A>, or by mailing a request for information to Mr. Tompkins at Registration Division (7505C), Office of Pesticide Programs, Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington, DC 20460-0001.

If you would like to request a waiver of the tolerance objection fees, you must mail your request for such a waiver to: James Hollins, Information Resources and Services Division (7502C), Office of Pesticide Programs, Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington, DC 20460-0001.

3. Copies for the Docket. In addition to filing an objection or hearing request with the Hearing Clerk as described in Unit IX.A., you should also send a copy of your request to the PIRIB for its inclusion in the official record that is described in Unit I.B.2. Mail your copies, identified by docket ID number OPP-2002-0016, 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-

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. Submission to Congress and the Comptroller General

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: September 11, 2002.
James Jones,
Acting Director, 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), 346(a) and 371.

2. Section 180.1222 is added to subpart D to read as follows:

Sec. 180.1222 Sucrose octanoate esters; exemption from the requirement of a tolerance.

An exemption from the requirement of a tolerance is established for residues of sucrose octanoate esters [([alpha]-D-glucopyranosyl-[beta]-D-fructofuranosyl-octanoate), mono-, di-, and triesters of sucrose octanoate]
in or on all food commodities when used in accordance with good agricultural practices.

[FR Doc. 02-24224 Filed 9-24-02; 8:45 am]
BILLING CODE 6560-50-S

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 FFDCA section 408(d), 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

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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 FFDCA section 408(n)(4). 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

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BIOPESTICIDES REGISTRATION ACTION DOCUMENT

Sucrose Octanoate Esters
(PC Code 035300)

U.S. Environmental Protection Agency
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division
Sucrose Octanoate Esters
(PC Code 035300)

b. Other Non-occupational Exposure

4. Occupational, Residential, School and Day Care Exposure and Risk Characterization
   a. Occupational Exposure
   b. Residential, School and Day Care Exposure and Risk Characterization
5. Acute and Chronic Dietary Risks for Sensitive Subpopulations Particularly Infants and Children
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C. ENVIRONMENTAL ASSESSMENT

1. Ecological Effects Hazard Assessment
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3. Ecological Exposure and Risk Characterization

D. EFFICACY DATA

IV. Risk Management Decision

A. DETERMINATION OF ELIGIBILITY FOR REGISTRATION

B. REGULATORY POSITION

1. Unconditional Registration
2. Tolerances for Food Uses and/or Exemptions
3. CODEX Harmonization
4. Nonfood Re/Registrations
5. Risk Mitigation
6. Endangered Species Statement

C. LABELING RATIONALE

1. Human Health Hazard
   a. Worker Protection Standard
   b. Non-Worker Protection Standard

-12 Executive Summary

A. IDENTITY

The technical grade active ingredient (TGAI)/manufacturing-use product (MUP), "Avachem Sucrose Octanoate Manufacturing Use Product," consists of 85.43% sucrose octanoate esters [(α-D-glucopyranosyl-β-D-fructofuranosyl-octanoate), mono-, di-, and triesters of sucrose octanoate], made from a caprylic fatty acid ester derived from an edible oil or fat, and sucrose, a sugar which is a regular part of the diet of humans and animals. The end-use product (EP), "Avachem Sucrose Octanoate [40%]," contains 40% sucrose octanoate esters.

B. USE/USAGE

Avachem Sucrose Octanoate [40%] is a spray for use on a) various crops to control soft-bodied insects and mites, b) mushroom growing media to control sciarid flies, and c) adult honey bees to control *Varroa* mites. The use is classified as a food crop application.

C. RISK ASSESSMENT

No unreasonable adverse effects on humans or the environment are anticipated from aggregate exposure to Avachem Sucrose Octanoate Manufacturing Use Product or Avachem Sucrose Octanoate [40%]. This includes all anticipated exposures for which there is reliable information.

1. Human Health Risk Assessment

a. Toxicological Endpoints

No toxicological endpoints are expected. Mammalian toxicology information from the open scientific literature and data were submitted to adequately satisfy data requirements to support

BIOPESTICIDES REGISTRATION ACTION DOCUMENT TEAM

Office of Pesticide Programs:

Biopesticides and Pollution Prevention Division

Biochemical Pesticides Branch

Sheryl K. Reilly, Ph.D.	Biologist, Branch Chief
Denise Greenway	Biologist, Regulatory Action Leader
Linda Hollis	Microbiologist, Team Leader
Russell S. Jones, Ph.D.	Biologist, Health Effects/Nontarget Organisms
Freshteh Toghrol, Ph.D.	Chemist, Senior Scientist

Data waivers were requested and granted for ecological testing requirements because no toxic endpoints are expected, based on submitted mammalian data and information from the open scientific literature. An acute contact honey bee toxicity study demonstrated that sucrose octanoate esters are practically non-toxic to honey bees ($LD_{50} > 80$ µg/bee).

b. Ecological Exposure

The active ingredient does not persist in the environment and biodegrades within approximately five days at approximately 20-27ºC, in both aerobic and anaerobic conditions.

c. Risk Assessment

Risk to other organisms is expected to be minimal due to the low chances of exposure to the environment. The Agency posits sucrose octanoate esters, used according to label directions, will not result in significant adverse effects to wildlife or other organisms.

-14 DATA GAPS / LABELING

There are no data gaps. Because of sucrose octanoate esters' Toxicity Category I for primary eye irritation, certain precautionary labeling is required to mitigate risks associated with proposed uses (see Labeling Rationale for details).

II. Overview

-15 ACTIVE INGREDIENT OVERVIEW

Common Name:	Sucrose octanoate esters
Chemical Name:	Sucrose octanoate esters [(α-D-glucopyranosyl, β-D-fructofuranosyl-octanoate), mono, di-, and triesters of

the registration. Submitted information and data for the TGAI/MUP and the end-use product indicate Toxicity Category IV for acute oral, acute dermal, and acute inhalation toxicity; and for primary dermal irritation. Neither the TGAI/MUP, nor the end-use product, is a dermal sensitizer. The data reported for primary eye irritation studies show that the test substance was moderately to severely irritating, and is thus a Toxicity Category I when the TGAI/MUP is used, and Toxicity Category II when the end-use product is tested.

b. Human Exposure

While exposure to the general population is expected to be low, worker exposure will occur. Appropriate protective wear and precautionary label language will mitigate vulnerability to the worker.

c. Risk Assessment

The Biopesticides and Pollution Prevention Division (BPPD) has not identified any subchronic, chronic, immune, endocrine, dietary or nondietary exposure issues with respect to sucrose octanoate esters as relates to children or the general U.S. population. Ocular risk to applicators is mitigated providing the label directions are followed. No toxicological endpoints are expected, and there is limited exposure of the general public to this product when used according to the label instructions. The Agency has considered sucrose octanoate esters in light of relevant safety factors in the Food Quality Protection Act (FQPA) of 1996 and under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and determined there will be no unreasonable adverse effects from the use of this product.

2. Ecological Risk Assessment

a. Ecological Toxicity Endpoints

water, with agitation. Maintain gentle agitation during application. The proposed label specifies application rates a) between 0.8% and 1.0% volume/volume (v/v) for foliarly applied spray, b) between 1.25% and 2.50% v/v for mushroom growing media, and c) of 0.625% v/v for application to honey bees.

Use Practice Limitations: Do not allow workers into treated areas for 48 hours following application.

Timing: Application to foliage or adult honey bees should be initiated as soon as the target pest is observed. Mushroom growing media applications are to be made prior to spawning.

C. ESTIMATED USAGE

Although the Experimental Use Permit (EUP) issued in 2000 allowed the application 25 gallons of active ingredient over 50 acres in the state of California, no sucrose octanoate esters were actually applied under the experimental program (due to the unexpected unavailability of the test plot acreage). The EUP issued in 2002 allowed the application of 33 gallons of active ingredient over 100 acres in the state of California.

D. DATA REQUIREMENTS

BPPD reviewed data requirements for granting this registration under Section 3(c)(5) of FIFRA. Mammalian toxicology and ecological effects data requirements for sucrose octanoate esters were fulfilled. Product analysis data requirements are adequately satisfied.

E. REGULATORY HISTORY

On February 23, 1999, EPA received an application from AVA Chemical Ventures, L.L.C. for two new products with the new active ingredient, sucrose octanoate esters. A notice of

	sucrose octanoate]
CAS Numbers:	42922-74-7 and 58064-47-4
Trade and Other Names:	Avachem Sucrose Octanoate Manufacturing Use Product, Avachem Sucrose Octanoate [40%]
OPP Chemical Code:	035300
Basic Manufacturer:	Manufactured for: AVA Chemical Ventures, L.L.C. 80 Rochester Avenue Suite 214 Portsmouth, NH 03801

B. USE PROFILE

Proposed uses and application methods for sucrose octanoate esters include the following:

Type of Pesticide: Biochemical insecticide/miticide

Use Sites: Sucrose octanoate esters is for field, greenhouse and nursery use on any type of agricultural commodity (including certain non-food ornamentals); as well as on mushroom growing media and on adult honey bees.

Formulation Types: Liquid

Method and Rates of Application: Most conventional ground spray application equipment may be used. Shake or stir before use, adding the appropriate quantity to

uses the term "sucrose octanoate esters" to mean sucrose octanoate esters [(α-D-glucopyranosyl-β-D-fructofuranosyl-octanoate), mono-, di-, and triesters of sucrose octanoate].

On August 14, 2000, EPA received an application from the United States Department of Agriculture's Agricultural Research Service (USDA/ARS) for an Experimental Use Permit (EUP) covering the use of sucrose octanoate esters to evaluate control of the glassy-winged sharp shooter on non-bearing grape vines. On September 15, 2000, the Agency granted the EUP (65 FR 76259) to use 25 gallons/year of the biochemical active ingredient sucrose octanoate esters on 50 acres in the state of California.

On April 24, 2002, EPA received an application from AVA Chemical Ventures, L.L.C., on the behalf of the USDA/ARS, for a new Experimental Use Permit (EUP) covering the use of sucrose octanoate esters to evaluate control of the glassy-winged sharp shooter on non-bearing/post harvest citrus in addition to non-bearing grape vines. On May 31, 2002, the Agency granted the EUP (67 FR 43598) to use 33 gallons/year of the biochemical active ingredient sucrose octanoate esters on 100 acres in the state of California.

F. CLASSIFICATION

On January 14, 1997, the Biochemical Classification Committee determined that the insecticide/miticide sucrose octanoate esters are functionally identical and structurally similar to naturally occurring sucrose fatty acid esters, and so are eligible for testing using the biochemical reduced data requirements. Following review of the full data set submitted in support of the registration applications, which demonstrated a non-toxic, indirect mode of action for the active ingredient, the committee on July 2, 2002, amended the report by granting the "biochemical pesticide" designation to sucrose octanoate esters (Ref. 1).

G. FOOD CLEARANCES/TOLERANCES

receipt of the application for registration of sucrose octanoate (C_8 fatty acid mono-, di- and triesters of sucrose octanoate and sucrose dioctanoate) (α-D-glucopyranoside, β-D-fructofuranosyl, monooctanoate and dioctanoate) was published in the Federal Register on August 11, 1999 (64 FR 43701) with a 30-day comment period. No comments were received following this publication.

Note that the Agency and the registrant agreed to represent the active ingredient name as sucrose octanoate esters [(α-D-glucopyranosyl-β-D-fructofuranosyl-octanoate), mono-, di-, and triesters of sucrose octanoate] on the product labels and Confidential Statements of Formula and the tolerance exemption expression. This name is synonymous with the name used in the Federal Register notice of receipt of August 11, 1999 (64 FR 43701).

On September 9, 1999, EPA published a Notice of Filing Pesticide Petitions to Establish a Tolerance for Certain Pesticide Chemicals (sucrose fatty acid esters) in or on Food (8E4926, 64 FR 49010) with a 30-day comment period. No comments were received.

The EPA determined that the designation "sucrose fatty acid esters" is too broad, in that it could include other compounds not intended by the registrant, and for which the Agency has not reviewed relevant data. The data and information submitted by the registrant in support of the petition cover an exemption from the requirement of a tolerance for sucrose octanoate esters, which have been identified as the specific type of sucrose fatty acid esters that act as the active ingredient in the registrant's pending products. EPA's general policy is to establish a tolerance or exemption from the requirement of a tolerance for the actual active ingredient contained in the registrant's products. Because the active ingredient for which the registrant actually is petitioning is technically defined as sucrose octanoate esters [(α-D-glucopyranosyl-β-D-fructofuranosyl-octanoate), mono-, di-, and triesters of sucrose octanoate], all discussions in this document (and the tolerance exemption expression established in the associated Final Rule for this new active ingredient) refer only to "sucrose octanoate esters [(α-D-glucopyranosyl-β-D-fructofuranosyl-octanoate), mono-, di-, and triesters of sucrose octanoate]." Hereinafter EPA

The physical and chemical characteristics of the TGAI/MUP and the end-use product were submitted to support the registration. They are summarized in Table 1.

Table 1. Product chemistry data requirements:

Product Chemistry (MRID 444880-01, as amended by 451974-01, 451974-02, 454103-01 and, 454103-02)	**TGAI/MP**	**EP**
151B-10 (880.1100): Product identity	Avachem Sucrose Octanoate Manufacturing Use Product, the technical product, consists of 85.43% sucrose octanoate esters and 14.57% other ingredients	Avachem Sucrose Octanoate [40%], the end-use product, contains 40% sucrose octanoate esters
151B-11 (880.1620): Formulation process	An acceptable description of the manufacturing process was submitted.	The product is formulated via a simple mixing process without any chemical reactions.
151B-12 (880.1400): Discussion of formulation of unintentional impurities	Acceptable nominal concentrations and certified limits were reported for the manufacturing impurities.	No impurities of toxicologic concern are formed during the formulation process.

On September 9, 1999, EPA published a Notice of Filing Pesticide Petitions to Establish a Tolerance for Certain Pesticide Chemicals in or on Food (8E4926, 64 FR 49010) with a 30-day comment period. No comments were received. A final rule establishing an exemption from the requirement of a tolerance is being published in association with this document. There are no Codex tolerances for sucrose octanoate esters.

III. Science Assessment

A. PHYSICAL/CHEMICAL PROPERTIES ASSESSMENT

All product chemistry data requirements for the technical grade/manufacturing-use product and the end-use product are met.

1. Product Identity and Mode of Action

a. Product Identity:

The technical grade active ingredient/manufacturing-use product, Avachem Sucrose Octanoate Manufacturing Use Product, consists of 85.43% sucrose octanoate esters [(α-D-glucopyranosyl-β-D-fructofuranosyl-octanoate), mono-, di-, and triesters of sucrose octanoate] and the end-use product, Avachem Sucrose Octanoate [40%], 40% sucrose octanoate esters.

b. Mode of Action:

The mode of action is physical and non-toxic; the surfactant effect of sucrose octanoate esters de-waxes the cuticle of the target insect, causing it to dessicate.

2. Physical And Chemical Properties Assessment

Product Chemistry (MRID 444880-01, as amended by 451974-01, 451974-02, 454103-01 and, 454103-02)	**TGAI/MP**	**EP**
		158.190
880.7300: Density, Bulk Density, or Specific Gravity	8.75 to 9.50 lbs/gal	8.50 to 9.00 lbs/gal
880.7840: Solubility	Forms an emulsion with water	Not required per 40 CFR § 158.190
880.7050: Vapor Pressure	<5 mm Hg	Not required per 40 CFR § 158.190
880.7370: Dissociation constant	NA	NA
880.7550: Octanol/water partition coefficient	Unknown	Not required per 40 CFR § 158.190
880.7000: pH	NA	7.0
880.6313: Stability	Stable below 40ΘC	Not required per 40 CFR §158.190
880.6314: Oxidizing or Reduction Action	NA, does not contain an oxidizing or reducing agent	NA, does not contain an oxidizing or reducing agent
880.6315: Flammability/Flame Extension	None; decomposes above 105ΘC	NA, does not contain a combustible liquid
880.6316: Explodability	NA, is not potentially explosive	NA, is not potentially explosive
880.6317: Storage Stability	NA	At least 1year at 40ΘC based on

Product Chemistry (MRID 444880-01, as amended by 451974-01, 451974-02, 454103-01 and, 454103-02)	**TGAI/MP**	**EP**
151B-13 (880.1700): Preliminary analysis	Data obtained from the five-batch analysis demonstrate that the analytical method is precise and accurate.	No five-batch preliminary analysis data were submitted, but none are required since the end-use product is not manufactured via an integrated system, and because the TGAI/MUP will be registered simultaneously with the EP.
880.1750: Certified limits	The certified limits for the active ingredient and other impurities are acceptable.	Acceptable nominal concentrations and certified limits were reported for the other (inert) ingredient in the formulation.
880.1800: Enforcement analytical method	The analytical method is liquid chromatography (HPLC).	An acceptable liquid chromatography (HPLC) analytical method was submitted.
Physical/Chemical Properties (MRID 444158-02, as amended by MRIDs 446101-02, 447634-01, and 451974-02)	**TGAI/MUP**	**EP**
880.6302: Color	Amber	Amber
880.6303: Physical State	Liquid	Liquid
880.6304: Odor	Faint sweet smell	Faint sweet smell
880.7200: Melting Point	NA, not a solid	Not required per 40 CFR § 158.190
880.7220: Boiling Point	Decomposes above 105ºC	Not required per 40 CFR §

Adequate mammalian toxicology information and data are available and support registration of the products containing the active ingredient sucrose octanoate esters. New studies were contracted by the registrant only for primary eye irritation and primary dermal irritation. Data waivers were requested and granted for all other toxicity data requirements. Publically available information/data were submitted, in lieu of studies, as part of the scientific justification necessary to support the data waiver requests (Refs. 2, 3, 6). In addition, the Agency has found additional relevant data from additional public sources, including the National Toxicology Program (NTP), which have been of value to the Agency's review of this application (Ref. 4). The submitted information/data, in combination, were found equivalent to what would normally be provided by guideline studies, and therefore would likely have been adequate to meet each toxicology requirement, had they been submitted as such pursuant to 40 CFR 152.90 (b)(4). More detailed analyses of these data and information can be found in specific Agency reviews of the studies and technical literature (Refs. 4, 5 and 7).

a. Acute Toxicity

The registrant submitted acceptable data and information from the open technical literature (Refs. 2 and 3) to justify the data waiver request and satisfy the requirement for acute toxicity studies. Based on the submitted information/data and additional relevant data found by the Agency from public sources, including the NTP (Ref. 4), BPPD has categorized both the manufacturing-use and end-use sucrose octanoate esters products as Toxicity Category IV for acute oral toxicity, acute dermal toxicity and acute inhalation toxicity. On the strength of a report showing no hypersensitivity responses or incidents among laboratory workers regularly exposed for up to six years to sucrose octanoate esters (aerosols or dried residues), BPPD has determined that neither product is a sensitizer, and has waived the hypersensitivity study (Ref. 5).

Following ocular instillation of 0.1 mL of undiluted manufacturing-use product into the eyes of rabbits, moderate to severe eye irritation and mild corneal opacity was observed in the treated eyes of all rabbits at 24 hours post-dosing and persisted in one rabbit to 21 days post-dosing. Mild iritis was exhibited in three rabbits at 24-hours post-dosing and persisted in one rabbit to 72

Product Chemistry (MRID 444880-01, as amended by 451974-01, 451974-02, 454103-01 and, 454103-02)	TGAI/MP	EP
		shelf-life tests
880.7100: Viscosity	Not required per 40 CFR §158.190	500 to 2000 CP at 25ºC
880.6319: Miscibility	NA, is not to be diluted with petroleum solvents	Totally miscible in water
880.6320: Corrosion Characteristics	NA	Non-corrosive to metals, plastics and glass
880.6321: Dielectric Breakdown Voltage	Not required per 40 CFR §158.190	NA, is not to be used around electrical equipment

B. HUMAN HEALTH ASSESSMENT

Information and data submitted to support the registration application of the technical grade/manufacturing-use product active ingredient, Avachem Sucrose Octanoate Manufacturing Use Product, and the end-use product, Avachem Sucrose Octanoate [40%], adequately satisfy the food and non-food use requirements set forth in 40 CFR 158.690 (c) for biochemical pesticides. Excepting ocular exposure, the overall toxicological risk from human exposure to sucrose octanoate esters is negligible.

1. Toxicology Assessment

Due to the low toxicity of sucrose octanoate esters (as demonstrated in the cited open technical literature (Refs. 2, 3, 5, 6 and 7)), the Agency granted waivers from all Subdivision M toxicity data requirements, including the immune response, 90-day feeding and teratogenicity studies. In addition, a sucrose octanoate esters constituent, octanoic acid, is considered a nonteratogenetic compound even at the very high dose rate of 18.75 mmoles/kg (Ref. 4).

Data Waivers (Refs. 2, 3 and 6) were requested for the following studies:

Acute oral toxicity (OPPTS 870.1100)
Acute dermal toxicity (OPPTS 870.1200)
Acute inhalation (OPPTS 870.1300)
Hypersensitivity study (OPPTS 870.2600)
Studies to detect genotoxicity (OPPTS 870.5300)
Immune response (OPPTS 880.3800)
Mammalian mutagenicity tests (OPPTS 870.5195)
90-Day Feeding (OPPTS 870.3100)
Teratogenicity (OPPTS 870.3700)

The registrant's rationale to support the waivers is that considerable sucrose octanoate esters safety data are available (Refs. 2, 3 and 6). The active ingredient, derived from edible

hours. This classifies Avachem Sucrose Octanoate Manufacturing Use Product as Toxicity Category I. Following ocular instillation of 0.1 mL of undiluted end-use product into the eyes of rabbits, moderate to severe eye irritation was observed in the treated eyes of all six rabbits at 72 hours post-dosing, was mild at seven days, and cleared by 14 days. Mild corneal opacity was observed in all six rabbits at 24 hours, and persisted to seven days in one rabbit, then cleared by 14 days post-dosing. Mild iritis persisted in four rabbits to 72 hours, then cleared. This classifies Avachem Sucrose Octanoate [40%] as Toxicity Category II.

Following dermal application of 0.5 mL of undiluted manufacturing-use product to the skin of rabbits, five rabbits exhibited very slight erythema and one exhibited well-defined erythema at one hour post-treatment. Very slight erythema persisted on four rabbits to 24 hours, then cleared. No edema was observed on any rabbit. Following dermal application of 0.5 mL of undiluted end-use product to the skin of rabbits, very slight erythema was exhibited by six rabbits at 0.5 hour post-treatment and five rabbits exhibited very slight to slight edema. All symptoms cleared by 24 hours. The results from these two studies place both the manufacturing-use and end-use sucrose octanoate esters products in Toxicity Category IV for primary dermal irritation. Based on the submitted information for hypersensitivity, sucrose octanoate esters is not a dermal sensitizer. Agency reviews are available in the docket (Ref. 5).

b. Genotoxicity and Mutagenicity

No guideline studies were submitted, but it was determined that none are required because the registrant submitted published information from the open, technical literature to scientifically justify waivers for these studies (Refs. 2 and 3). The submitted data/information demonstrate that sucrose octanoate esters are not genotoxic and/or mutagenic, nor is the active ingredient structurally and/or chemically similar to known mutagens or known classes of mutagens (Ref. 5). A study reported by the NTP shows a sucrose octanoate esters constituent, octanoic acid, to be negative for genotoxicity/mutagenicity (Ref. 4).

c. Other Subdivision M Toxicity Data Requirements

GUIDELINE NO.	STUDY	RESULTS	MRID NO.
152-10, 870.1100	Acute oral toxicity in rats and mice	Data waiver granted (see text for details) Toxicity Category IV (MUP and EP)	444158-03 and Amendment No. 1
152-11, 870.1200	Acute dermal toxicity	Data waiver granted (see text for details) Toxicity Category IV (MUP and EP)	444158-03 and Amendment No. 1, and 444158-04
152-12, 870.1300	Acute inhalation toxicity	Data waiver granted (see text for details) Toxicity Category IV (MUP and EP)	None; not a likely pathway of exposure
152-13, 870.2400	Primary eye irritation in rabbits	Toxicity Category I (MUP)	446101-05
		Toxicity Category II (EP)	446101-06
152-14, 870.2500	Primary dermal irritation in rabbits	Toxicity Category IV (MUP)	446101-03

vegetable oils, edible tallow or hydrogenated edible tallow, has been FDA-approved for use as emulsifiers in certain processed foods and as post-harvest protective coatings for certain fruits since 1983. In 1995, FDA expanded the range of foods in which sucrose octanoate esters are permitted, to include use in emulsifiers, stabilizers, and texturizers in chewing gum, confections, and frostings; texturizers in surimi-based fabricated seafood products; and emulsifiers in coffee and tea beverages with added dairy ingredients and/or dairy product analogs (60 FR 44755). Sucrose octanoate esters' constituent sugars and fatty acids are normal parts of the human diet, and the Agency knows of no instance where they have been associated with any toxic effects related to the consumption of food. Due to this knowledge of sucrose octanoate esters' presence in the human diet (Ref. 4), the summarized safety data (Ref. 2), the NTP data (Ref. 4), and the recent primary eye and primary dermal irritation testing, EPA believes sucrose octanoate esters are unlikely to be carcinogenic or have other long-term toxic effects. See also memos from R. S. Jones to D. Greenway, February 14, 2000 (Ref. 5) and D. Greenway to R. S. Jones, August 7, 2002 (Ref. 7).

Mammalian toxicity data for sucrose octanoate esters are summarized in Table 2.

Table 2. Toxicity data requirements

GUIDELINE NO.	STUDY	RESULTS	MRID NO.
152-23, 870.3700	Teratogenicity	Data waiver granted (see text for details)	NA

d. Effects on the Endocrine System

EPA is required under the FFDCA, as amended by FQPA, to develop a screening program to determine whether certain substances (including all pesticide active and other ingredients) "may have an effect in humans that is similar to an effect produced by a naturally-occurring estrogen, or other such endocrine effects as the Administrator may designate." Following the recommendations of its Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), EPA determined that there was scientific basis for including, as part of the program, the androgen- and thyroid hormone systems, in addition to the estrogen hormone system. EPA also adopted EDSTAC's recommendation that the Program include evaluations of potential effects in wildlife. For pesticide chemicals, EPA will use FIFRA and, to the extent that effects in wildlife may help determine whether a substance may have an effect in humans, FFDCA authority to require the wildlife evaluations. As the science develops and resources allow, screening of additional hormone systems may be added to the Endocrine Disruptor Screening Program (EDSP).

Based on the weight of the evidence of available data, no endocrine system-related effects have been identified for sucrose octanoate esters.

GUIDELINE NO.	STUDY	RESULTS	MRID NO.
		Toxicity Category IV (EP)	446101-04
152-15, 870.2600	Dermal sensitization	Data waiver granted (see text for details) Not a sensitizer	444158-04
152-17, 870.5300	Studies to detect genotoxicity	Data waiver granted (see text for details)	NA
152-18, 870.8700	Cellular immune response	Data waiver granted (see text for details)	NA
152-19, 870.5195	Mammalian mutagenicity test	Data waiver granted (see text for details)	NA
152-20, 870.3100	90-Day Feeding	Data waiver granted (see text for details)	NA

by the registrant demonstrating extremely low mammalian toxicity (Toxicity Category IV) indicate that risk associated with acute exposures by the oral, dermal and inhalation routes would be low to non-existent.

-13 Drinking Water

No drinking water exposure is expected, as sucrose octanoate esters are not soluble in water, do not persist in the environment and biodegrade within approximately five days at approximately 20-27ºC, in both aerobic and anaerobic conditions (Ref. 5). Because sucrose octanoate esters have extremely low toxicity, have been approved for food use by FDA, and are present as direct food additives in many foods, should exposure through drinking water occur, no risk is anticipated.

-12 Other Non-occupational Exposure

The potential for non-dietary exposure to sucrose octanoate esters residues for the general population, including infants and children, is unlikely because potential use sites are commercial, agricultural, and large-scale horticultural. Sucrose octanoate esters constituent sugars and fatty acids are normal parts of the human diet. While there exists a great likelihood of prior exposure for most, if not all, individuals, any increased exposure due to the proposed products would be negligible.

4. Occupational, Residential, School and Day Care Exposure and Risk Characterization

2. Dose Response Assessment

No toxicological endpoints are expected.

3. Dietary Exposure and Risk Characterization

-11 Dietary

-12 Food

Because sucrose octaonate esters are the mono-, di- and tri-esters of sucrose with fatty acids and are derived from sucrose and edible tallow or edible vegetable oils, there is a great likelihood of exposure to sucrose octanoate esters' components for most, if not all individuals, including infants and children. Thus, sucrose octanoate esters may be considered a normal part of the human diet. Because the sucrose octanoate esters' constituent sucrose (table sugar, to which humans and animals are regularly exposed) is the primary photosynthetic product of all higher plants, and the constituent octanoic acid (caprylic acid) is a common fatty acid in plants, any residues of sucrose octanoate esters on treated plants would be indistinguishable from background levels of the compounds (Ref. 4). Toxicological endpoints are not expected; therefore, risk from the consumption of residues is not expected for the general population, including infants and children. An acceptable daily intake (ADI) of sucrose octaonate esters for humans was estimated to be up to16 mg/kg body weight/day, which is equivalent to 2.82 lb (1.28 kg) of sucrose octaonate esters per day for a 176 lb person. In studies with rats ands humans, it was demonstrated that sucrose octaonate esters were rapidly hydrolyzed and absorbed by the body (Ref. 5). To date, there have been no reports of any hypersensitivity incidents or reports of any known adverse reactions in humans resulting from exposure to sucrose octanoate esters. Even if there is a significant increase in exposure to sucrose octanoate esters due to its use as a pesticide, the acute toxicity information and data submitted

Sucrose octanoate esters' components are found naturally in many foods already consumed by infants and children. And, as no toxic endpoints are expected, any hazard is impossible to determine (other than ocular). As a result, EPA has not used a margin of exposure approach to assess the safety of sucrose octanoate esters.

6. Aggregate Exposure from Multiple Routes Including Dermal, Oral, and Inhalation

Aggregate exposure to sucrose octanoate esters by field workers and applicators may occur via oral and dermal routes. These risks are measured via the acute toxicity studies and information submitted to support registration. The oral toxicity information and data for sucrose octanoate esters showed no toxicity (Toxicity Category IV); the risks anticipated from oral exposure are considered minimal. Because the inhalation route is not a likely pathway of exposure, and based on sucrose octanoate esters safety data from the open, technical literature, the risks anticipated for this route of exposure are also considered minimal (Toxicity Category IV).

BPPD concluded that the submitted acute dermal toxicity information indicated no toxicity (Toxicity Category IV). Study results also demonstrated no significant dermal irritation (Toxicity Category IV). Furthermore, BPPD has concluded that sucrose octanoate esters are not skin sensitizers. Based on these results, the anticipated risks from dermal exposure are also considered minimal. Therefore, the risks from aggregate exposure via oral, dermal and inhalation exposure are a compilation of three low risk exposure scenarios and are negligible, when appropriate protective clothing is used.

Aggregate exposure to sucrose octanoate esters by the consumer would include other sources in addition to the limited amount on the agricultural products. Sucrose octanoate esters constituent sugars and fatty acids are normal parts of the human diet. While there exists a great likelihood of prior exposure for most, if not all, individuals, any increased exposure due to the proposed products would be negligible.

Significant additional human exposure to sucrose octanoate esters is not expected in residential, school and day care areas since uses are limited to commercial, agricultural and large-scale horticultural settings.

a. Occupational Exposure

Agricultural use of sucrose octanoate esters is subject to the Worker Protection Standard (WPS), requiring Personal Protective Equipment (PPE), *i.e.*, a long-sleeved shirt, long pants, shoes plus socks, and protective eyewear; and a 48 hour Restricted Entry Interval (REI).

b. Residential, School and Day Care Exposure and Risk Characterization

Because toxicological endpoints are not expected, risk from the consumption of residues is not expected for populations, including infants and children, in residential, school and day care settings.

5. Acute and Chronic Dietary Risks for Sensitive Subpopulations Particularly Infants and Children

FFDCA section 408 provides that EPA shall apply an additional tenfold margin of exposure (safety) for infants and children in the case of threshold effects to account for pre- and post-natal toxicity and the completeness of the database, unless EPA determines that a different margin of exposure will be protective for infants and children. Margins of exposure are often referred to as uncertainty or safety factors. In this instance, based on all the available information, the Agency concludes that sucrose octanoate esters are practically non-toxic to mammals, including infants and children. Thus, there are no threshold effects of concern and, as a result the provision requiring an additional margin of safety does not apply. Further, the provisions of consumption patterns, special susceptibility, and cumulative effects do not apply.

A request for a waiver from the non-target insect studies requirement was adequately supported by a) an acute contact honey bee toxicity study from which the Agency determined that the active ingredient may be classified as practically non-toxic to honey bees (LD_{50} is > 80 μg active ingredient/bee, Ref. 8), and b) three supplemental non-target insect studies obtained from the open technical literature which indicate that sucrose octanoate esters are relatively non-toxic to certain non-target, beneficial, insects (Ref. 5).

As a result of BPPD's assessment of the information and data described above, organism/ecological effects studies were waived for these particular uses of Avachem Sucrose Octanoate [40%]. However, standard precautionary label statements under "Environmental Hazards" are presented on the label.

2. Environmental Fate and Ground Water Data

The need for environmental fate and groundwater data (Tier II, (40 CFR Section 158.690(d)(2)(vii through xv)) was not triggered because the Tier I studies were waived. Risk is minimal due to the lack of exposure, low toxicity, use pattern, and application methods.

3. Ecological Exposure and Risk Characterization

The active ingredient does not persist in the environment and biodegrades within approximately five days at approximately 20-27ºC, in both aerobic and anaerobic conditions. Minimal potential for exposure exists to insects, fish and other non-target wildlife as a result of Avachem Sucrose Octanoate [40%] use.

D. EFFICACY DATA

7. Cumulative Effects

Except through ocular exposure, sucrose octanoate esters are not toxic and it is not anticipated there would be cumulative effects from common mechanisms of toxicity. Risks to eyes can be prevented by the use of required protective eyewear (goggles or face shield).

-18 Risk Characterization

The Agency has considered sucrose octanoate esters in light of the relevant safety factors in FQPA and FIFRA. A determination has been made that no unreasonable adverse effects to the U. S. population in general, and to infants and children in particular, will result from the use of Avachem Sucrose Octanoate [40%] when label instructions are followed.

C. ENVIRONMENTAL ASSESSMENT

1. Ecological Effects Hazard Assessment

The end use product Avachem Sucrose Octanoate [40%] is intended for agricultural and large-scale horticultural use. When applied according to the proposed label directions, no direct exposure of birds or aquatic organisms to Sucrose Octanoate [40%] is expected to occur. Acceptable information/data were submitted from the open technical literature to support the data requirements for avian acute oral toxicity, avian dietary toxicity, freshwater fish LC_{50}, freshwater invertebrate LC_{50}, and non-target plants. Based on the data, the Agency concludes that it is unlikely that any toxic effects will occur in birds, freshwater fish, freshwater aquatic invertebrates, and/or non-target plants when the product containing sucrose octanoate esters is used according to label directions (Ref. 5).

All data requirements have been fulfilled and/or waived by the Agency and the Biopesticides and Pollution Prevention Division recommends unconditional registration of products which contain sucrose octanoate esters as their sole active ingredient.

-12 Tolerances for Food Uses and/or Exemptions

EPA received a pesticide petition (8E4926) from AVA Chemical Ventures, L.L.C., proposing [pursuant to section 408(b)(2)(D) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. section 346], to amend 40 CFR Part 180 by establishing an exemption from the requirement of a tolerance for the biochemical pesticide, sucrose fatty acid esters, in or on all food commodities.

EPA determined the designation "sucrose fatty acid esters" to be too broad. The active ingredient for which the registrant actually petitioned is technically defined as sucrose octanoate esters [(α-D-glucopyranosyl-β-D-fructofuranosyl-octanoate), mono-, di-, and triesters of sucrose octanoate]. Per section II. E. of this document, the tolerance exemption expression established in the associated Final Rule for this new active ingredient will be for "sucrose octanoate esters [(α-D-glucopyranosyl-β-D-fructofuranosyl-octanoate), mono-, di-, and triesters of sucrose octanoate]."

3. CODEX Harmonization

There are no CODEX values for sucrose octanoate esters.

4. Nonfood Re/Registrations

There are no non-food issues at this time. The non-food uses are listed in Appendix A, Table 4.

5. Risk Mitigation

No efficacy data are required, because no public health uses are involved. However, acceptable product performance data were submitted, and demonstrated activity against aphids, pear pyslla and whitefly.

IV. Risk Management Decision

A. DETERMINATION OF ELIGIBILITY FOR REGISTRATION

Section 3(c)(5) of FIFRA provides for the registration of new active ingredients if it is determined that (A) its composition is such as to warrant the proposed claims for it; (B) its labeling and other materials required to be submitted comply with the requirements of FIFRA; (C) it will perform its intended function without unreasonable adverse effects on the environment and (D) when used in accordance with widespread and commonly recognized practice it will not generally cause unreasonable adverse effects on the environment.

To satisfy criteria "A" above, the fatty acid composition of sucrose octanoate esters accounts for its surfactant-type physical mode of action against the target pests, and is not expected to cause unreasonable adverse effects when used according to label instructions. Criteria "B" is satisfied by the current label and by the data presented in this document. It is believed that this new pesticidal active ingredient will not cause any unreasonable adverse effects, and will act as a pesticide to control soft-bodies insects, satisfying Criteria "C." Criteria "D" is satisfied by the data submitted and the products' low toxicity when used according to the label directions.

Therefore, sucrose octanoate esters are eligible for registration. The uses are listed in Table 4, Appendix A.

B. REGULATORY POSITION

1. Unconditional Registration

The Agency has examined the toxicological data base for Avachem Sucrose Octanoate Manufacturing Use Product and Avachem Sucrose Octanoate [40%] and concluded that the proposed precautionary labeling (*i.e.*, Signal Word, First Aid and other label statements) adequately mitigates any risks associated with the proposed uses.

Technical Product Precautionary Labeling: For Avachem Sucrose Octanoate Manufacturing Use Product - "DANGER"

Hazards to Humans and Domestic Animals:
DANGER: CORROSIVE. Causes irreversible eye damage. Do not get in eyes or on clothing. Wear protective eyewear (goggles or face shield). Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash clothing before reuse.

First Aid:

If in eyes:
-Hold eye open and rinse slowly and gently with water for 15-20 minutes.
-Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye.
-Call a poison control center or doctor for treatment advice.

If swallowed:
-Call a poison control center or doctor immediately for treatment advice.
-Have person sip a glass of water if able to swallow.
-Do not induce vomiting unless told to do so by the poison control center or doctor.
-Do not give anything by mouth to an unconscious person.

There exits a risk from ocular exposure. Risks to workers are mitigated by label language requiring protective clothing and a 48-hour re-entry interval.

6. Endangered Species Statement

Given the species-specific action of this biochemical pesticide, the intended use pattern, the results of toxicity and exposure data from the public scientific literature and data submitted by the applicant, the Agency has determined that this action will have no effect on currently listed endangered and threatened species.

C. LABELING RATIONALE

It is the Agency's position that the labeling of Avachem Sucrose Octanoate [40%] and the technical grade active ingredient/manufacturing-use product, Avachem Sucrose Octanoate Manufacturing Use Product, containing, respectively, 40% and 85.43% sucrose octanoate esters, complies with current pesticide labeling requirements.

1. Human Health Hazard

a. Worker Protection Standard

This end-use product comes under the provisions of the Worker Protection Standards (WPS). PPE (long-sleeved shirt and long pants, shoes plus socks, and protective eyewear) and REI (48-hour) required.

b. Non-Worker Protection Standard

There are no non-WPS human health hazard issues.

c. Precautionary Labeling

It is the Agency's position that the labeling for the pesticide product containing sucrose octanoate esters complies with current pesticide labeling requirements. The Agency has not stipulated a maximum number of applications for the active ingredient. The proposed label specifies application rates a) between 0.8% and 1.0% volume/volume (v/v) for foliarly applied spray, b) between 1.25% and 2.50% v/v for mushroom growing media, and c) of 0.625% v/v for application to honey bees. The diluent is water. For foliar uses, the finished spray solution may be applied at seven to ten day intervals, up to and including the day of harvest. Mushroom growing media (casing and/or compost) is to be treated prior to spawning. Applications to adult honey bees may be repeated three times per infestation (the limit stipulated by the applicant), at seven to ten day intervals.

D. LABELING

(1) Product name: **Avachem Sucrose Octanoate Manufacturing Use Product**

Active Ingredient:
Sucrose Octanoate Esters (α-D-Glucopyranosyl,β-D-fructofuranosyl-octanoate), mono, di-, and triesters of sucrose octanoate..... .....85.43%

Other Ingredients:. 14.57%

Total 100.00%

Signal word is "DANGER." Ocular exposure risk precautions are appropriate.

The product shall contain the following information:
- Product Name
- Ingredient Statement

End-Use Product Precautionary Labeling: For Avachem Sucrose Octanoate [40%] - "WARNING."

Hazards to Humans and Domestic Animals:

Causes substantial but temporary eye injury. Do not get in eyes or on clothing. Wear protective eyewear (goggles or face shield). Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash clothing before reuse.

First Aid:

If in eyes:
-Hold eye open and rinse slowly and gently with water for 15-20 minutes.
-Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye.
-Call a poison control center or doctor for treatment advice.

If swallowed:
-Call a poison control center or doctor immediately for treatment advice.
-Have person sip a glass of water if able to swallow.
-Do not induce vomiting unless told to do so by the poison control center or doctor.
-Do not give anything by mouth to an unconscious person.

2. Environmental Hazards Labeling

End-Use Product Environmental Hazards Labeling: Although sucrose octanoate esters are considered non-toxic to the environment, the environmental hazards statement is nevertheless required on the end-use product's label.

3. Application Rate

Table 4 lists the use sites for the end-use product. The labels for both products are also attached.

Table 4. End-Use Product Name, Use Sites, Registration/Reregistration

Avachem Sucrose Octanoate [40%] Use Sites: Field Crops (including certain non-food ornamentals), Mushroom Growing Media, Adult Honey Bees	Official date registered:

VII. References

11 USEPA; Amendment of the January 14, 1997, Classification Committee decision on sucrose fatty acid esters. R. S. Jones, July 2, 2002.

12 Barrington, T. and C. L. Hartman. Sucrose Fatty Acid Esters-Safety Data in Support of Petition Proposing a Temporary (sic) Exemption From the Requirement of a Tolerance for Use in All Food Commodities (MRID 444158-03), October 2, 1997.

13 Barrington, T. and W. L. Biehn. Sucrose Fatty Acid Esters-Safety Data in Support of Petition Proposing an Exemption From the Requirement of a Tolerance for Use in All Food Commodities, Amendment No. 1 to MRID 444158-03, July 13, 1998

14 USEPA; Brief Summary of Toxicity Information to Support Registration/Tolerance Exemptions for Sucrose Octanoate. R. S. Jones to D. Greenway; August 8, 2002.

- Registration Number
- Signal Word (DANGER)

(2) Product name: **Avachem Sucrose Octanoate [40%]**

Active Ingredient:
Sucrose Octanoate Esters (α-D-Glucopyranosyl,β-D-fructofuranosyl-octanoate), mono, di-, and triesters of sucrose octanoate..... .40.0%
Other Ingredient:. 60.0%

Total 100.00%

Signal word is "WARNING." Ocular exposure risk precautions are appropriate.

The product shall contain the following information:
- Product Name
- Ingredient Statement
- Registration Number
- Signal Word (WARNING)

V. Actions Required by Registrants

There are no data requirements, label changes or other responses necessary for the reregistration of the end-use product since the product is being registered after November 1984 and is, therefore, not subject to reregistration. There are also no existing stocks provisions at this time.

VI. Appendix A

Ingredient Fact Sheets
Registration Tools
Plant Incorporated Protectants (PIPs)
Regulation of Biotechnology
Other Biopesticides

Sucrose Octanoate Esters (035300)

| Fact Sheet | Technical Doc | Products | Registrants | Regulatory Activity | FR Notices | Bibliography |

SUMMARY

Sucrose octanoate esters are intended to control mites and certain soft-bodied insects (e.g., aphids) at three distinct commercial sites: food and non-food crops, including certain ornamentals; media for growing mushrooms; and adult honey bees. These kinds of sucrose esters are found naturally in plants, and were originally isolated from the hairs present on tobacco leaves. Since 1983, the US Food and Drug Administration (FDA) has allowed sucrose octanoate esters to be added to certain processed foods. No harmful effects to humans or the environment are expected from the use of sucrose octanoate esters in pesticide products.

Issued: September 2002

I. DESCRIPTION OF THE ACTIVE INGREDIENT

Sucrose octanoate esters exist as an amber-colored liquid. The mixture of esters is manufactured from two biochemicals–sucrose (table sugar) and an octanoic acid ester (commonly found in plants and animals). Sucrose esters were isolated when researchers investigated the insecticidal properties of the leaf hairs on tobacco leaves. The active ingredient acts by dissolving the waxy protective coating (cuticle) of target pests, causing the insect or mite to dry out and die. (CAS Nos. 42922-74-7 and 58064-47-4)

II. TARGET PESTS, USE SITES, AND APPLICATION METHODS

Target Pests and Use Sites:

1) Mites and soft-bodied insects on food and non-food crops, including certain ornamentals. 2) Immature forms of certain species of gnats found in media used for growing mushrooms. Whether a substance poses a risk to humans or other organisms depends on two factors: how toxic the substance is, and how much of it an organism is exposed to. Therefore, the EPA considers toxicity data and exposure data in deciding whether to approve a pesticide for use. 3) Varroa mites on adult honey bees.

15 USEPA; Science review in support of registration of sucrose octanoate esters. R. S. Jones to D. Greenway, February 14, 2000.

16 Barrington, A., Waiver Request; July 12, 2002.

17 USEPA; Sucrose Octanoate Esters; a Request for Concurrence on a Decision to Waive the Requirement for 90-Day Feeding (152-20) and Teratogenicity (152-23) Studies, Based on the Registrant's Correspondence of July 12, 2002. D. Greenway to R.S. Jones; August 7, 2002.

18 USEPA; Science review in support of registration of sucrose octanoate esters. R. S. Jones to D. Greenway, January 23, 2001.

Last updated on Wednesday, January 15th, 2003
URL: http://www.epa.gov/pesticides/biopesticides/ingredients/factsheets/factsheet_035300.htm

Application Method: Spray with ground equipment.

III. ASSESSING RISKS TO HUMAN HEALTH

No risks to humans are expected from the use of sucrose octanoate esters as a pesticide active ingredient. Sucrose octanoate esters are not toxic to mammals, but in high concentrations are corrosive to the eye. To avoid irreversible eye damage, exposed workers are required to wear appropriate protective clothing.

IV. ASSESSING RISKS TO THE ENVIRONMENT

No risks to the environment are expected from the use of sucrose octanoate esters in pesticide products because a) the esters biodegrade rapidly and therefore do not persist in the environment, b) the esters are not toxic to mammals or other non-target organisms, c) organisms are already exposed because these sucrose esters are found in plants, and d) the tiny amounts used in pesticide products are not expected to substantially increase the amount of these esters in the environment.

V. REGULATORY INFORMATION

Year initially registered as an active ingredient: September, 2002
Number of end-use products (September, 2002): 1
End-use product name: Avachem Sucrose Octanoate [40.0%]

VI. REGISTRANT INFORMATION

AVA Chemical Ventures, L.LC.
80 Rochester Avenue Suite 214
Portsmouth, NH 03801
(603) 431-4242

VII. FOR FURTHER INFORMATION, CONTACT

Denise L. Greenway
Regulatory Action Leader
Biopesticides and Pollution Prevention Division (7511C)
Office of Pesticide Programs
Environmental Protection Agency
1200 Pennsylvania Avenue NW
Washington, DC 20460
Phone (703) 308- 8263 (or 308-8712)
Fax (703) 305-0118 or (703) 308-7026
e-mail: greenway.denise@epa.gov

The EPA Biopesticides Website is: http://www.epa.gov/pesticides/biopesticides

DISCLAIMER: *The information in this Biopesticide Fact Sheet is a summary only. Consult the person listed above or the Biopesticides Web Site for more information.*

eral) Spray Oil (Safer, Newton, MA), and a detergent-like acylsugar extracted from *Nicotiana gossei* Domin obtained from the Phytochemistry Research Laboratory, USDA–ARS, Athens, GA, and prepared as recommended (L. Smith, personal communication) (Liu & Stansly 1994). A pyrethroid, bifenthrin (Brigade 10 WP [wettable powder], FMC, Middleport, NY), was tested for comparison and purified tap water (7 ppm dissolved solids) was used as a control. The concentrations of each insecticide for each whitefly stage varied based on our preliminary tests: M-Pede, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, and 8.0% vol:vol; Sunspray Oil, 0.025, 0.05, 0.1, 0.5, 1.0, 2.0, and 3.0% vol:vol; *N. gossei* extract, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, and 10.0 g (AI)/liter; and bifenthrin, 0.015, 0.03, 0.06, 0.12, 0.24, and 0.48 g (AI)/liter (see related tables). The extremely high and low rates were applied to have high and low whitefly mortalities for the probit analyses of LC_{50} and LC_{90}.

Whiteflies and Plants. *B. argentifolii* used in this study was obtained from D. Schuster in Bradenton, FL, in 1990, and was identified as *B. tabaci* 'Biotype B' in 1992 (T. M. Perring, University of California at Riverside, personal communication) and as *B. argentifolii* in 1994 (A. C. Bartlett, USDA–ARS, Phoenix, AZ, personal communication). The colony was maintained in established greenhouse culture on potted tomato plants, *Lycopersicum esculentum* Miller, 'Lanai' (one in each 15-cm pot), grown in Metro-Mix 300 growing medium (Grace Sierra, Horticultural Products, Milpitas, CA). Plants were fertilized with a slow-release fertilizer (N:P:K, 12:8:6) (Diamond R Fertilizer, Winter Garden, FL).

Adults. Tomato leaves bearing whitefly pupae were collected 3 d before the test and placed in a wooden framed cage (30 by 30 by 30 cm) with sides covered in 60-mesh nylon screen and the top covered with clear vinyl film. For the bioassay, newly emerged whitefly adults were collected using an aspirator and were placed into 0.9-liter, clear, plastic cup cages with a 9-cm screened opening on top and a corked access hole (1.2 cm in diameter) on the side.

Immature Stages. Whitefly-free tomato plants were placed in the whitefly colony and infested with adults by agitating adjacent plants. After an oviposition period of 24 h, the newly infested plants were removed from the colony and cleaned of adults using a hand-held vacuum cleaner (AC Insect Vac, BioQuip, Gardena, CA). The egg-bearing leaves were incubated in whitefly-free cages at 25 ± 2°C, 75% RH, and a photoperiod of 14:10 (L:D) h until the appropriate nymphal stages were ready for treatments. Three whitefly developmental stages were obtained and used in the tests: eggs (24 h old), young nymphs (7 d old, most were first instars), and old nymphs (14 d old, most were third instars).

Bioassays. Tomato leaves (trifoliates) were treated by dipping for 5 s in the appropriate solutions, then air-dried for 2 h, and placed individually into glass vials (petiole down) filled with 20 ml of water. A vial was secured in the center of a cup cage with double-stick cellophane tape. For residue toxicity bioassays of adults, 15 unsexed individuals were introduced into the cup-cage following a 1, 2, or 5 d waiting period. Each treatment had eight cup cages (one cage as a replicate) with a total of 360 whiteflies. The experiment was repeated three times. Live and dead adults were recorded after 24 h under a stereo microscope. Adults were considered dead if no movement was detected when touched with a needle.

For bioassay of young nymphs, the treated leaf was placed in the vial filled with water inside the cage for 4 d, and later examined using a dissecting microscope. An average of 54 (SD = 14) young nymphs per leaf were examined. Nymphs which had dried or detached from the leaf surface were considered dead. For bioassays of old nymphs (third instars or older), treated leaves were caged for 10–14 d to allow surviving nymphs to pupate before scoring for dead and live nymphs. Number of old nymphs on each leaf was averaged 67 (SD = 33). Each treatment had eight leaves, and the experiment was repeated three times.

For bioassays of whitefly eggs, treated egg-bearing leaves were incubated individually in vials placed in cages and incubated for 7 d. Number of eggs on each leaf was averaged 94 (SD = 63). The experiment had eight replicates and was repeated three times. An egg was considered to have hatched when microscopic examination revealed that the crawler had successfully eclosed and separated itself from the chorion.

All experiments were conducted in a laboratory, and all treated leaves were then kept in an insectary at 25 ± 2°C, 70 ± 5% RH, and illuminated with fluorescent lights set for a photoperiod of 14:10 (L:D) h.

Data Analysis. Mortalities (percentages) of adults and nymphs were transformed to the arcsine square root [arcsine (percent mortality/100)$^{1/2}$] before analysis to stabilize error variance (Steel & Torrie 1960, Gomez & Gomez 1984), and mean mortalities were analyzed using analysis of variance (ANOVA), and were separated using the least significant difference (LSD) test following a significant F test. Although all tests of significance were based on the transformed data, we report the untransformed percent mortality (percent mean ± SD). Regression analysis (PROC REG procedure) was used for toxicity test data for eggs, and slopes of two insecticides were compared using PROC GLM procedure with two dummy variables. LC_{50} and LC_{90} were computed using a probit procedure (PROC PROBIT LOG10, SAS Institute 1988).

Results and Discussion

Adults. Residues of Sunspray oil at concentrations of 0.5, 1.0, and 2.0% proved to be the most

Toxicity of Biorational Insecticides to *Bemisia argentifolii* (Homoptera: Aleyrodidae) on Tomato Leaves

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J. Econ. Entomol. 88(3): 564–568 (1995)

ABSTRACT Bioassays were conducted to test the toxicity of insecticide leaf residue to adults, and contact toxicity to eggs and nymphs of silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring. Four insecticides were tested: insecticidal soap (Sunspray oil), mineral oil (M-Pede), extract of *Nicotiana gossei* Domin (a detergent-like acylsugar), and bifenthrin (a pyrethroid). Purified tap water was used as a control. Bioassays of adults were conducted by dipping whitefly-free tomato leaves into serial dilutions of the insecticides, air-drying for prescribed periods, and exposing adults to leaves in large cup cages for 24 h. Residues of Sunspray oil caused greatest mortality to adults for up to 5 d after treatment, and the LC_{50} of 24 h residue to adults was 0.029%. Two-hour leaf residues of bifenthrin at the field rate (0.06 g [AI]/liter) or higher (0.12–0.24 g [AI]/liter) gave >68% mortality of adults, but efficacy was reduced with residues of 24 h (LC_{50} = 0.034 g [AI]/liter) or older. Dried residues of insecticidal soap and *N. gossei* extract were not effective on adults. Contact bioassays were also conducted on tomato leaves infested with uniform cohorts of eggs or nymphs. Response patterns to insecticides were similar among developmental stages of the whitefly, with young nymphs being the most susceptible, followed by older nymphs and eggs. LC_{50}s of Sunspray oil to young and old nymphs were 0.032 and 0.088%, and of bifenthrin were 0.001 and 0.106 g (AI)/liter, respectively. Insecticidal soap and *N. gossei* extract were all effective on young nymphs, even at very low rates (LC_{50}, 0.15% and 0.08 g [AI]/liter, respectively), but had no significant effect on eggs. *N. gossei* extract was effective on older nymphs at low rates (LC_{50} = 0.14 g [AI]/liter), whereas insecticidal soap was not (LC_{50} = 0.51%).

KEY WORDS sweetpotato whitefly, silverleaf whitefly, insecticide

SILVERLEAF WHITEFLY,[1] *Bemisia argentifolii* Perring & Bellows, formerly known as sweetpotato whitefly, *B. tabaci* (Gennadius) strain B, is a key insect pest of vegetables, field crops, and ornamental crops in the southern United States. Damage results from plant debilitation, sooty mold growth, and, in tomato, irregular ripening and transmission of tomato mottle geminivirus (TMoV) (Stansly & Schuster 1990). Crop damage was estimated at >500 million dollars in the United States in 1991 (Perring et al. 1993), and yield reduction from irregular ripening and geminivirus plus control costs for Florida tomato alone were estimated at $125 million for the 1990–1991 season (Schuster et al. 1995). Intensive use of broad-spectrum insecticides incurs economic, health, and environmental costs and may cause pest resurgence and secondary pest outbreaks through decimation of natural enemies. Furthermore, documented loss of susceptibility by *B. tabaci* to some of the most commonly used insecticides suggests that their efficacy will be of limited duration (Prabhaker et al. 1985, 1992; Stansly & Schuster 1992). Therefore, it is necessary to develop insecticides with alternative modes of action that do not obviate the activity of natural enemies.

Mineral oils, detergents, and insecticidal soaps have demonstrated efficacy against *B. tabaci* on cotton and several vegetable crops under field conditions (Butler et al. 1988, 1989, 1993; Stansly & Vavrina 1993). These biorationals were used to control greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), on vegetable and ornamental crops under greenhouse conditions (Larew & Locke 1990, Buta et al. 1993). However, their activity on particular whitefly stages has not been reported in detail. The aim of this study was to evaluate the residual toxicity of potential biorational insecticides on adults and the contact toxicity on eggs and nymphs of *B. argentifolii* on tomato plants under laboratory conditions.

Materials and Methods

Insecticides. Three biorational insecticides were used: M-Pede, an insecticidal soap (49% potassium salt of a naturally derived fatty acid) (Mycogen, San Diego, CA), Sunspray Ultra-Fine (min-

[1] The name has not been approved for use by the ESA Committee on Common Names of Insects.

0022-0493/95/0564–0568$02.00/0

Table 3. Summary of toxicity of insecticides to *B. argentifolii* on tomato leaves in laboratory bioassays

Insecticide	*n*	Slope ± SEM	LC_{50}	95% FL	LC_{90}	95% FL	χ^2
			Adults				
Bifenthrin[a]	2,160	0.90 ± 0.11	0.034	0.023–0.045	0.906	0.524–2.123	6.8
Sunspray oil	2,160	2.12 ± 0.46	0.290	0.130–0.620	1.180	0.570–1.440	18.9[b]
M-Pede[c]	NA	NA	NA	NA	NA	NA	NA
N. gossei extract[d]	2,160	1.53 ± 0.26	5.878	3.727–12.47	40.60	16.97–56.30	14.0[b]
			Young nymphs				
Bifenthrin	5,694	0.87 ± 0.09	0.001	0.001–0.002	0.032	0.023–0.059	0.9
Sunspray oil	4,992	1.01 ± 0.10	0.032	0.018–0.050	0.594	0.348–1.293	10.4
M-Pede	4,992	1.71 ± 0.15	0.149	0.110–0.197	0.836	0.577–1.400	9.4
N. gossei extract	4,581	1.35 ± 0.09	0.076	0.061–0.091	0.678	0.526–0.925	6.2
			Old nymphs				
Bifenthrin	5,980	1.23 ± 0.11	0.106	0.087–0.132	1.171	0.759–2.146	2.0
Sunspray oil	4,112	1.35 ± 0.16	0.088	0.051–0.139	0.783	0.454–1.841	10.5
M-Pede	3,607	2.22 ± 0.18	0.507	0.433–0.584	1.918	1.589–2.436	0.7
N. gossei extract	3,793	1.66 ± 0.17	0.142	0.098–0.199	0.841	0.541–1.622	8.3

NA, not applicable.
[a] Units: bifenthrin and *N. gossei* extract are in g (AI)/liter, and Sunspray oil and M-Pede are in percent concentration (vol:vol).
[b] $\chi^2 > 12.6$ (tabular χ^2 with df = 6, $P = 0.05$).
[c] Mortalities were too low to compute LC_{50} and LC_{90} values.
[d] Concentration of 0.59% would cause severe phytotoxicity on tomato leaves.

= 0.15%) but only at high rates to old nymphs (LC_{50} = 0.51%). *N. gossei* extract was effective against both young and old nymphs (LC_{50}s ≈0.1 g [AI]/liter).

All test insecticides at recommended field rates gave excellent control to young nymphs with mortalities >90% (Table 4). Bifenthrin, however, gave the lowest mortality (38.1%) to old nymphs, followed by M-Pede (72.1%). Sunspray oil and *N. gossei* extract gave best control on old nymphs.

We observed that the nymphs treated with M-Pede and *N. gossei* extracts dried quickly and detached from the leaf surface, with dorsal and ventral surfaces of the body compressed together. Nymphs killed by bifenthrin also dried eventually though not as quickly. These nymphs did not detach from the leaf surface, nor did the dorsal and ventral surfaces of the body compress together. Effectiveness of all four insecticides on old nymphs was similar to young nymphs except for bifenthrin and low rates of M-Pede. Thus our impression was that M-Pede and *N. gossei* extract were killing nymphs by desiccation in contrast to bifenthrin where the nymphs appeared to desiccate subsequent to death.

Table 4. Toxicity of insecticides to *B. argentifolii* nymphs at recommended field rates on tomato leaves in laboratory

Treatment	Rate	% mortality ± SD	
		Young nymphs	Old nymphs
Bifenthrin	0.06 g (AI)/liter	92.1 ± 5.0a	38.1 ± 11.1c
Sunspray oil	1.0%	90.4 ± 8.6a	88.8 ± 5.9a
M-Pede	2.0%	97.0 ± 3.3a	72.1 ± 9.7b
N. gossei extract	1.0 g (AI)/liter	92.0 ± 8.4a	88.7 ± 3.1a
Water		3.0 ± 3.2b	6.4 ± 4.7d

Means followed by the same letter for each insecticide in the same column are not significantly different ($P > 0.05$, LSD, SAS Institute 1988).

The highest rate (3%) of Sunspray oil and the higher rates (>0.2%) of *N. gossei* extract caused obvious phytotoxicity to young tomato leaves producing irregular chlorotic spots, desiccated margins, or total desiccation. Severe phytotoxicity led to dried leaves. No phytotoxicity was noticed on the leaves from any other treatments.

We were able to achieve a uniform, standardized, and repeatable index of contact toxicity of insecticides with a wide range of activities using the leaf dip method. Rosenheim & Hoy (1986) and Spollen & Hoy (1993) have claimed that the leaf dip method yields reliable predictions of the relative field mortality of different insecticides. Our results also indicate the potential usefulness of oils and surfactants for control of *B. argentifolii*. We believe these materials could play an important role in the integrated pest management *B. argentifolii* due to their distinct modes of action to conventional insecticides and their possible selective characteristics. Additional research is needed to compare results from leaf-dip bioassays with bioassays employing spray techniques which achieve different degrees of coverage, and to test the effects of these materials on natural enemies of *B. argentifolii*.

Acknowledgments

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Table 1. Residual toxicity of insecticides to *B. argentifolii* adults at various intervals after exposure for 24 h on tomato leaves dipped in insecticide solutions

Treatment	Rate	% mortality after treatments ± SD			
		2 h	1 d	2 d	5 d
Bifenthrin	0.03	53.3 ± 19.1d	44.4 ± 15.9bc	38.9 ± 20.9bc	43.9 ± 12.5cd
(g [AI]/liter)	0.06	68.9 ± 12.5c	52.2 ± 14.4bc	49.4 ± 18.3bc	49.4 ± 10.8cd
	0.12	86.7 ± 9.4b	63.3 ± 12.9b	60.0 ± 15.8bc	61.7 ± 7.6bc
Sunspray oil	0.5%	97.8 ± 4.3a	87.2 ± 12.2a	81.7 ± 20.5a	84.4 ± 13.4ab
(vol:vol)	1.0%	98.3 ± 5.8a	87.8 ± 11.3a	83.3 ± 13.8a	77.8 ± 21.5ab
	2.0%	99.4 ± 1.9a	94.4 ± 6.2a	92.8 ± 8.3a	85.0 ± 15.3a
M-Pede (vol:vol)	0.5%	6.7 ± 7.5g	8.3 ± 8.1f	7.2 ± 10.4d	8.9 ± 5.2e
	1.0%	10.0 ± 17.2g	12.8 ± 14.3ef	10.0 ± 9.6d	9.4 ± 8.7ef
	2.0%	10.6 ± 9.2g	14.4 ± 10.9ef	12.8 ± 12.2d	6.1 ± 9.2ef
N. gossei extract	0.5	20.0 ± 11.7f	9.4 ± 7.2ef	7.8 ± 8.0d	8.3 ± 9.9ef
(g [AI]/liter)	1.0	36.7 ± 16.2e	17.8 ± 8.7de	19.4 ± 10.4cd	14.4 ± 8.9e
	2.0	52.2 ± 20.3d	29.4 ± 13.5cd	34.4 ± 13.9bc	26.7 ± 11.4d
Water	—	8.5 ± 10.8g	3.1 ± 5.0f	4.3 ± 7.1d	7.0 ± 6.8f

Mean mortalities (%) followed by the same lowercase letters in the same column are not significantly different, based on analysis of transformed data ($P > 0.05$, LSD, SAS Institute 1988).

effective treatments against whitefly adults for up to 5 d after treatment (Table 1). Sunspray oil was effective against adults giving LC_{50} and LC_{90} values of 0.29 and 1.20%, respectively (Table 2). However, the chi-square value was greater than tabular value (12.6 at df = 6, $P = 0.05$) indicating that the data did not fit the probit model.

Adult response to Sunspray oil departed most from the model between the concentrations of 0.25 and 0.5% which caused 23.3 and 87.2% mortality, respectively. We found large number of adults contacting the residue were trapped and died. Butler et al. (1989) made a similar observations. Possibly, there is a threshold thickness of the oil film, below which whiteflies are not trapped. We found dead adults on both upper and lower leaf surfaces, suggesting that death occurred after contact with oil residues. Two-hour leaf residues of bifenthrin caused 86.7% mortality at the highest rate (0.12 g [AI]/liter), but as time increased, effectiveness decreased slightly. Mortality at lower rates of bifenthrin (0.03 and 0.06 g [AI]/liter) from leaf residues older than 24 h caused only 40–60% mortality. Toxicity bioassays of bifenthrin gave similar results (LC_{50} = 0.034 g [AI]/liter). Dry residues of M-Pede had little or no effect on adults compared with the water treatment. Previous studies (Butler et al. 1993, Liu & Stansly 1994) showed that soap and other surfactants function primarily when wet. All rates at 2 h, two higher rates at 1 d and the highest rate at 2 and 5 d of *N. gossei* extract still gave significantly higher mortality than the water controls. However, goodness-of-fit test indicated that the data set did not fit the probit model ($\chi^2 > 12.6$). The lack of fit was resulted from the poor rate response even at high concentrations (29.4% mortality at 4.0 g [AI]/liter).

Eggs. The egg was the least susceptible stage to all test insecticides (Table 2). However, Sunspray oil gave 63.6% egg mortality at recommended field rate of 1.0%, and 28.9% at 0.1%. We found that many first-instar crawlers died while attempting to eclose from oil-sprayed leaves, indicating that activity was not primarily upon the egg itself. Bifenthrin had slight toxicity against eggs, causing 30.5% mortality at recommended field rate of 0.06 g (AI)/liter (equivalent to 0.05 lb[AI]/100 gal/acre). M-Pede was not effective to eggs except for the highest rate (3.0%), which caused 25.3% mortality. Regression analysis indicated the relationship between the concentrations ($\log_{10}$[c]) and percent mortalities in M-Pede were not significant ($P > 0.05$). Slopes among the three insecticides were not significantly different (F values: 0.03–0.64; $P > 0.05$).

Nymphs. All test insecticides were effective on young nymphs (Table 3). Bifenthrin was extremely toxic to young nymphs with LC_{50}s = 0.001 g (AI)/liter, but less toxic to old nymphs (LC_{50}s = 0.106 g [AI]/liter). Sunspray oil gave excellent control of both young and old nymphs with LC_{50}s < 0.1%. M-Pede was effective against young nymphs (LC_{50}

Table 2. Toxicity of insecticides to *B. argentifolii* eggs on tomato leaves in the laboratory (concentrations [c] were transformed to log10[c])

Insecticide	Intercept ± SEM	Slope ± SEM	R^2	df	P
Bifenthrin (g [AI]/liter)	47.3 ± 5.3	19.2 ± 4.1	0.88	118	0.018
Sunspray oil (%, vol:vol)	55.0 ± 2.6	26.6 ± 4.8	0.91	118	0.012
M-Pede (%, vol:vol)	14.2 ± 2.3	10.1 ± 5.5	0.53	118	0.164

Slopes of insecticides are not significantly different with F values at 0.25–0.73; df = 1, 236; $P > 0.05$ (SAS Institute 1988).

Activity of Sugar Esters Isolated from Leaf Trichomes of *Nicotiana gossei* to Pear Psylla (Homoptera: Psyllidae)

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ABSTRACT Insecticidal activity of a sugar ester fraction isolated from leaf trichomes of wild tobacco, *Nicotiana gossei* Domin, to egg, nymph, and adult stages of pear psylla, *Cacopsylla pyricola* Foerster, was determined in replicated laboratory bioassays. Aqueous solutions of sugar ester concentrations ranging from 62 to 1,000 ppm (mg/liter) were applied by an ultra-low-volume spray device to petri dishes containing eggs, nymphs, or adults. Data were recorded on nymphal mortality rates 1, 3, 5, and 7 d after treatment, on the percentage of eggs hatched 7 d after treatment, on the mortality rates of eclosed nymphs 3 d after eclosion (10 d after treatment), and on adult mortality rates 1 d after treatment. Mortality rates did not differ significantly for sugar ester concentrations of 500 and 1,000 ppm, which both produced a mortality rate of ≥94% for nymphs and adults 1 d after treatment. Lethal concentration values for sugar esters to pear psylla nymphs and adults differed significantly 1 d after treatment for the LC_{50} (90 versus 200 ppm, respectively), but not for the LC_{90} (300 versus 400 ppm, respectively). Nymphal mortality rates for each sugar ester concentration did not increase over time significantly 1 d after application, which suggests that the sugar ester is a contact insecticide that is active mainly in the liquid state. The percentage of eggs hatched was not affected by any of the sugar ester treatments 7 d after application. In contrast, the mortality rates of newly eclosed nymphs ranged from 18.7 to 67% for sugar ester concentrations of 62–1,000 ppm. Our data suggest that both nymphs and adults would be equally controlled with sugar ester concentrations high enough to obtain a mortality rate of >90%. These results will be useful in determining the range of sugar ester concentrations for field trials. In addition, an inexpensive ultra-low-volume spray device used in the bioassays is described that is capable of applying microliter amounts of candidate insecticidal materials to target insects.

KEY WORDS *Cacopsylla pyricola*, sugar ester, spray device

PLANT TRICHOMES CONFER resistance to insects by a variety of mechanisms including feeding and ovipositional deterrence, toxic effect, or insect entrapment (Norris & Kogan 1980). The Solanaceous plant, *Nicotiana gossei* Domin, possesses glandular leaf trichomes that confer resistance to the green peach aphid, *Myzus persicae* (Sulzer) (Thurston & Webster 1962), the tobacco hornworm, *Manduca sexta* (L.) (Farr & Thurston 1968, Thurston 1970), and the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Neal et al. 1987). Resistance was thought to be attributed to alkaloids such as nicotine, nornicotine, and anabasine within the trichome exudate (Thurston et al. 1966). However, more detailed chemical analyses and bioassays on constituents of the trichome exudate from *N. gossei* has determined that the primary active compound was a sugar ester fraction comprised of two glucose esters and two sucrose esters. Buta et al. (1993) identified the two sucrose esters as 2,3-di-O-acyl-6′-O-acetylsucrose and 2,3-di-O-acyl-1′,6′-diacetylsucrose. Later, Severson et al. (1994) identified the glucose esters as 1-O-acetyl-2,3-di-O-acylglucose, and 2,3-di-O-acylglucose, with the acyl groups comprised of 5-methylheptanoic acid. Each of these individual sugar ester compounds was equally toxic to *T. vaporariorum* nymphs and produced mortality rates of ≥94% when concentrations of 0.1% in aqueous solution were topically applied (Buta et al. 1993). When the sugar ester fraction containing a mixture of all four sugar esters was applied at 0.1% concentration, it was also found to produce mortality rates of ≥94% for *Bemesia tabaci* (Grennadius) and *T. vaporariorum* nymphs and *M. persicae* adults (Neal et al. 1994, Severson et al. 1994). Although the mode of action is not known, topical applications of crude trichome exudates from *N. gossei* rendered the cuticle of *M. sexta* larvae transparent; this was followed by rapid loss of body fluids before death

This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by USDA.

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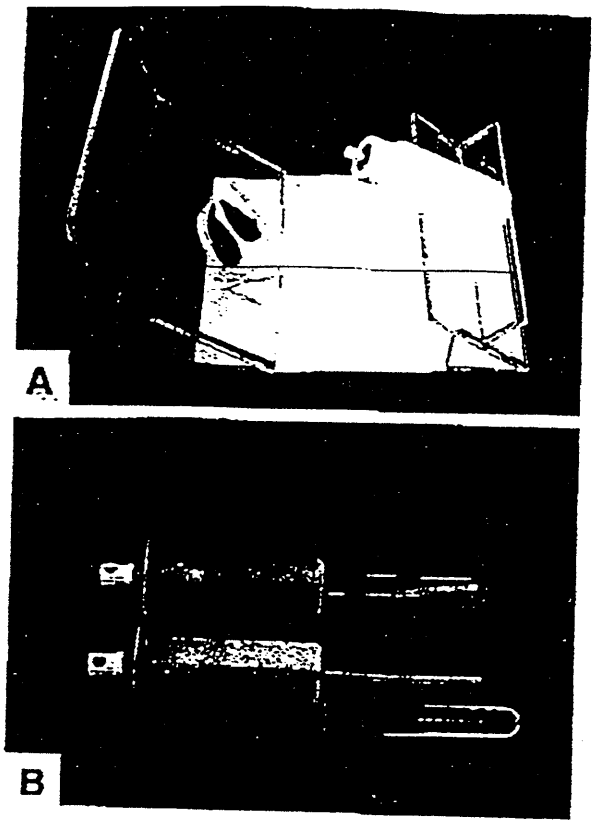

Fig. 1. Components of the ultra-low-volume spray device. (A) Spray platform with pressurizable spray bottle and petri dish with infested leaves held at the proper distance and angle to ensure consistent repeatable applications. (B) Spray bottle nozzle-pump body fitted with test tube (12 by 75 mm) that holds test solutions (blueprints available from G.J.P.).

tized. They were provided a moisture source by adhering one-half of a piece of Whatman No. 3 filter paper dipped in sterile ddH_2O to the petri dish lid. The adult bioassay dishes were not sealed with parafilm to prevent moisture condensation from entrapping and killing the adults. Treated bioassay dishes were kept in an environment of 25°C, and a photoperiod of 16:8 (L:D) h was used for the duration of the study.

Data were recorded on nymphal mortality rates 1, 3, 5, and 7 d after treatment, on the percentage of eggs hatched 7 d after treatment, on the mortality rates of eclosed nymphs ≤3 d after eclosion (10 d after treatment), and on adult mortality rates, 1 d after treatment. Six replications of each egg-nymph or adult combination of sugar ester treatments were conducted in a completely randomized block experimental design replicated in time.

Statistical Analysis. Concentration–mortality probit regressions for nymph and adult data were calculated for 1 d after treatment using SAS PROBIT (SAS Institute 1988). Lethal-dose ratios were used to determine significant differences between nymph and adult LC_{50}s and LC_{90}s at $P = 0.05$ (Robertson & Preisler 1992). Analysis of variance (ANOVA) for a completely randomized block design (SAS Institute 1988) was conducted on egg, nymph, and adult data. Significant differences in mortality rates among sugar ester concentrations within a life stage on 1 d after treatment was determined using the least significant difference (LSD) method at $\alpha = 0.05$ (SAS Institute 1988) after the data was transformed by arcsine $\sqrt{Y}$ to standardize mean percentages (Gomez & Gomez 1984).

Table 1. Pear psylla nymphal and adult mortality rates 1 d after treatment with the sugar ester in the laboratory

Sugar ester concn (ppm)	% nymphal mortality rate ± SEM	% adult mortality rate ± SEM
1,000	99.0 ± 1.2a	99.3 ± 1.6a
500	95.0 ± 2.5ab	94.3 ± 7.3a
250	84.0 ± 8.7b	83.6 ± 5.3b
125	60.3 ± 8.0c	23.6 ± 3.8c
62	30.2 ± 11.2d	10.7 ± 4.3d
0	2.3 ± 3.8e	1.3 ± 2.0e
	LSD = 11.2	LSD = 5.1

Six replications. Means within columns followed by the same letter are not significantly different ($P > 0.05$, LSD).

Results and Discussion

Nymphal and Adult Mortalities. All sugar ester concentrations caused significant nymphal and adult mortalities compared with the water controls 1 d after treatment (Table 1). Mortality rates did not differ significantly for sugar ester concentrations of 500 and 1,000 ppm, which both produced mortality rates of ≥94% for nymphs and adults 1 d after treatment. The psylla mortality rate obtained from 1,000 ppm sugar ester was considerably higher than the 73% mortality rate for this same sugar ester fraction and concentration used against *T. vaporariorum* nymphs (Buta et al. 1993). However, that study obtained nymphal mortality rates of ≥94% when each of the four sugar ester components were individually evaluated. Sugar ester solutions at concentrations of 500 and 1,000 ppm had remarkable surfactant properties in that the solutions spread uniformly over the leaf or Petri dish surface; the lower concentrations beaded on the leaf surface. This may be one reason why the sugar ester was not as effective at the lower concentrations. Lethal concentration values for sugar esters on pear psylla nymphs and adults differed significantly 1 d after treatment for the LC_{50}, but not for the LC_{90} (Table 2). This suggests that both nymphs and adults would be equally controlled with sugar ester concentrations high enough to obtain mortality rates of >90%.

Egg and Eclosed Nymphal Mortalities. Eggs were treated ≈3 d after oviposition, and egg hatch began 4 d after treatment. Approximately 80% of the eggs in the treatments had hatched at the time

(Parr & Thurston 1968). *M. persicae* died by rapid desiccation after contact with trichomes (Thurston & Webster 1962), suggesting that the insect's cuticle is affected.

The pear psylla, *Cacopsylla pyricola* Foerster, is a serious pest of pear throughout the United States and has developed resistance to all classes of currently labeled chemical insecticides (Follet et al. 1985, Pree et al. 1990, van de Baan & Croft 1991). Therefore, it is important that alternative control agents be identified and developed. Knowledge of the insecticidal activity of this plant compound to insects other than tobacco pests is limited. Furthermore, information is lacking on dose–mortality and time–mortality relationships, as well as on residual activity. Such information is essential to the understanding of the fundamental properties of this natural insecticidal compound (Severson et al. 1994). The objectives of this study were to determine the insecticidal activity of sugar ester fraction to a nonpest of tobacco, pear psylla, and to elucidate residual activity and dose– and time–mortality relationships. A laboratory bioassay was developed for testing microliter amounts of potential insecticidal agents on all life stages of pear psylla that would be applicable to other insects.

Materials and Methods

Insect Colony. The pear psylla colony was established by collecting adults from pear at Kearneysville, WV, in August 1992 and placing them on 'Bartlett' seedlings grown in 21-cm diameter pots. Infested seedlings were covered by Lexan plastic cylinder cages (21-cm diameter by 60-cm height) that were topped with fine mesh polyester Noseeum netting (Recreational Equipment, Seattle, WA). Each colony was allowed to increase for four generations (≈4 mo) at a temperature of 25°C and a photoperiod of 16:8 (L:D) h before the study began.

Sugar Ester Extraction. The sugar ester isolate used in this study was a mixture of the four sugar esters that naturally occur in exudate of *N. gossei* leaf trichomes (Buta et al. 1993). Sugar ester isolate was obtained by washing the trichome exudate from the leaves with methylene chloride. The methylene chloride extract was dried over sodium sulfate, filtered, and the solvent removed. The extract was redissolved in acetonitrile and partitioned against hexane to remove aliphatic hydrocarbons and esters. The sugar ester-enriched acetonitrile solution was again concentrated to dryness, redissolved in methylene chloride, and partitioned against an aqueous 1N tartic acid solution. The tartaric acid extracted all alkaloids, mainly nicotine, into the aqueous solution. The acetonitrile solution was dried over anhydrous, crystalline sodium sulfate and concentrated on a rotary evaporator. This alkaloid-free sugar ester fraction was chromatographed with a chloroform-methanol gradient on a preparative Sephadex LH-20 gel column to yield a highly purified sugar ester isolate (Severson 1994). The purity of the sugar ester fraction was determined to be 98%, based on gas chromatography that used an SE-54 capillary column.

Sugar Ester Bioassay. The sugar ester solutions were prepared in concentrations of 1,000, 500, 250, 125, and 62 ppm in ddH_2O (mg/liter) and sonicated for 20 min to ensure complete homogenization. These solutions, plus a ddH_2O control, were applied to pear psylla eggs, nymphs, and adults. Both eggs and nymphs were evaluated together by transferring first and second instars onto pear leaves that contained eggs ≤24 h old. The bioassay consisted of 1–2 sterilized pear leaves placed in a standard plastic petri dish that contained two sterile pieces of 7.0-cm Whatman No. 3 filter paper dipped in sterile ddH_2O. Leaves with eggs were obtained by caging 100 psylla adults on a pear seedling for 48 h. These leaves with eggs were sterilized by soaking them for 3 min in 0.05% sodium hypochlorite then rinsing them three times with sterile ddH_2O. Small leaf sections that contained first and second instars were also sterilized in this manner and placed on leaves with eggs within a Petri dish. The leaf pieces were removed in 24 h, and the total number of first and second instars that hatched and walked onto the detached leaves was recorded. Additional nymphs were transferred by single-haired probe in those rare cases when ≥25 nymphs did not walk onto and settle on the detached leaves.

Bioassays on adult pear psylla were similar to the egg-nymph bioassay. Adults (n = 25 per dish) were gently blown into a Petri dish through a 10-mm access hole in the lid. The access hole was plugged by a cotton ball, which allowed CO_2 gas to be piped through to anesthetize the adults before the treatments were applied by the spray device.

The sugar ester solutions were applied using a specially designed ultra-low-volume spray device that consisted of a spray platform that holds a pressurizable spray bottle (Nalgene Aerosol Spray Bottle #2430-200, Rochester, NY) and Petri dish at the proper distance and angle (Fig. 1A, blueprints available from G.J.P.). Measured amounts (200 μl) of each sugar ester concentration were placed in a glass test tube (12 × 75 mm). The spray bottle siphon tube was placed in the test tube so that the test tube fit into the nozzle-pump body and was held in place with a piece of adhesive tape (Fig. 1B). The smallest-size spray nozzle of the three sizes provided with the spray bottle was used to deliver a fine spray. The bottle was pressurized to ≈10 psi with 20 strokes of the pump mechanism for each application. Leaves with nymphs and eggs were sprayed with 200 μl of sugar ester solution each to the adaxial and abaxial surfaces of the infested leaves. The petri dishes were sealed with parafilm after treatment to maintain a high humidity, which kept the detached leaves in excellent condition for >7 d. Adults were also sprayed with 200 μl of each sugar ester solution while anesthe-

this spray device is that the modified spray bottle gives complete coverage of the detached leaf while only using 200 μl of test solution. Using small amounts of candidate insecticidal material can be a very important concern in bioassays when these materials are difficult and expensive to obtain or costly to destroy. This bioassay system could be applied to other leaf-feeding insects as well. This bioassay system was used with satisfactory results in another study in which fungal pathogens were evaluated for virulence to pear psylla nymphs (Puterka et al. 1994).

In conclusion, we found that the 1,000 ppm sugar ester fraction was as effective against pear psylla nymphs as it was against *B. tabaci* nymphs, *M. persicae* adults (Severson et al. 1994), and *T. vaporariorum* nymphs (Buta et al. 1993). Furthermore, we documented that the sugar ester isolate was effective against pear psylla adults, had residual activity to newly eclosed nymphs but had no ovicidal activity. Based on these results, the sugar ester fraction appears to have insecticidal activity that is not just limited to tobacco pests. Neal et al. (1994) found that this sugar ester was ineffective against two other nontobacco pests, *Leptinotarsa decemlineata* (Say) and *Frankliniella occidentalis* (Pergande). Therefore, this sugar ester compound will be quite selective in activity against various insect species. Another important note is that the range of sugar ester concentrations had no apparent phytotoxicity to pear leaves under our laboratory test conditions. Our results in the laboratory will be useful in determining the range of sugar ester concentrations to use in field trials. Additional studies on the mode of action of this natural plant compound and its components as well as bioassays on other tobacco and nontobacco insect pests will lead to an understanding of the ecological function of this plant defense chemical and the spectrum of insects against which it will be effective.

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Table 2. LC_{50} and LC_{90} values (mg/ml) for the sugar ester used against pear psylla nymphs and adults [illegible] treatment in the laboratory

Insect stage	LC_{50} (95% FL)[a] ppm	LC_{90} (95% FL) ppm	Slope ± SEM	Intercept ± SEM	χ^2 of[b] slope	Regression χ^2[c]
Nymph	90 (0.006–0.010)a	300 (0.02–0.04)a	2.45 ± 0.21	5.01 ± 0.42	129.9*	14.2
Adult	200 (0.015–0.025)b	400 (0.03–0.06)a	3.45 ± 0.26	5.89 ± 0.48	170.2*	36.4**

[a] LC values within a column followed by the same letter are not significantly different ($P = 0.05$) using the lethal dose ratio method (Robertson & Preisler 1992).

[b] Chi-square of the slope of the regression equation is significant ($P > 0.0001$, df = 1) when followed by an asterisk (*).

[c] Pearson chi-square goodness-of-fit test on the regression equation; chi-square values followed by asterisks (**) are significantly different ($P < 0.05$, df = 23). Variances and covariances were multiplied by the heterogeneity factor (H) when the chi-square values indicated significant departures from the model.

egg hatch, and eclosed nymphal mortalities were obtained (≤3 d after eclosion). The percentage of eggs hatched was not affected by any of the sugar ester treatments 7 d after application, which implied that the sugar ester has no ovicidal activity (Table 3). In contrast, mortality rates of newly eclosed nymphs ranged from 18.7 to 67% for 62–1,000 ppm sugar ester concentrations. Why dry sugar ester residues did not cause significant nymphal mortalities beyond 1 d after treatment yet caused significant newly eclosed nymphal mortalities 7 d after application to leaves is an intriguing question. Newly eclosed nymphs appeared to have died soon after they walked on the treated leaf surface and often died within 2 mm of their egg shells. This rapid death was characteristic of the mortalities observed when nymphs were sprayed by aqueous sugar ester solutions. Therefore, we propose that the moist bodies of the newly eclosed nymphs probably activated the dry sugar ester residues, which caused their rapid death. Dry sugar ester residues on eggplant leaves were also observed to cause significant rates of mortality to *Tetranychus urticae* Koch. (Neal et al. 1994).

Residual Activity. Data on nymphal mortalities over time indicated that mortalities did not increase significantly 1 d after application (Fig. 2). Therefore, there appears to be no significant long-term insecticidal activity to treated nymphs. The mode of action of the sugar ester fraction has not yet been determined. However, the lack of chronic toxic effects and the phloem feeding nature of this pest suggests that the sugar ester is a contact insecticide that is mainly active in the liquid state. Psylla death was very rapid, and most of the total mortality that resulted in a treatment occurred within 2 h after application. The dead insects were not wrinkled from desiccation as was observed for dead *B. tabaci* treated with sugar ester (Neal et al. 1994). Instead, dead nymphs appeared to be swollen, suggesting that the cuticle was compromised and allowed water to be absorbed from the humid environment of the petri dish. Therefore, our observation supports Neal et al. (1994) assertion that the insect cuticle is adversely affected by the sugar ester.

Bioassay System Using Ultra-Low-Volume Spray Device. The spray device and bioassay method was presented in detail because it can be made from materials readily obtainable at a low cost (<$50), and the modified spray bottle completely dispenses premeasured candidate insecticidal agents (Fig. 1 A and B). Moreover, the bioassay system was found to cause far less mortality in the control than in another detached-leaf bioassay that was attempted that used water agar and required the insects to be drenched or sprayed by air brush, air dried, and then returned to the detached leaf (Yokomi & Gottwald 1988). Psylla mortalities occurred mainly when they became entrapped in the water agar. Another advantage of

Table 3. Residual effect of various sugar ester concentrations on egg hatch and nymphal mortality ≤3 d after eclosion, 10 d after treatment in the laboratory

Sugar ester concn (ppm)	% egg hatch ± SEM	% nymphal mortality rate ± SEM
1,000	88.2 ± 7.0a	67.3 ± 11.2a
500	79.3 ± 6.2a	43.2 ± 10.7b
250	76.4 ± 3.7a	36.7 ± 8.7bc
125	79.2 ± 5.8a	30.9 ± 4.8bc
62	81.8 ± 6.9a	18.7 ± 7.3cd
0	76.2 ± 2.4a	1.1 ± 1.1d
	LSD = 26.5	LSD = 18.8

Six replications. Means within columns followed by the same letter are not significantly different ($P > 0.05$, LSD).

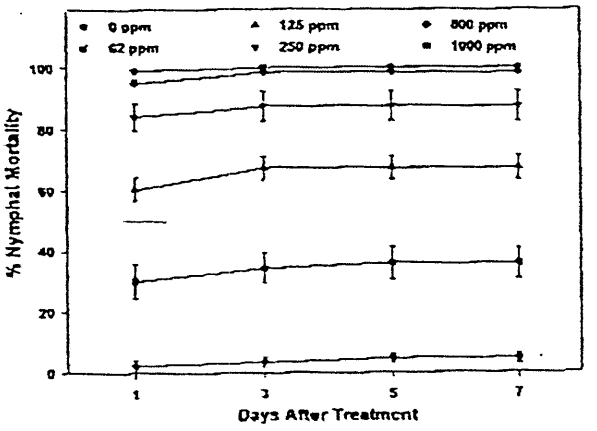

Fig. 2. Time–mortality relationships for pear psylla nymphal mortality when nymphs were treated with various concentrations of sugar ester in aqueous solutions.

and damaging whiteflies (over $200 million losses annually in the United States alone) have shown that sugar esters are a new class of "natural" insecticides that should be exploited for commercial use.

As the sugars are produced in the glandular secretions of leaf hairs (trichomes) of the *Nicotiana* plants, their levels on *Nicotiana* species leaf surfaces are very small, being generally less than 100 $\mu g/cm^2$ of leaf surface (Severson et al., 1991). Our recent studies (to be published) on yields of sugar esters from large scale field productions showed that the best producer of active sucrose esters was *Nicotiana trigonophylla*, yielding sugar esters at 158 $\mu g/cm^2$ (2.8 g/kg of plant material). Therefore, plants cannot serve as sources of commercial quantities of the insecticidal sucrose esters. As more and more studies are showing the potency of naturally-occurring sugar esters as pesticides, the need exists to identify and synthesize specific synthetic sugar ester pesticides for use against whiteflies and other soft-bodied arthropod pests, which are damaging our agricultural products.

MATERIALS AND METHODS

Sucrose Esters (SE) Synthesis. To maximize the formation of diacyl sucrose esters, 1 mol of sucrose was reacted with 2.25 mol of acid chloride. Thus, for example, sucrose was dissolved in dimethylformamide at a concentration of 54.8 g (0.16 mol) of sucrose/100 mL of DMF (in a 1 L Erlenmeyer flask), with gentle heating (up to 100 °C) and stirring on a magnetic stirrer/hot plate, until the sucrose dissolved. Then, 40 mL of pyridine was added, and the solution was cooled to 65 °C. The flask was returned to a magnetic stirrer plate, a thermometer was inserted into the flask, and the solution was stirred vigorously as the acid chloride solution was added. Acid chloride (0.36 mol) was dissolved in 150 mL of acetonitrile and poured into a separatory funnel, and this solution was added at a fast drop rate (over a 45 min period) to the sucrose solution, while stirring vigorously. (Acid chlorides ranged from hexanoyl to dodecanoyl chloride.) Acid chlorides must be added as a CH_3CN solution, otherwise extensive degradation of SE to glucose esters will occur. The reaction temperature was maintained at 65 °C, with cooling of the flask in a water bath, if needed. After addition of the acid chloride, the reaction mixture was stirred for 1 h at 65 °C, cooled to about 40 °C, and poured into 200 mL of acetone. About 34 g of sodium bicarbonate (0.4 mol) was mixed with 5 mL of water, and the paste was added slowly into the reaction mixture to decompose the pyridine hydrochloride product. After the evolution of CO_2 ceased, anhydrous, crystalline sodium sulfate (200 g) was added. At this point, the reaction mixture liquid was clear and pale yellow. The reaction mixture was then filtered and evaporated to dryness on a rotary evaporator, with the water bath temperature below 40 °C. A vacuum pump was required to remove any residual solvents (such as dimethylformamide). The yield of total SE was generally 85–90%, in addition to about 5% glucose esters, 5% unreacted sucrose, and smaller amounts of α- and β-D-glucose. The SE mixture was generally composed of 20–30% monoacyl sucroses, 35–45% diacyl sucroses, 14–25% triacyl sucroses, and 5–10% tetraacyl sucroses, as determined by GC.

Chromatographic Separation of Sucrose Esters on Silicic Acid (SA). The reaction products, dissolved in chloroform, were separated on activated SA using a solvent system of increasing percentages of methanol in methylene chloride. About 300 g of 100–200 mesh silicic acid (Unisil SA from Clarkson Chemical Co. or 100 mesh silicic acid from Sigma Chemical Co.) was required to separate 15–20 g of reaction product. The silicic acid, slurried in methylene chloride, was packed into a glass column (90 × 4 cm) equipped with a 500 mL reservoir and a ball joint at the top of the reservoir to allow the use of air or nitrogen pressure and clamps. The reaction product (15 g in 60 mL of $CHCl_3$) was added to the top of the SA column. Air pressure, at 2 psi, was used to push the solvents rapidly through the column. The column was eluted with 500 mL volumes of the following percentages of methanol in methylene chloride: 0%, 1%, 2%, 2.5%, 3%, 3.5%, 4%, 4%, 5%, 5%, 5.5%,6%, 6%, 6.5%, 7%, 7%, 7.5%, 8%, 10%, 12%, 14%, and 16%. (The small increases in the percentages of methanol were required to separate the individual groups of sucrose esters.) The resulting chromatographic fractions were concentrated to dryness on a rotary evaporator (40 °C) in round bottom flasks. Methanol (5 mL) was added to each fraction to redissolve the residue, and 2–3 μL was removed for gas chromatographic analysis.

Spinning thin-layer chromatography was performed on a chromatotron, model B #7924T (Harrison Research Inc., 840 Moana Ct., Palo Alto, CA). A circular (9.5 in.) glass plate (rotor) was coated with a 2 mm layer of silica gel 60 (EM Science). The SE sample was applied as a methylene chloride solution, and the plate was dried. The eluting solvent system was a gradient of 0–10% methanol in methylene chloride, pumped at a rate of 3 mL/min. Fractions (10 mL) were collected.

Gas Chromatography (GC). The sucrose esters obtained in the SA fractions as well as in the original reaction products were characterized by GC of their trimethylsilyl (TMS) ether derivatives. To form the volatile GC derivatives, sugar esters were derivatized by reacting them with *N,O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) and dimethylformamide (DMF) in GC autosampler vials, which were sealed and heated at 75 °C for 1 h (Severson et al., 1984). One microliter samples were injected into a 0.32 mm × 30 m glass capillary GC column, coated with 0.1 μm of DB 5HT (J&W Scientific Co.). The GC oven was programmed from 80 to 390 °C at 5 °C/min, the injection port and detector of the instrument (Hewlett Packard 5890) were set to 350 °C, and the carrier gas (H_2) flow rate was set at 35 cm/s.

Mass Spectrometry. Total SE reaction products, as well as SA or other liquid chromatography fractions, were analyzed as their TMS derivatives with a Hewlett Packard 5989A GC–MS instrument. Total ion chromatograms were obtained. The GC–MS interface temperature was 280 °C, the ion source temperature was 250 °C, and the electron impact (EI) ionization energy was 70 eV for each analysis. Other MS conditions for the analyses were scan range of 40–650 Da, 0.88 scans/s, and electron multiplier voltage of 1866 volts. The GC column and conditions were the same as for the GC analyses.

Magnetic Resonance Spectrometry. All proton (^1H) and carbon (^{13}C) experiments were performed with a Brucker 400 MHz instrument (Aspect 3000) interfaced to a Brucker FDD 280 data system. Multiplicity, broad-band decoupling, COSY, HETCOR, and *J*-resolve experiments were done according to the Brucker operations manual and techniques described by Nakanishi (1990) and Derome (1990). The substituted sugars were in acetone-d_6 solutions contained in 5 mm tubes.

Whitefly Bioassay. SE products or individual SE groups (10.0 mg) were placed into 20 mL scintillation vials and dissolved in 500 μL of methanol. Water (9.5 mL) was added, and the vial was sonicated for 10 min. Methanol (5%)–water was used as control. Adult whiteflies (*Bemisia tabaci* Gennadius, B. strain) were knocked from sweet potato plants onto yellow Sticky strips (Olson Products Inc., Medina, OH) on damp paper towels in flat plastic boxes, in a bioassay first devised by G. W. Pittarelli (personal communication). Each strip was 3 cm × 14 cm with two 3 cm square areas of sticky surface exposed, onto which approximately 30 adults/square adhered. Two strips were used per treatment. Treatment applications were replicated on different dates. The strips were sprayed with test compound solutions (2 mL), using an airbrush (Badger 2000), with a fine-mist nozzle setting from a distance of 30 cm, in a laboratory fume hood. Counts for mortality were made 2 h after spraying using a binocular microscope.

Tobacco Aphid Bioassay. Aqueous dispersions of sugar ester fractions, obtained from the column chromatographic step, or the total reaction SE products, were sprayed on greenhouse-reared apterous (wingless) aphids. Sugar ester products or individual SE groups (10.0 mg) were placed into 20 mL scintillation vials and dissolved in 500 μL of acetone.

Syntheses and Characterizations of Insecticidal Sucrose Esters

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New types of sucrose esters have been synthesized and shown to be potent insecticides against sweet potato whiteflies. On the basis of the structures of natural sucrose esters isolated from various *Nicotiana* species and which were shown to be potent whitefly insecticides, it was decided to synthesize similar sucrose esters. Specific conditions were worked out for the reaction of acid chloride with sucrose to yield a series of mono-, di-, tri-, and tetraacyl sucroses. As the active sucrose esters of *Nicotiana* species contain mainly heptanoic and octanoic acids esterified to sucrose, C_6−C_{12} aliphatic acid sucrose esters were prepared. Capillary gas chromatography of their TMS derivatives showed that distinct groups of isomers were produced. Separation by silicic acid chromatography produced fractions containing individual groups of monoacyl sucroses, diacyl sucroses, triacyl sucroses, etc. Evaluations of individual groups of the C_6−C_{12} acid sucroses showed that diheptanoyl sucroses, dioctanoyl sucroses, and dinonanoyl sucroses were most active against whiteflies and aphids. Details of syntheses, separations, GC and NMR data, and whitefly assays are presented.

Keywords: *Sugar esters; insecticides; whiteflies; aphids; chromatography; syntheses; bioassays; correlation*

INTRODUCTION

As sugars have a number of free alcoholic hydroxyl groups, their reactions with aliphatic or aromatic acids produce sugar esters having one or more acyl groups in the sugar ester molecule. Sugar esters have been found to occur naturally in plants and are being commercially produced for the food industry.

Extensive research in the early 1960s led to the production of large quantities of stearic and palmitic sucrose esters for use as emulsifiers in cakes, biscuits, chocolates, candy, ice cream, etc., and as stabilizers and wetting agents, while lauric and oleic acid sugar esters were produced for use as detergent surfactants (Kosaka and Yamada, 1977). The food applications of sucrose esters (Walker, 1984) have been extended to their use as solubilizers for poorly water soluble drugs (Hahn and Sucker, 1989). As more than one fatty acid could be added to sucrose, the preparations of sucrose esters ranged from monoesters to sucrose polyesters. In recent years, work on sucrose polyesters has concentrated on their use as low-calorie fat (Olestra) and oil substitutes (Akoh and Swanson, 1990) leading to a world market of about $75 million for these additives or substitutes (Elsner et al., 1991).

On the other hand, sucrose esters obtained from plants have yet to be developed to such an extent. Plant sucrose or glucose esters are composed of the lower fatty acids (C_2−C_{10}) and possess very interesting biological properties. Sucrose esters have been found in wild tomato and wild potato species (King et al., 1988, 1993 and references therein) and have been related to aphid resistance (Neal et al., 1990) and antifungal properties (Holley et al., 1987). Exudates from the trichomes of tomato leaves have revealed the presence of glucose esters in the polar lipids (Burke et al., 1987; Goffreda et al., 1990). More recently, our work has shown the presence of glucose and sucrose esters in petunias (Kays et al., 1994), and their structures will be reported shortly.

Perhaps, the most interesting plants are those of the *Nicotiana* family, whose species, including *Nicotiana tabacum*, the commercial tobacco plant, have been the source of a large and diverse group of both glucose and sucrose esters. One of our laboratory's works characterized the levels and compositions of both glucose and sucrose esters of 50 *Nicotiana* species (Severson et al., 1991). Acids esterified to sucrose or glucose were generally methyl-branched and ranged from C_2 to C_8 aliphatic acids, with methyl groups on the 2, 3, or 4 carbon of the acids. The most predominant sucrose esters had acyl groups on the hydroxyl groups of the 2, 3, and 4 carbons of the glucose portion. Such structures have been deduced from ^{13}C-NMR and mass spectrometry data (Arrendale et al., 1990; Matsuzaki et al., 1991). One of the most interesting species that has been extensively examined is *Nicotiana gossei* (Severson et al., 1994), mainly due to the fact that its sucrose esters have shown potent toxicity against the greenhouse whitefly (Buta et al., 1993). The subsequent patent (Pittarelli et al., 1993) on the whitefly toxicities of sugar esters of the *Nicotiana* species indicated that the activity was due to 2,3-di-*O*-acyl-1′,6′-di-*O*-acetylsucroses, with the acyl groups being mainly 5-methylhexanoyl and 5-methylheptanoyl groups. Such potent insecticidal activities of natural sucrose esters against the persistent

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Table 1. Percent Distribution of SE Groups in Chromatographic Fractions from an Octanoyl SE Preparation[a]

fraction (%) of CH_3OH in CH_2Cl_2	SE group 1	2	3	4	5
2			10	79	10
2.5			13	71	8
3			80	18	
3.5		7	93		
4		35	64		
4		87	24		
5		90	8		
5		87			
5.5		99			
6		95			
6		100			
6.5		100			
7		100			
7	6	94			
7.5	37	58			
8	80	20			
10	94	6			
12	99	1			
14	100				
16	100				

[a] Calculated from peak areas of GC data for each fraction.

(27), such as 2,3-, 2,4-, 2,6-, 3,4-, 3,6-, 4,6-, 1′,2-, 1′,3-, 1′,4′-, 1′,6-, etc., dioctanoyl sucrose. (Glucose carbons are numbered 1−6; fructose carbons are 1′−6′.) Similarly group 3 SE could have a large number of possible isomers. As seen from Figure 1, the reaction only produced a few of the possible isomers. There are only three major monooctanoyl sucrose compounds in group 1, one very predominant dioctanoyl sucrose with two lesser dioctanoyl sucroses in group 2, one major trioctanoyl sucrose and four minor trioctanoyl sucroses in group 3, and two major and several minor tetraoctanoyl sucrose esters in group 4. The acylation of sucrose has been studied and shown to be selective for certain hydroxy groups (Chowdhary et al., 1984), and therefore a much smaller number of isomers in each group is obtained.

In order to determine the structures of these sucrose compounds as well as their insecticidal activities, it was necessary to effect a major chromatographic separation. For this purpose, a total octanoyl sucrose ester reaction product was subjected to column chromatography on silicic acid. Elution with increasing percentages of methanol in methylene chloride yielded a series of fractions that were analyzed by gas chromatography. For example, the developed elution program shown in Table 1 produced a successful separation of SE groups 1−5. The higher-substituted, but less polar SE, groups eluted first followed by the increasingly polar lower groups. It was apparent that the presence of three or four octanoyl groups greatly reduced the polarity of the total sucrose molecule, even though four or five of the original hydroxyls were still present. By contrast, group 2 SE eluted over a range of polar fractions. The small increases in the percent of methanol were selected on purpose in order to obtain fractions that were 100% pure dioctanoyl sucrose esters. As shown by the GC data, this objective was achieved. The elution scheme could be modified to obtain pure trioctanoyl SE. Individual groups of monooctanoyl, dioctanoyl, and trioctanoyl sucrose esters could now be tested for biological activity against whiteflies and aphids. At this time, heptanoyl sucrose esters (C_7SE), nonanoyl sucrose esters (C_9SE), decanoyl sucrose esters (C_{10}SE), and didodecanoyl sucrose esters (C_{12}SE) were also synthesized by the same method and then separated into individual groups by SA column chromatography, as was done for the C_8SE.

Table 2. Bioassay Results of Different SE against Tobacco Aphids

SE[a]	no. of tests	mortality[b] (%)
monoheptanoyl sucrose	1	17
diheptanoyl sucrose	3	88
triheptanoyl sucrose	2	16
water	3	5
monooctanoyl sucrose	1	11
dioctanoyl sucrose	3	88
trioctanoyl sucrose	2	27
water	3	5
monononanoyl sucrose	1	16
dinonanoyl sucrose	2	64
trionanoyl sucrose	2	13
water	3	5
monododecanoyl sucrose	1	47
didodecanoyl sucrose	2	23
tridodecanoyl sucrose	2	15
water	3	5

[a] Tested at 1 mg of SE/mL of aqueous spray solution, mean values for number of tests shown. [b] After 24 h, standard deviations ranged from 5% to12%.

Table 3. Bioassay Results of the Total SE Reaction Product against Tobacco Aphids

reaction products[a]	mortality[b]	reaction products[a]	mortality[b]
total heptanoyl SE	95	total decanoyl SE	64
total octanoyl SE	85	control (water)	5
total nonanoyl SE	75		

[a] Tested as an aqueous dispersion at 1 mg/mL (0.1%). [b] After 24 h, mean values of two tests, standard deviation of 8−14%.

Bioassays of the total SE reaction products as well as of the individual groups of SE were conducted using first the tobacco aphid and then the sweet potato whitefly. Tables 2 and 3 show the percent mortality of aphids treated by the individual sucrose ester groups and the total SE reaction products. The results (Table 2) indicated that diheptanoyl and dioctanoyl sucrose esters produced the highest percent aphid mortality. Sucrose esters of hexanoic acid (not shown) were also tested and gave low percent mortality (about 23−43%), while SE of higher aliphatic acids (C_9, C_{10}, C_{12}) produced progressively lower mortalities. It was most interesting to see (Table 3) that the total reaction SE products derived from the heptanoyl and octanoyl sucrose esters were also highly active against aphids. (In a commercial application, the use of the total reaction SE product would be more economical than SA fractions.) Thus, heptanoyl and octanoyl SE preparations, as well as their group 2 SE, are potent pesticides against tobacco aphids.

Bioassay tests with the total SE mixtures were conducted also against the adult sweet potato whitefly (Table 4). After only 2 h, assay results indicated high toxicity for all of the total sucrose ester reaction products, with the highest whitefly mortality produced by the C_8SE product. It is expected that higher concentrations (above 1 mg/mL) of the other SE products would also yield high toxicities against soft-bodied arthropods.

As the bioassay tests had established that the diacyl sucroses, such as the dioctanoyl sucroses, were the most toxic compounds against whiteflies, the next step was to determine their structures. Accordingly, efforts were first concentrated on determining the structure of the major dioctanoyl sucrose. Silicic acid fractions of the C_8SE product were selected for their high content of the

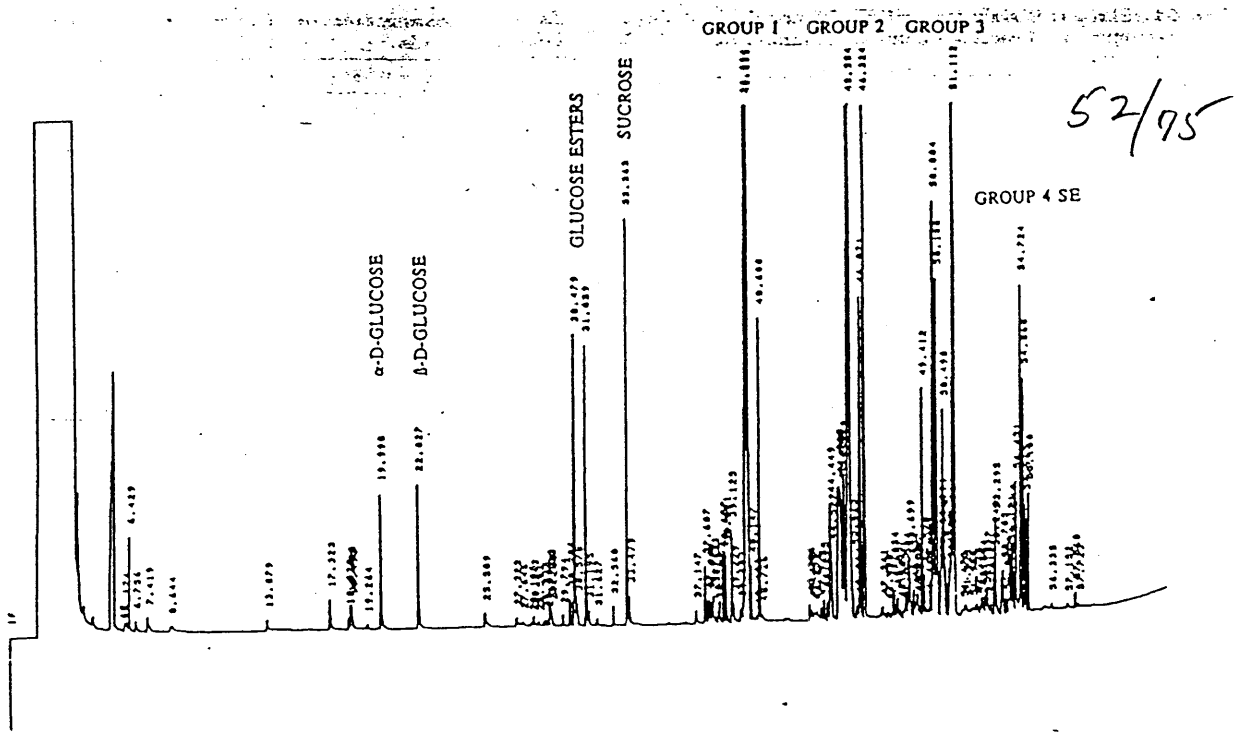

Figure 1. Gas chromatogram of total octanoyl sucrose ester reaction products (TMS derivatives).

Distilled water (9.5 mL) was added to each vial and sonicated for 10 min just prior to treatment applications. Acetone (5%)–water was used as a control. Small tobacco bud leaves (5–9 cm) infested with apterous tobacco aphid (Myzus *nicotiana* Blackman) nymphs were collected from greenhouse plants. Each leaf was considered one replication and sprayed with a designated sucrose ester at a rate of 1 mg/mL of water and placed in a petri dish (9 cm × 1.5 cm) fitted with moistened filter paper. Four replications were used for each treatment. Aphids were treated with an airbrush (Binks-B) using a fine-mist nozzle setting by spraying both sides of each leaf with the test solutions from a distance of 8 cm. Leaves were sprayed to run off with test compound solutions by passing the airbrush across each leaf surface four times while spraying. Percent mortality was determined after 24 h using a binocular microscope.

RESULTS AND DISCUSSION

The sucrose esters were prepared under specially developed conditions but according to the standard reaction of acid chlorides and alcohols to form esters. The synthetic conditions developed as follows. Generally, esterifications are conducted under anhydrous conditions by adding a solution of an acid chloride to a solution of the alcohol. Unfortunately, sugars, such as sucrose, cannot be dissolved in standard solvents such as chloroform, acetone, acetonitrile, or benzene, and polar solvents such as methanol or ethanol cannot be used as they would compete in the reaction to form methyl or ethyl esters of the acids. The literature revealed a large variety of methods for the formation of palmitic, stearic, or oleic esters of sucrose. Generally, these sucrose esters were prepared by transesterification of fatty acid methyl esters with sucrose with catalysts such as K_2CO_3, molten sodium, or lithium or potassium soaps at high temperatures (180 °C) and with or without solvents (Kurtz, 1966; Feuge et al., 1970; Akoh and Swanson, 1989; Osipow and Rosenblatt, 1967; Rizzi and Taylor, 1978). As these conditions appeared rather drastic and generally produced mostly monoesters, another approach was needed.

As most natural sucrose esters that exhibit insecticidal activities are di- and triacyl sucroses, where the acyl groups are heptanoic or octanoic acids, it was decided to modify the reaction as to prepare mostly di- and trioctanoyl sucroses. The most logical approach appeared to be the addition of 3 mol of acid chloride to 1 mol of sucrose in solution, with pyridine present to neutralize the HCl from the reaction. This seemed reasonable as a literature method (Youngs, 1958) described the formation of tristearin by heating 3 mol of steroyl chloride with glycerol at 100 °C and 2 mm pressure. The problem of a suitable solvent for sucrose was overcome by using dimethylformamide. Solutions of sucrose in DMF were readily prepared by slowly heating sucrose with DMF, with vigorous stirring, to 100 °C. Then the pyridine and octanoic acid chloride were added. Although, sugar esters were formed, they were mostly monooctanoyl sucroses. The final syntheses was developed after much trial and error. The best reaction conditions involved the slow addition of 2.25 mol of acid chloride in acetonitrile to a solution of 1 mol of sucrose in DMF and pyridine at 65 °C. (Higher molar ratios yielded more of the tri-, tetra-, and pentaacyl sucroses.) These conditions yielded mono-, di-, tri-, and tetraacyl sucroses, as shown by the gas chromatogram in Figure 1.

As sucrose has eight free hydroxyl groups, esterification with octanoic acid could result in the formation of eight groups of sucrose esters: monooctanoyl sucroses (called "group 1"), dioctanoyl sucroses (called "group 2"), trioctanoyl sucroses (called "group 3"), tetraoctanoyl sucroses (called "group 4"), etc., all the way up to octaoctanoyl sucrose. For monooctanoyl sucroses, octanoic acid can attach to any one of the eight hydroxyls of sucrose to give eight different positional isomers. Group 2 SE, which have two acids esterified to two hydroxyls, could also have a large number of isomers

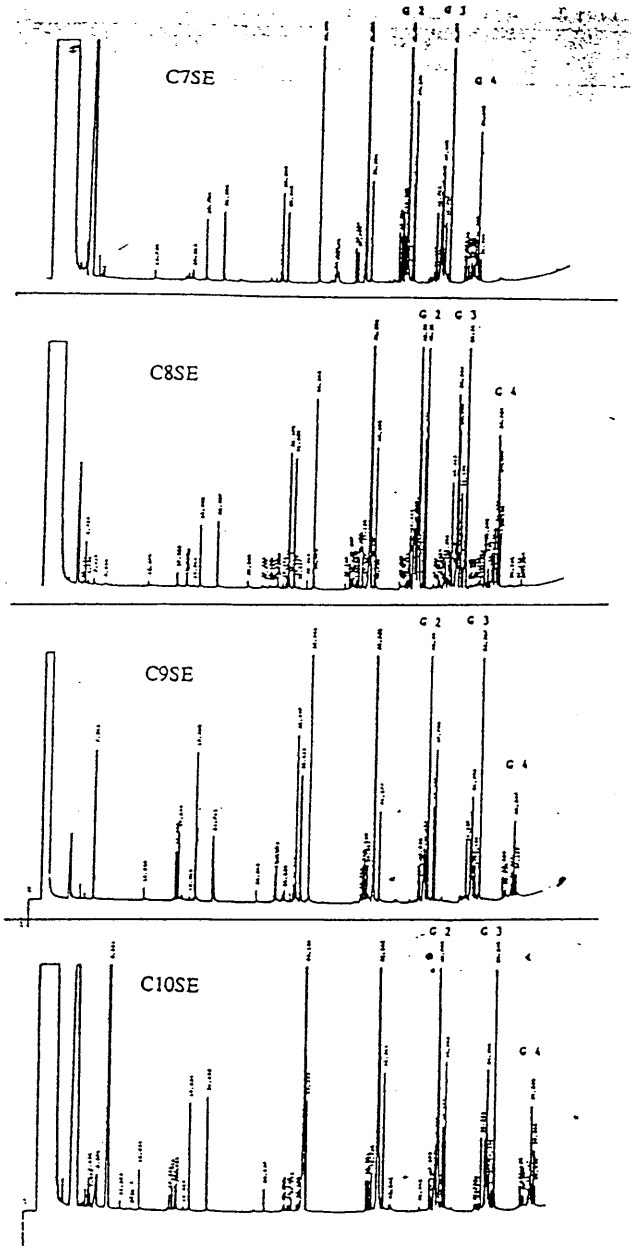

Figure 2. Gas chromatograms of heptanoyl, octanoyl, nonanoyl, and decanoyl sucrose esters products (TMS derivatives).

from the more hindered 1′-hydroxyl group. Similarly, the other dioctanoyl sucroses are probably the 6,1′ and 1′,6′ compounds. However, nature does not follow the rules of conformational stereochemistry, as most of the natural sucrose esters have acid groups on the 2, 3, and/or 4 carbon of glucose (Severson et al., 1991).

The gas chromatograms of the heptanoyl, nonanoyl, decanoyl, and dodecanoyl SE products were identical with that of the octanoyl sucroses, showing the same product distributions. As expected, higher retention times with increasing molecular weights of the SE were observed (Figure 2), that is, group 2 SE for C_{12}SE eluted later than C_{10}SE, which were later than C_9SE, etc., as expected for compounds of increasing aliphatic chain lengths. Thus, the synthesis produced the same distribution of SE for each aliphatic acid.

It was concluded that this synthetic method produced highly toxic SE products for the control of whiteflies and possibly other soft-bodied arthropods. The simpli and reproducibility of the developed synthesis and large quantity of toxic SE that can now be produce a rapid manner indicate that this synthetic met should be readily adapted for commercial productio environmentally-friendly insecticides against the hig destructive whiteflies and aphids. The future of th sucrose esters appears to be bright. A patent appli tion has been filed on the use of these SE for the con of soft-bodied arthropods. Three commercial compar have signed cooperative research and developm agreements and are committing extensive funds a expertise for the testing of these compounds, and o a dozen cooperators are field testing these SE agai various insects on various agricultural and ornamen crops.

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Table 4. Toxicities of Total SE Reaction Products against the Sweet Potato Whitefly

reaction products[a]	sweet potato whiteflies	
	no. of tests	mortality[b] (%)
total hexanoyl SE	3	80
total heptanoyl SE	3	95
total octanoyl SE	3	99
total nonanoyl SE	3	92
total decanoyl SE	3	80
control (water)	3	5

[a] Tested at 1 mg/mL of aqueous spray solution. [b] After 2 h, mean values from three tests, four repititions each, standard deviation ranged from 4 to 7%.

major group 2 SE compound, and these were further subjected to a chromatotron (spinning thin-layer chromatography plate) separation to yield a fraction highly enriched in the major dioctanoyl sucrose ester. Subsequent LH-20 chromatography, using a chloroform–methanol solvent gradient (Severson et al., 1994), yielded the pure compound. Our past GC–MS experience in characterizing the SE of the *Nicotiana* species (Arrendale et al., 1990; Severson et al., 1984, 1994) proved most helpful in determining the structure of the synthetic compound. MS data showed an m/z fragment at 505, indicating the presence of only monooctanoylglucose or monooctanoylfructose fragments. This meant that the original dioctanoyl sucrose ester had one octanoyl group on each half of the sucrose molecule. This proposed structure was confirmed by subsequent NMR experiments. The literature has over a dozen references on NMR data of natural sucrose and glucose esters (Severson et al., 1985; Nishida et al., 1986; Matsuzaki et al., 1988, 1989, 1991, 1992; King et al., 1993; Ohya et al., 1994). Using these data combined with our NMR analyses, which included proton NMR, ^{13}C-NMR, broad-band decoupling, and *J*-resolve experiments, it was proven that this major group 2 compound was 6,6′-dioctanoyl sucrose. The proton NMR spectrum of this SE presented considerable difficulty in interpretation. The sucrose portion of the spectrum was complex, and many peaks overlapped. COSY experiments allowed connectivity for G1 to G2, G2 to G3, and G3 to G4, but G4 to G5 and G5 to G6 were confusing because of overlap with the fructose protons. The fructose proton assignments were also confused by the overlapping resonances. Broad-band proton decoupling experiments did not seem to resolve the issue. Final assignments of protons and carbons required HETCOR *J*-resolve experiments (Nakanishi, 1990) in which all protons were decoupled and correlated to carbon-13 resonances. The values for these correlations are shown in Tables 5 and 6.

As the next major compound in abundance in the SE product was the triacyl sucrose, it was of interest to determine the structure of the trioctanoyl sucrose ester. In addition to the 505 ion representing monooctanoylglucose or monooctanoylfructose fragments, the GC–MS data also showed a 559 ion, indicating that there were two C_8 groups on fructose or glucose. This showed that the trioctanoyl sucrose ester had one octanoyl group on one half of the sucrose molecule and two octanoyl groups on the other half, but it was not clear as to which half of the sucrose molecule had the two C_8 groups. In view of the fact that sucrose has three primary hydroxyl groups on the 6, 1′, and 6′ carbons, acylation at these positions is much more likely to occur than on the more hindered, secondary hydroxyls on the 2, 3, 4, 3′, and 4′ carbons. This was confirmed by NMR experiments that

Table 5. ^{1}H-NMR Shift (δ) Data for Major Synthetic SE

	6,6′-di-*O*-octanoylsucrose	6,1′,6′-tri-*O*-octanoylsucrose
G1	5.38	5.36
G2	3.46	3.42
G3	3.77	3.72
G4	3.27	3.26
G5	4.15	4.06
G6a	4.26	4.26
G6b	4.46	4.4
F1a	4.27	4.24
F1b	4.44	–
F3	4.71	4.76
F4	4.07	4.16
F5	3.94	3.9
F6a	4.40	4.28
F6b	4.43	4.38

Table 6. ^{13}C-NMR Shift (δ) Data for Major Synthetic SE

	6,6′-di-*O*-octanoylsucrose[b]	6,1′,6′-tri-*O*-octanoylsucrose[a]
G1	92.42	92.94
G2	71.52	71.42
G3	74.30	74.18
G4	71.43	71.26
G5	77.03	76.03
G6	66.36	62.97
F1	64.61	65.90
F2	104.80	104.00
F3	79.97	78.35
F4	72.89	72.48
F5	80.62	80.40
F6	64.74	64.54

[a] The carbonyl carbons had values of 172.8, 173.2, and 173.7 ppm for the three acyl substituents. [b] The carbonyl carbons had values of 173.7 and 173.8 ppm for the two acyl substituents.

showed the structure of the triacyl sucrose to be 6,1′,6′-trioctanoyl sucrose. The ^{1}H-NMR assignments for the sugar portion of the triester were determined by 2-D COSY experiments and are listed in Table 5. The pattern of substitution for the three ester moieties was established by the assignments of protons to the various carbons of sucrose by the downfield shift of those protons attached to the *O*-acylated carbon hydroxyl group. Connectivity between protons on adjacent carbons was easily established for the glucose and fructose portions of the molecule except for the anomeric F2 carbon, which has no connectivity to other carbons due to a lack of protons. Chemical shift values for protons on carbons containing free or *O*-acyl substituted hydroxyl groups were compared to those taken from various literature sources. They reflected and were in agreement with values of downfield shift caused by *O*-acyl-substitution. The ^{13}C-NMR assignments for the triester were determined by the normal and the multiplicity experiments (Table 6). The anomeric F2 carbon resonance appeared at 103.8 ppm and disappeared in the multiplicity experiment (confirming no hydrogens). Three resonances for carbonyl were found at 172.8, 173.2, and 173.7 ppm and also disappeared in the multiplicity experiment confirming the triacyl nature of the molecule. All 12 resonances of the sucrose structure were accounted for and could be assigned to specific carbons.

In line with the structural determinations for the dioctanoyl and trioctanoyl sucrose esters, it is most logical to assume that the structures of the monooctanoyl SE are 6-octanoyl sucrose, 6′-octanoyl sucrose, and 1′-octanoyl sucrose. Thus, the two large peaks (doublet) in the group 1 gas chromatogram probably are the 6- and 6′-octanoyl sucrose, while the smaller peak corresponds to the 1′-octanoyl sucrose that is formed

Insecticidal Activity of Natural and Synthetic Sugar Esters Against *Bemisia argentifolii* (Homoptera: Aleyrodidae)

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ABSTRACT Insecticidal activities of natural sugar ester isolates of *Nicotiana* spp. and synthetic sugar esters were tested against *Bemisia argentifolii* Bellows & Perring in laboratory bioassays and a tomato field trial on staked tomato. A mixture of the pyrethroid cyfluthrin and methamidophos, as well as the juvenile analog pyriproxyfen, were used for comparison in the field trial. Mortality of adults immobilized on yellow sticky cards and sprayed to run-off (≈100% coverage) with sugar ester isolates of *Nicotiana* spp. (including *N. gossei*) approached 100%. In contrast, mortality of immobilized adults treated in a Potter spray tower (≈70% coverage) with the same concentrations of *N. gossei* was <50%. Sugar ester isolates of *N. gossei, N. amplexicaulis, N. glutinosa, N. langsdorffii, N. trigonophylla,* and *N. palmeri* and a synthetic sucrose ester were more toxic to 2nd-instar nymphs at a rate of 1 g (AI)/liter than were isolates of *N. cavicola, N. simulans, N. pauciflora, N. plumbaginifolia, N. noctiflora,* and *N. otophora.* Whitefly populations on tomato sprayed weekly in the field with a sugar ester isolate of *N. trigonophylla* or 4 synthetic preparations were reduced by 40–98% for immatures and 43–73% for adults compared with untreated plants. Sugar ester isolate and synthetic sugar esters in the field tomato trials compared favorably with commercial insecticides for whitefly control.

KEY WORDS Bemisia argentifolii, Bemisia tabaci, *Nicotiana* sugar ester isolates, botanical insecticides, synthetic sugar esters

A GROUP OF natural sucrose and glucose esters from sugar ester isolates of *Nicotiana gossei* Domin and other *Nicotiana* species have been demonstrated to be highly effective against nymphal stages of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), and *Bemisia tabaci* (Gennadius) (*Bemisia argentifolii* Bellows & Perring) (Neal et al. 1987; Buta et al. 1993; Neal et al. 1994; Liu and Stansly 1995a, b, c). These results have aroused interest in sugar ester isolates from additional *Nicotiana* species as well as synthetic sugar esters and also the ability of these materials to reduce populations of *B. argentifolii* when applied in the field with conventional spray equipment. We tested the insecticidal activity of sugar ester isolates from 11 species of *Nicotiana* and a synthetic preparation against adults and immatures of *B. argentifolii* in laboratory bioassays and also demonstrated the ability of natural and synthetic sugar esters to reduce whitefly populations in the field significantly.

Materials and Methods

***Nicotiana* Plant Cultivation.** Plants were grown in replicated field plots (300 plants each entry) under flue-cured tobacco production conditions at the following 3 sites: University of Georgia Coastal Plains Experimental Station, Tifton, GA; the Crop Research Laboratory, Oxford, NC; and the Pee Dee Research and Education Center, Clemson University, Florence, SC. All species were grown at each site, and extracts from different sites were combined.

Whiteflies and Host Plants. *Bemisia argentifolii* were cultured in an air-conditioned greenhouse at the Southwest Florida Research and Education Center (SWFREC), Immokalee FL, on potted tomato, *Lycopersicon esculentum* Miller, 'Florida Lanai'; collard, *Brassica oleracea* L. var. *acephala,* 'Georgia LS'; salvia, *Salvia splendens* L.; eggplant, *Solanum melongena* L, 'Black Beauty'; hibiscus, *Hibiscus rosa-sinensis* L.; and sweet potato plants, *Ipomoea batatas* L. (1 per 15-cm pot) using Metro-Mix 300 growing medium (Grace Sierra, Horticultural Products Company, Milpitas, CA). Plants were watered with 0.4% (wt.:vol.) of Stern's Miracle-Gro (an all-purpose water-soluble plant food with N/P/K: 15:30:15) (Stern's Miracle-Gro Products, Port Washington, NY) once per week.

Sugar Ester Isolates. Cuticular extracts were obtained by dipping whole, cut-off plants into isopropyl alcohol (1.5 l/kg of plant material) in the field as previously described by Severson et al. (1994). Plants were allowed to regrow and were

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Table 2. Mortality of *B. argentifolii* adults treated with *Nicotiana* SE isolates applied with a hand pump to runoff (≈100% coverage)

Sugar esters	% mortality ± SE		
	1.0 g (AI)/liter	0.5 g (AI)/liter	*F*
N. amplexicaulis	94.4 ± 4.7a	94.4 ± 6.5ab	0.08
N. glutinosa 24	96.1 ± 4.0a	98.1 ± 2.2ab	0.57
N. glutinosa 24A	94.7 ± 6.3a	94.2 ± 4.0ab	0.04
N. glutinosa 24B	95.3 ± 5.3a	96.4 ± 4.7ab	0.20
N. gossei	95.5 ± 6.8a	96.6 ± 4.5ab	0.01
N. langsdorffii	100.0 ± 0.0a	93.2 ± 6.4b	16.57**
N. trigonophylla	100.0 ± 0.0a	100.0 ± 0.0a	0.00
Water + 1% acetone	1.4 ± 2.6b	3.0 ± 3.5c	0.52
F	59.0**	44.5**	—
LSD	6.5	6.5	—

**, $P = 0.01$. Means in the same column followed by different letters differ significantly (SAS Institute 1988).

Trial 2. Whitefly-bearing sticky cards were sprayed as above with 0.25, 0.5, 1, and 2 g (AI)/liter *N. gossei* sugar ester or with 2 ml of each solution using the Potter spray tower (Burkard Manufacturing, Rickmansworth, Hertfordshire, England) at 7 kg/cm^2 pressure.

Nymph Leaf-Dip Bioassays. For all except the 2nd bioassay, young whitefly-free sweet potato leaves were collected and inserted into individual root cubes (3.75 by 3.75 by 3.75 cm) (0ASIS Growing Media, [Smithers-Oasis; USA Grower Products, Kent, OH], petiole down. Root cubes with sweet potato leaves were kept in plastic trays and immersed in water (2 cm in depth) into which 1 g/liter of Miracle-Gro was added once per week. Rooted sweet potato leaves maintain their quality and therefore supply a convenient medium for testing effects on nymphs. For the 2nd of 3 experiments, tomato leaves were used as a substrate because whitefly control on tomato was the ultimate objective and was to be used in the field experiment. Leaves (trifoliates) were placed individually into glass vials (petiole down) filled with 20 ml of water. Male and female whiteflies (40–60 per leaf) were introduced onto the sweet potato or tomato leaves in a large cage (60 by 60 by 60 cm, screened). After an oviposition period of 24 h, the newly infested leaves were removed from the large cage, and the whiteflies were extracted using a hand-held vacuum cleaner (AC Insect Vac [Bio-Quip, Gardena, CA]. Egg-bearing leaves were incubated in whitefly-free cages at 25 ± 2°C, 75% RH, and a photoperiod of 14:10 (L:D) h for 10 d when most had developed to 2nd instar. Whitefly-bearing leaves were dipped in appropriate sugar ester concentrations for 5 s, then air-dried for 1 h on paper towels. Treated leaves were incubated in whitefly-free cages (60 by 60 by 60 cm) at 25 ± 2°C, 55–60% RH, and a photoperiod of 14:10 (L:D) h for 3–4 d. An average of 54 ± 14 (mean ± SD) small nymphs per leaf were observed using a stereoscopic dissecting microscope. Nymphs that had dried or detached from the leaf surface were considered dead. The 1st and 2nd experiments were conducted comparing different sugar ester isolates of *Nicotiana,* and a 3rd experiment compared a synthetic sugar ester with a *N. gossei* sugar ester isolate. A randomized complete block design was employed with 8 replicates, and each experiment was repeated 3 times.

Table 3. Mortality of *B. argentifolii* adults treated with *N. gossei* SE isolate using the Potter spray tower (≈70% coverage)

Rate, g (AI)/liter[a]	% mortality ± SE
2.00	50.7 ± 5.5a
1.00	47.9 ± 4.0a
0.50	28.8 ± 3.3b
0.25	23.2 ± 3.1b
Water + 1% acetone	7.5 ± 1.2c
F	32.5[b]
LSD	9.6

[a] 2 ml solution, 0.7 kg/cm^2 pressure.
[b] Significant at $P = 0.01$. Mean percentages in the same column followed by different letters differ significantly (SAS Institute 1988).

A 4th bioassay was conducted to evaluate effects of coverage. We treated 2nd-instar nymphs on sweet potato leaves with *N. gossei* sugar ester isolate by either dipping the leaves in 1 g (AI)/liter concentrations (≈100% coverage) or spraying the whitefly-bearing leaves with the Potter spray tower (2 ml solution at 0.7 kg/cm^2) (≈70% coverage; Liu and Stansly 1995a). Mortality was examined 4 d after treatment. Three concentrations and the water control were tested for each treatment, with 8 replicates at each concentration.

Field Trials. Tomato ('Agriset') seedlings (15–20 cm high) were exposed for 5 d to a greenhouse colony of *B. argentifolii* for infestation with whitefly eggs. Seedlings were planted on 27 February 1995 in sandy soil at SWFREC, 46 cm between in beds (81 cm wide) fumigated with 220 lb methyl bromide–choropicran 67/33 and covered with black polyethylene mulch following standard procedures for southwestern Florida staked tomato production. A randomized complete block design was used with 4 replications, and treatments included 5 sugar ester isolates—the 2 commercial

Table 1. Constituents and their structures of sugar ester isolates tested

Sugar ester sources	Sucrose esters		Glucose esters	
	Acyl groups[a]	Acetyl groups[b]	Acyl groups	Acetyl groups
N. amplexicaulis	2, 3	1′, 6′	2, 3	1
N. cavicola	4, 3′	6, 4′, 6′	—	—
N. glutinosa 24	2, 3, 4	3′	—	—
N. glutinosa 24A	2, 3, 4	—		
N. glutinosa 24B	2, 3, 4	3′		
N. gossei	2, 3	1′, 6′		
N. langsdorffii	2, 3, 4	3′		
N. noctiflora	Unknown	—		
N. otophora	2, 3, 4	6		
N. otophora 38A	2, 3, 4	6		
N. otophora 38B	2, 3, 4	6		
N. otophora 38C	2, 3, 4	6	—	—
N. palmeri	2, 3, 4	—	2, 3, 4	—
N. pauciflora	2, 3, 4	6, 1	2, 3, 4	6
N. plumbaginifolia	2, 3, 4	3′	—	—
N. simulans	Unknown	—	—	—
N. trigonophylla	2, 3, 4	3′	2, 3, 4	—

Major components of synthetic OTC7SE, OTC8SE, OTC9SE, and OTC10SE were 6-, 6′-, and 1′ monoacyl SE; 6,6′-, 6,1′-, and 1′,6′-diacyl SE; 6,1′,6′-triacyl SE (based on GC/MS data and NMR data).

[a] Glucose carbons are 1–6, fructose carbons are 1′–6′; acyl groups range from propionic to octanoic acids.

[b] Acetyl groups are generally on fructose carbon hydroxyls.

then cut back and dipped into solvent to extract the cuticular components. This procedure was repeated 3 or 4 times. Sugar ester isolates were obtained from the cuticular extracts by a previously described solvent partitioning procedure (Severson et al. 1991, 1994). This scheme was designed to remove aliphatic hydrocarbons and wax esters with a hexane extraction and to remove alkaloids with an aqueous tartaric acid solution, leaving an acetonitrile fraction that contained the purified sugar esters.

Sugar ester isolates were characterized using gas chromatography–mass spectrometry (GC/MS) by converting samples to volatile trimethylsilyl derivatives and separating on SE-54 or DB-5 glass capillary GC columns (Arrendale et al. 1990). Sugar ester isolates in *Nicotiana* spp. and synthetic preparations generally contained glucose and sucrose of different types and proportions (Table 1). The sugar ester isolate of *N. gossei* consisted of 2 major types of glucose esters (2,3 di-acyl-1-acetyl glucose and 2,3-acyl-glucose) and two major types of sucrose esters (2,3 di-acyl-1′-acetyl sucrose and 2,3-di-acyl-1′,6′-di-acetyl sucrose) (Severson et al. 1994). The sugar ester isolate of *N. gossei* has been extensively investigated (Buta et al. 1993), and the 2 sucrose ester compounds have been patented (Pittarelli et al. 1993). The 2 major acyl groups on the sugar esters have been determined to be 5-methylhexanoyl and 5-methylheptanoyl (Pittarelli et al. 1993). Isolates of *N. glutinosa* 24A contained large amounts (85%) of labdanes along with sugar esters (11%), in contrast to the other *N. glutinosa* accession. All other *Nicotiana* isolates contained ≥98% sugar ester and no significant amounts of labdanes.

Synthetic Sucrose Esters. Synthetic sugar esters were prepared by reacting sucrose with acid chlorides according to the method recently described by Chortyk et al. (1996). Sucrose esters of heptanoic, octanoic, nonanoic, and decanoic acids were prepared. The total reaction product, consisting of nonoacyl sucroses, diacyl sucroses, and triacyl sucroses, was used directly for testing. Heptanoyl sugar ester were labeled OTC7SE, octanoyl SE were labeled OTC8SE, and so on.

Spray Dilution Preparations. Aqueous dispersions of sugar ester isolates were prepared for either spray or leaf-dip application as described by Liu and Stansly (1995b). In brief, the natural or synthetic sugar esters were dissolved in 20 times of acetone (wt.:vol.) to make up a 5% stock solution. When used, the concentrated solution was slowly mixed into vigorously stirred water on a magnetic stirring plate (Model 11-498-7SH [Fisher Scientific, Philadelphia, PA] for 2 min, giving a cloudy emulsion. Acetone (1%) water mixtures were used as controls. All experiments were conducted in the laboratory at 25 ± 2°C, 70 ± 5% RH, and illuminated with fluorescent lights (≈40 μmol· m^{-2} s^{-1} light intensity) set at a photoperiod of 14:10 (L:D) h.

For field application, 23 g of sugar ester isolates were dissolved with 100 ml of acetone, 100 ml of methanol, and 28 ml of Latron CS-7 spray adjuvant (Rohm-Haas, Philadelphia, PA). The sugar ester solution was then poured into rapidly stirred water (7.6 liter) to make a spray dilution of 3 g (AI)/liter (0.3%). Two commercial standards, as follows, were included for comparison: (1) a mixture of a pyrethroid, cyfluthrin (Baythroid 2EC [Bayer, Kansas City, MO] at 49.0 g (AI)/ha (1st 3 weekly sprays) or at 25.5 g (AI)/ha (5 remaining sprays) plus a synthetic organic phosphate, methamidophos (Monitor 4EC [Bayer, Kansas City, MO] at 841.4 g (AI)/ha, and (2) pyriproxyfen (an insect growth regulator, S-71639 [Knack 0.83 EC] [Sumitomo, Osaka, Japan] at 49.36 g (AI)/ha.

Adult Bioassays. Yellow sticky polyethylene cards (Olson products, Medina, OH) were used to immobilize whitefly adults. The sticky cards were cut into pieces (4 by 4 cm) with square area (2 by 2 cm) of sticky surface exposed and attached to a bamboo stick (15 cm long). Infested foliage in the greenhouse was gently shaken over the cards to capture 20–50 whiteflies per card.

Trial 1. Whitefly-bearing cards were sprayed to runoff with 2 concentrations (0.5 and 1 g (AI)/liter) of 7 sugar ester isolates including *N. gossei,* using a hand-spray pump (Spritzer [Bel-Art Products, Pequannock, NJ]. Cards were air-dried for 1 h and then held in a plastic ice chest (100% RH for 4 h) after treatment. Whiteflies were examined under a stereoscopic microscope and considered dead when no movement was observed after gentle probing with a camel's-hair brush.

Table 6. Toxicity of a *N. gossei* sugar ester isolate and a synthetic sugar ester applied as a dip to 2nd-instar nymphs of *B. argentifolii* on sweet potato leaves

Rates, g (AI)/liter	% mortality ± SE		
	N. gossei sugar ester	Synthetic SE (OTC8SE)	*F*
1.00	95.6 ± 5.2a	89.5 ± 10.7a	2.13
0.50	87.1 ± 8.5ab	80.1 ± 9.0ab	2.55
0.25	81.5 ± 2.2b	72.5 ± 12.4a	2.13
Water + 1% acetone	4.2 ± 3.2c	3.2 ± 2.6c	0.12
F	185.5**	128.3**	—
LSD	8.7	9.7	—

**, $P = 0.01$. Means in the same column followed by different letters differ significantly (SAS Institute 1988).

Table 7. Mortality response of 2nd-instar nymphs of *B. argentifolii* on sweet potato leaves to *N. gossei* sugar ester isolate applied as a leaf dip and with spray in the Potter spray tower

Rates, g (AI)/liter	*n*	% mortality ± SE		
		Dipped	Sprayed	*F*
1.00	1,300	93.8 ± 0.8a	52.8 ± 4.6a	99.9**
0.50	1,347	87.2 ± 1.5b	46.3 ± 5.2b	63.3**
0.25	751	84.1 ± 1.8b	35.9 ± 2.8b	52.1**
0.00	921	2.0 ± 1.1c	2.0 ± 0.8c	0.2
F	—	533.1**	37.0**	—
LSD	—	3.3	12.2	—

**, $P = 0.01$. Mean percentages in the same column followed by different letters differ significantly (SAS Institute 1988).

[a] ≈100% coverage.

[b] 2 ml of solution at 0.7 kn/cm^2; ≈70% coverage.

Nymph Leaf-Dip Bioassays. Whitefly nymphs treated with *Nicotiana* sugar ester isolates and the synthetic sugar ester quickly dried and detached from the leaf surface, with dorsal and ventral surfaces of the body compressed together as reported by Neal et al. (1994). Significant differences in mortality response of 2nd-instar *B. argentifolii* to both rates of 11 natural sugar ester isolates of *Nicotiana* species were observed in the 1st test ($P < 0.001$) (Table 4). At the rate of 1 g (AI)/liter, sugar ester isolates of *N. gossei* and *N. palmeri* caused greatest mortality (96.7 and 89.9%, respectively), whereas at the rate of 0.5 g (AI)/liter, the highest mortality (89.6%) was seen with *N. gossei*. Mortality response of nymphs to other materials tested was weak (18.2–58.8%).

Greater than 95% mortality of 2nd-instar nymphs was observed in response to sugar ester isolates at 1 g (AI)/liter of *N. amplexicaulis, N. glutinosa, N. langsdorffii, N. trigonophylla,* and *N. gossei* when tested on tomato leaves (Table 5). The same concentration of sugar ester isolate from *N. glutinosa* 24A caused only 31.2% mortality to 2nd-instar whiteflies, probably because of low (11%) content of sucrose esters. Mortality responses of 2nd-instar nymphs exposed by leafdip to 3 concentrations of *N. gossei* sugar ester isolate and the synthetic sugar ester was statistically indistinguishable (Table 6), but mortalities within rates of each material were significantly different for both synthetic sugar ester and *N. gossei* sugar ester isolates ($P < 0.001$). Mortality of whitefly nymphs were significantly less when leaves were sprayed than when dipped for all 3 rates of *N. gossei* sugar ester isolate (Table 7).

Field Trial. Whitefly populations were greater than experienced by local commercial tomatoes that season but were more typical of previous seasons before the widespread use of imidacloprid to control whitefly (Stansly 1996).

Effects on Immatures. The mean number of whitefly nymphs sampled before treatments commenced were 1.8 ± 0.4 and not significantly different among replicates ($F = 0.7$, df = 4, 138, $P > 0.05$). Posttreatments differences were most pronounced in larger instars, reflecting accumulated effects over instars. All stages (eggs, small nymphs, large nymphs, and pupae) were significantly less on treated plants compared with the control, except for the synthetic octanoyl sugar ester (OTCSE) and the cyfluthrin–methamidophos mixture against pupae (Table 8). Only these plants receiving these 2 treatments and the control had significantly more pupae than plants treated with pyriproxyfen. Parasitization of whitefly pupae by *Encarsia* spp. and *Eretmocerus* spp. at the end of the field trial averaged 19 ± 4.4% ($N = 165$ pupae) with no significant differences between treatments ($F = 1.09$; df = 7, 35; $P = 0.39$).

Effects on Adults. Significantly fewer adults were observed from plants treated with sugar ester isolates compared with untreated controls on all 3 sample dates, corresponding approximately to 3 generations of whiteflies (Table 9). There were no significant differences in results among sugar ester treatments. In comparison, there were no difference between the untreated control and pyriproxyfen in the 1st generation or the cyfluthrin–methamidophos mix in the 2nd and 3rd generations. Numbers of adults in the untreated plots were >3 times than in plots treated with sugar ester isolates at the end of the trial.

Discussion

The *N. gossei* sugar ester isolate was the most active natural sugar ester extract tested against whitefly nymphs, although some synthetic sucrose esters showed similar activity. The sugar ester isolates of the *N. glutinosa* accessions—*N. glutinosa* 24 and *N. glutinosa* 24B, which contained 55% and 90% sugar ester respectively—were highly toxic to whitefly nymphs. In contrast, the sugar ester isolate of *N. glutinosa* 24A with 11% sugar ester and 89% labdane terpenoids gave a very weak response. Neal et al. (1994) found that sugar ester isolates of *N. gossei, N. benthamiana* Domin, and *N. bigelovii* (Torrey) and 17 *Nicotiana* species were highly active against 2nd- and early 3rd-instar whitefly nymphs. Weak response of 2nd-instar

Table 4. Toxicity of sugar ester isolates of *Nicotiana* spp. applied as a dip to 2nd-instar nymphs of *B. argentifolii* on sweet potato leaves

Sugar ester isolates	% mortality ± SE		
	1.0 g (AI)/liter	0.5 g (AI)/liter	*F*
N. cavicola	40.1 ± 9.7e	44.3 ± 11.4bc	0.43
N. gossei	96.7 ± 3.5a	89.6 ± 6.5a	5.53*
N. noctiflora	46.4 ± 13.9cde	20.5 ± 3.3e	19.74**
N. otophora 38	53.3 ± 12.9bcd	28.9 ± 14.7de	9.33*
N. otophora 38A	40.2 ± 15.3de	22.3 ± 7.5e	6.48*
N. otophora 38B	58.0 ± 11.4bc	33.6 ± 6.4cd	20.32**
N. otophora 38C	32.2 ± 5.9e	18.2 ± 9.9e	8.57*
N. palmeri	89.9 ± 12.1a	63.0 ± 11.3b	12.20**
N. pauciflora	42.9 ± 7.9e	18.7 ± 8.3e	23.11**
N. plumbaginifolia	58.8 ± 13.6bc	36.7 ± 14.7bcd	7.31
N. simulans	52.1 ± 25.9b	46.7 ± 6.7b	0.43
Water + 1% acetone	4.7 ± 2.0f	4.3 ± 1.9f	0.08
F	17.5**	42.7**	—
LSD	14.6	10.9	—

*, $P = 0.05$; **, $P = 0.01$. Means in the same column followed by different letters differ significantly (SAS Institute 1988).

standards mentioned above and an untreated control. Blocks ran east and west and plots were 7.4 m long and 3 rows (1.8-m centers) wide. Plants were sprayed weekly for 8 wk starting the 4th week after the transplanting (except for pyriproxyfen, which was sprayed every other week at the manufacture's recommendation). Applications were made in the early morning around 0700–0900 hours (March-May, 1995) with a tractor-drawn high-clearance sprayer fitted with 4–8 Albuz yellow hollow cone ceramic nozzles per row (depending on plant height) operating at 14 kg/cm^2 pressure and 3.2 km/h (2 mph). Delivery rates were 309 liter/ha (33 gal/acre) with 4 nozzles (first 3 wk), 570 liters/ha (61 gal/acre) with 6 nozzles (4th wk), and 758 liters/ha (81 gal/acre) with 8 nozzles (remaining 4 wk).

A pretreatment sample of whitefly nymphs and pupae was taken on 17 March 1995. Posttreatment samples (8) of whitefly adults, small nymphs (1st and 2nd instars), large nymphs (3rd and 4th instars), pupae, and parasitized pupae were taken weekly thereafter. Whitefly adults from 6 plants in the center row in each plot were sampled by striking a black baking pan (24 by 33 by 2.5 cm) against the vegetation and counting whiteflies trapped in a thin coating of soybean oil (Publix brand) and detergent (Dawn [Procter & Gamble, Cincinnati, OH] mixture (oil–detergent, 30:1 [vol.:vol.]. Whitefly immatures were sampled from 4 randomly selected plants of each of the 3 rows by removing a trifoliate from the 6th node from the top of each plant for a total of 12 trifoliates per plot. All whitefly stages falling within a 0.5-cm^2 template placed twice on each side of the midvein of the terminal leaflet of the trifoliate were counted with a stereoscopic microscope, giving 4 cm^2 of leaf area per trifoliate.

Data Analysis. Percentage mortality (bioassay) of whitefly adults and nymphs were transformed to the arc sine square root [arsine (percentage mortality/100)$^{1/2}$] before analysis of variance (ANOVA) to stabilize error variance (Gomez and Gomez 1984), although untransformed mean percentage mortality (±SE) is reported. Sources of variation for this analysis were insecticides, replicate, repetition, and insecticides × replicate. The error term used to test insecticide effects was the mean square for the insecticide × replicate interaction (Freund et al. 1986). Means were separated using the least significant difference (LSD) test following a significant *F* test (SAS Institute 1988).

Table 5. Toxicity of sugar ester isolates of *Nicotiana* spp. (1.0 g [AI]/liter) applied as a dip to 2nd-instar nymphs of *B. argentifolii* on tomato leaves

Sugar ester isolate	% mortality ± SE
N. amplexicaulis	99.0 ± 2.1a
N. glutinosa 24	96.0 ± 3.6a
N. glutinosa 24B	97.6 ± 4.0a
N. glutinosa 24A	31.2 ± 7.3b
N. gossei	98.5 ± 2.5a
N. langsdorffii	96.1 ± 4.9a
N. trigonophylla	95.0 ± 4.9a
Water + 1% acetone	4.1 ± 2.9c
F	239.9**
LSD	4.8

**, $P = 0.01$. Means in the same column followed by different letters differ significantly (SAS Institute 1988).

Results

Adult Bioassays. Sugar ester isolates of *N. amplexicaulis, N. glutinosa, N. langsdorffii, N. trigonophylla,* and *N. gossei* induced strong mortality responses in immobilized whitefly adults sprayed to runoff (Table 2). In contrast, mortality response of adult *B. argentifolii* to *N. gossei* sugar ester isolate applied with the Potter spray tower were feeble (Table 3). Rate response was significant ($P < 0.001$), but only between the concentrations of 0.5 and 1 g (AI)/liter. These results indicated that complete coverage of adult whiteflies with these materials was necessary to achieve high levels of adult mortality.

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Table 8. Populations of immature *B. argentifolii* on tomato foliage in the field after 8 weekly sprays with selected insecticides

Sugar esters	No./10 cm² ± SE			
	Eggs	Small nymphs (1st–2nd)	Large nymphs (3rd–4th)	Pupae
N. trigonophylla	1.9 ± 0.8b	2.7 ± 1.2bc	0.5 ± 0.3b	0.3 ± 0.1cd
OTC7SE	0.3 ± 0.2b	1.3 ± 0.4bc	0.6 ± 0.3b	0.7 ± 0.3bcd
OTC8SE	1.4 ± 0.6b	3.8 ± 1.3bc	0.8 ± 0.3b	1.8 ± 0.5abc
OTC9SE	1.0 ± 0.4b	2.3 ± 0.6bc	0.2 ± 0.1b	1.1 ± 0.6bcd
OTC10SE	1.3 ± 1.0b	2.9 ± 1.0bc	0.3 ± 0.2b	0.8 ± 0.4bcd
Pyriproxyfen	0.9 ± 0.6b	0.6 ± 0.3c	0.1 ± 0.1b	0.1 ± 0.1d
Cyfluthrin + methamidophos	8.9 ± 4.8ab	7.9 ± 1.9b	1.8 ± 0.8b	2.1 ± 0.6ab
Untreated	12.3 ± 7.7a	17.3 ± 7.1a	7.8 ± 4.3a	3.5 ± 1.4a
F	1.88*	4.00**	2.84**	3.37**
LSD	8.71	7.22	4.13	1.70

*, $P = 0.05$; **, $P = 0.01$. Means in the same column followed by different letters differ significantly (LSD; SAS Institute 1988).

nymphs to 98% sugar ester isolates of *N. cavicola, N. simulans, N. pauciflora, N. plumbaginifolia, N. noctiflora,* and *N. otophora* are probably caused by differences in sugar ester structure or composition and remain to be investigated.

Among sugar ester isolates from different species of *Nicotiana,* those from *N. gossei, N. amplexicaulis, N. glutinosa, N. langsdorffii,* and *N. trigonophylla* were highly active when sprayed to runoff against immobilized whitefly adults on yellow sticky cards, although untreated adults could hardly be soaked this way by a field application. Less mortality was seen when adults were sprayed with a Potter spray tower, which gives even but incomplete coverage (Liu and Stansly 1995c); dried residues of *N. gossei* sugar ester isolate were ineffective as toxicants or repellents (Liu and Stansly 1995a,b). Sugar esters of *N. gossei* also were not toxic to eggs of *B. argentifolii* (Liu and Stansly 1995a,b,c). Therefore, the effects of sugar ester sprays observed on adult and egg populations in the field probably results largely from mortality to nymphs. Treatment with pyriproxyfen also reduced the numbers of adults in the 2nd and 3rd generations, in this case, because of suppression of embryogenesis and formation of adults (Ishaaya and Horowitz 1992). Therefore, movement of adults between plots mush have limited.

Buta et al. (1993) and Neal et al. (1987, 1994) also reported that mixtures of sucrose and glucose esters from extracts of *N. gossei* caused >90% mortality against 2nd- and early 3rd-instar nymphs of *T. vaporariorum* and *B. tabaci* (= *B. argentifolii*) as well as the green peach aphid, *Myzus persicae* (Sulzer), and the two-spotted spider mite, *Tetranychus urticae* Koch. They were only weakly toxic to the western flower thrips, *Frankliniella occidentalis* (Pergande), and non-toxic to the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). Concentrations of 0.2 g (AI)/liter of *N. gossei* sugar ester isolates were innocuous to all developmental stages of *Nephaspis oculatus* (Blatchley), and leaf residues did not affect adults of *Encarsia pergandiella* Howard, predator and parasitoid of *B. argentifolii,* respectively (T.-X.L. and P.A.S, unpublished data). The selective toxicity of some natural sugar esters and synthetic sugar esters to a number of plant pests make them potentially attractive biorational alternatives for many management applications.

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Table 9. Adult *B. argentifolii* sampled in the field with a beat pan from tomato plants sprayed weekly with selected insecticides

Sugar esters	No. adults/pan ± SE		
	27 March (1st generation)	21 April (2nd generation)	17 May (3rd generation)
N. trigonophylla	2.0 ± 0.6b	2.9 ± 0.5b	44.7 ± 6.6b
OTC7SE	2.6 ± 0.5b	3.3 ± 0.6b	35.7 ± 3.6b
OTC8SE	3.2 ± 0.5b	3.8 ± 0.8ab	53.0 ± 9.2b
OTC9SE	2.3 ± 0.6b	3.8 ± 0.6ab	44.5 ± 8.4b
OTC10SE	3.3 ± 0.6b	2.8 ± 0.6b	46.8 ± 13.0b
Pyriproxyfen	5.3 ± 1.0a	2.2 ± 0.5b	24.8 ± 3.6b
Cyfluthrin + methamidophos	2.3 ± 0.3b	5.3 ± 0.9a	161.0 ± 19.4a
Untreated	6.9 ± 0.9a	5.8 ± 0.9a	165.8 ± 21.5a
F	6.55**	2.92**	19.96**
LSD	1.81	1.95	33.72

**, $P = 0.01$. Means in the same column followed by different letters differ significantly (SAS Institute 1988).

Table 1. Humectants evaluated for enhancement of the efficacy of sugar esters to tobacco aphids in this study

Trade name and synonyms	CTFA Name[a]	Manufacturer[b]
Amino collagen 25	Collagen amino acids	Maybrook
Collagen native extra 1%	Soluble collagen 010193 R1	Maybrook
Elas-Tein 10	Hydrolyzed elastin	Maybrook
May-Tein AF	Cocoyl hydrolyzed collagen	Maybrook
Aqua-Tein C	Collagen acids and acetamide MEA and propylene glycol	Maybrook
Hydrolactin 2500	Hydrolyzed milk protein	Croda
Tritisol	Soluble wheat protein (proposed)	Croda
Cropeptide W	Hydrolyzed wheat protein and wheat oligosaccharides	Croda
Collasol	Soluble collagen	Croda
Cromoist O-25	Hydrolyzed oats	Croda
Crosilk 10,000	Hydrolyzed silk	Croda
Cromoist CS	Chondroitin sulfate and hydrolyzed animal protein	Croda
Incromectant AMEA 100	Acetamide MEA	Croda
Incromectant LMEA	Lactamide MEA	Croda
Incromectant LAMEA	Acetamide MEA and lactamide MEA	Croda
Incromectant AMEA 70	Acetamide MEA	Croda
Incromectant AQ	Acetamidopropyl trimonium chloride	Croda
Incromectant LQ	Lactamidopropyl trimonium chloride	Croda
Volpo G-31	Glycereth-31	Croda

[a] Cosmetic, Toiletry, and Fragrance Association, 1110 Vermont Avenue N.W., Washington, DC 20005.
[b] Maybrook, P. O. Box 68, 570 Broadway, Lawrence, MS 01842; Croda, 7 Century Drive, Parsippany, NJ 07054–4698.

Materials and Methods

Sugar Esters. Sugar esters used in this study included a mixture of 2 sucrose esters (2,3-di-*O*-acyl-6′-*O*-acetylsucrose and 2,3,-di-*O*-acyl-1′,6′-diacetylsucrose) and 2 glucose esters (1-*O*-acetyl-2,3-di-*O*-acylglucose and 2,3-di-*O*-acylglucose) from *N. gossei*; a mixture of 2,3,4-tri-*O*-acyl-sucrose (TAS) and 3′-TAS from *N. glutinosa*; and a mixture of TAS, 3′-TAS, 4′-TAS, and 3′,4′-TAS from *N. palmeri* (Chortyk et al. 1993). Four synthetic sucrose esters were prepared by the method described by Chortyk et al. (1996). Each of the 4 synthetic sucrose esters was a mixture of 6-, 6′-, or 1′-monoacyl sucroses, 6,6′-, 6,1′-, or 1′,6′-diacyl sucroses, and 6, 1′, 6′-triacyl sucroses. Sugar esters were dissolved in acetone/methanol (9:1, vol:vol) in the proportion of 1 g (AI): 50 ml (wt:vol) to make stock solutions. Sugar esters and acetone–methanol solutions were then formulated in distilled water (for laboratory tests) or tap water (for field tests) to the desired concentrations immediately before applications.

Tobacco Aphids. Tobacco aphids used in this study were the red color form, and apterous adults and immatures were tested together. Aphids used in laboratory tests were collected from a colony maintained on tobacco plants in the greenhouse at the Clemson University Pee Dee Research and Education Center in Florence, SC. The colony was started from field plants and reared on potted 'K326' tobacco plants at temperatures ranging from 15 to 30°C under a natural photoperiod. Aphids used in field tests were of similar age and were from natural infestations from a nearby field of 'K346' tobacco plants.

Humectants. The humectants used were obtained from commercial sources (Table 1). No attempt was made to purify the humectants before use, and no correction was made for differences in percentage active ingredient among the humectants. Concentrations of humectants in this study were based on the formulated products (vol:vol).

Laboratory Experiments. A leaf spray technique was used for laboratory evaluations. Tobacco leaves infested with numerous tobacco aphids (minimum 100 per leaf) were collected from potted tobacco plants immediately before treatment. Treatments were applied to each tobacco leaf or leaf section by using a hobby-type air brush (General Electric, Fort Wayne, IN) at a pressure of ≈1.06 kg/cm^2 (15 psi). Both sides of each leaf or leaf section were sprayed by passing the sprayer over each leaf surface 5 times at a distance of 12 cm from the leaf surface, achieving an application rate of ≈39 μl/cm^2 of spray mixture. The application rate was determined by spraying tobacco leaf sections of a predetermined area with water in the manner described and weighing the leaf sections before and after spraying. The volume of water per square centimeter on the given area was obtained by converting the weight increase in milligrams to microliters. When there were too many aphids on a leaf, we cut a leaf into sections (≈12–15 cm^2) and applied the sugar ester solution to each leaf section by using the leaf spray technique described earlier. After spray application, leaves were dried immediately under an electric fan for 30 min and placed in petri dishes (9 cm diameter) containing dry filter paper. Each petri dish containing 1 leaf or leaf section was considered a replication, and each treatment was replicated 4 times. Petri dishes were maintained in an environmental chamber (Sherer-Gillett Company, Marshall, MI) at a temperature of 25 ± 2°C and relative humidity of 30 ± 10%. Although our preliminary tests showed that most aphid mortality occurred within 6 h after application, some mortality occurred 6 h after application. Therefore, aphid mortality was deter-

FIELD AND FORAGE CROPS

Enhanced Toxicity of Sugar Esters to the Tobacco Aphid (Homoptera: Aphididae) Using Humectants

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ABSTRACT Eighteen humectants were evaluated in the laboratory and in the field to determine if they enhanced the insecticidal activity of natural and synthetic sugar esters against the tobacco aphid, *Myzus nicotianae* Blackman. Sugar esters included 3 types of mixtures of sucrose esters and glucose esters extracted from *Nicotiana gossei* Domin, *N. palmeri* Gray, and *N. glutinosa* L., and 4 types of synthetic sucrose esters. A leaf spray technique was used to apply sugar esters and treatment combinations in the laboratory. Sugar esters from *N. gossei* also were evaluated alone and in combination with certain humectants in the field by using a CO_2 hand-held sprayer and a high-clearance sprayer fitted with a compressed air system. Toxicities of all sugar esters to tobacco aphids were significantly improved by certain humectants. Improved efficacy was related to the concentration of humectants and the type of sugar esters and humectants. The addition of humectants Volpo G-31, Incromectant AMEA 100, Aqua-Tein C, Incromectant AMEA 70, Incromectant LQ, Incromectant LAMEA, Incromectant AQ, Hydrolactin 2500, Crosilk 10,000, and Cromoist CS at a 5% concentration to a solution of *N. gossei* sugar esters at a concentration of 1 mg/ml resulted in >90% aphid mortality. Aphid mortality from the sugar esters alone was only 12%. Aphid mortality increased as the concentration of humectants with sugar esters increased. Field evaluations showed that 5 humectants also were effective in enhancing the toxicity of *N. gossei* sugar esters against the tobacco aphid. Sugar esters applied with a CO_2 hand-held sprayer in the field gave higher aphid control than a high-clearance sprayer. Humectants alone were not toxic to tobacco aphids.

KEY WORDS *Myzus nicotianae*, *Nicotiana* spp., humectants, sugar esters, sucrose esters, glucose esters

RECENT RESEARCH ON plant resistance to insect pests of *Nicotiana* spp. (Johnson et al. 1992); wild tomato, *Lycopersicon hirsutum* F. *glabratum* (Dimock and Kennedy 1983); *Solanum* spp. (Tingey 1991); and other plants (Juniper and Southwood 1986, King and Calhoun 1988) has demonstrated that glandular trichomes and the exudates they produce contribute to insect resistance in these plants. Toxicity, repellency, and physical entrapment have been reported as the modes of action of various trichome exudates against insect pests (Johnson and Severson 1982, 1984; Dimock and Kennedy 1983; Duffey 1986; Walters et al. 1990). Studies have shown that sugar esters, including sucrose esters and glucose esters secreted by glandular trichomes, were the main chemicals responsible for aphid resistance in tobacco (Severson et al. 1985) and other plants (Walters and Steffens 1990). Sugar esters have been extracted from certain *Nicotiana* species. Bioassays have shown that sugar esters are toxic to the tobacco aphid, *Myzus nicotianae* Blackman (Severson et al.1991), and other insect pests (Buta et al. 1993, Neal et al. 1994, Chortyk and Nottingham 1995, Puterka and Severson 1995). However, data from early field tests have suggested that sugar ester efficacy against the tobacco aphid in the field is much lower than in the laboratory (Xia and Johnson 1997). Recently, we found that ambient relative humidity and leaf surface moisture were important factors affecting the efficacy of *N. gossei* sugar esters (Xia and Johnson 1997). Humectants are hygroscopic materials that attract water, largely from the surrounding air, effectively binding moisture at the site of application (Coupland and Smith 1986). Humectants are widely used in the cosmetic industry. They also can change the solubility and penetration of pesticides after field application, thereby enhancing pesticidal activity by increasing pesticide uptake by plants and fungi (Matsumoto et al. 1992). The addition of humectants that promote the retention of moisture could improve the effectiveness of sugar esters. Consequently, we conducted field and laboratory tests to determine if humectants could improve the toxicity of sugar esters from *N. gossei* and other *Nicotiana* species as well as certain synthetic sugar esters against tobacco aphids.

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Table 2. Effects of humectants on the toxicity of *N. gossei* sugar esters and synthetic octanoyl sucrose esters against the red color form of adult and immature tobacco aphids in the laboratory using a leaf spray technique

Humectant	% aphid mortality after 12 h mean ± SEM					
	1 mg/ml *N. gossei* sugar esters and humectant at a concn of			1 mg/ml synthetic octanoyl sucrose esters and humectant at a concn of		
	0.5%	1%	5%	0.5%	1%	5%
Sugar esters only	11 ± 2.1 bc,a	11 ± 4.1 abc,a	12 ± 3.4a,a	9 ± 2.1a,A	9 ± 2.0abc,A	10 ± 2.0bcd,A
Elas-Tein 10	11 ± 3.7bc,a	13 ± 3.9abc,a	13 ± 2.9a,a	11 ± 4.1a,A	10 ± 2.6bc,A	13 ± 3.4cd,A
CNE	10 ± 2.7ab,a	14 ± 3.1bc,a	16 ± 5.2a,a	12 ± 3.1a,A	14 ± 1.8cd,A	24 ± 5.5e,B
Collasol	11 ± 2.8bc,a	12 ± 2.3abc,a	17 ± 3.6a,b	10 ± 3.4a,A	9 ± 0.8abc,A	9 ± 2.7abc,A
Cromoist 0-25	14 ± 1.4cde,a	13 ± 2.1abc,a	61 ± 6.2c,b	10 ± 3.9a,A	10 ± 2.4bc,A	29 ± 6.9e,B
Tritisol	10 ± 1.9b,a	17 ± 5.9cd a	80 ± 9.1d,b	13 ± 3.5a,A	25 ± 5.9fg,B	62 ± 7.8h,C
Cropeptide W	13 ± 2.5bcd,a	19 ± 4.7c,a	55 ± 8.8b,b	10 ± 2.2a,A	14 ± 3.9cd,A	26 ± 7.3e,B
AC	11 ± 2.9b,a	18 ± 2.1c,b	77 ± 6.5d,c	11 ± 3.1a,A	12 ± 3.9cd,A	40 ± 13.8f,B
Cromoist CS	12 ± 3.9bcd,a	21 ± 3.9c,b	91 ± 5.4e,c	10 ± 3.3a,AB	9 ± 2.9abc,A	15 ± 3.8d,B
Crosilk 10,000	18 ± 3.0ef,a	47 ± 8.1d,b	91 ± 2.0ef,c	12 ± 3.6a,A	23 ± 8.0f,B	41 ± 6.7f,C
Aqua-Tein C	16 ± 3.5de,a	58 ± 7.9ef,b	96 ± 3.1fg,c	13 ± 3.6a,A	49 ± 4.4i,B	78 ± 6.4i,C
Hydrolactin	20 ± 2.6f,a	55 ± 17.9de,b	92 ± 3.5ef,c	11 ± 3.4a,AB	10 ± 2.6abc,A	25 ± 5.1e,B
IAQ	25 ± 2.5g,a	67 ± 16.6fg,b	92 ± 3.6ef,c	13 ± 3.9a,A	49 ± 13.2i,B	93 ± 5.0l,C
ILAMEA	29 ± 6.1h,a	49 ± 19.3de,b	90 ± 5.9e,c	9 ± 2.7a,A	12 ± 3.6cd,B	28 ± 4.7e,C
ILQ	29 ± 5.9h,a	67 ± 17.0fg,b	91 ± 4.0ef,c	13 ± 5.0a,A	40 ± 8.7h,B	91 ± 4.6kl,C
IAMEA 70	30 ± 4.0h,a	51 ± 12.5de,b	91 ± 4.1ef,c	12 ± 2.6a,A	22 ± 6.0ef,A	66 ± 10.5h,B
ILMEA	29 ± 5.4h,a	51 ± 19.6de,b	88 ± 3.6e,c	14 ± 3.3a,A	38 ± 9.1h,B	79 ± 11.4ij,C
IAMEA 100	31 ± 3.3hi,a	68 ± 20.2fg,b	96 ± 1.3fg,c	10 ± 1.2a,A	17 ± 7.4de,A	49 ± 8.3g,B
Volpo G-31	33 ± 3.4f,a	74 ± 18.55g,b	98 ± 2.0g,c	12 ± 2.2a,A	29 ± 5.9g,B	85 ± 6.9jk,C

Values followed by the same letter are not significantly different within a column (letters before comma) or a row (letters after comma). Lower case letters and capital letters after comma are for *N. gossei* sugar esters and synthetic octanoyl sucrose esters, respectively ($\alpha = 0.05$, Waller–Duncan k ratio t-test [SAS Institute 1990]). CNE, Collagen Native Extra 1%; AC, Amino Collagen 25; IAQ, Incromectant AQ; ILQ, Incromectant LQ; ILAMEA, Incromectant LAMEA; ILMEA, Incromectant LMEA; IAMEA 70, Incromectant AMEA 70; IAMEA 100, Incromectant AMEA 100; Crosilk, Crosilk 10,000; Hydrolactin, Hydrolactin 2500.

gossei sugar esters was not different from the addition of Collasol, Cromoist O-25, Tritisol, and Cropeptide W at concentrations of 0.5 and 1%, but it was different at concentrations of 1 and 5% (Table 2).

Aphid mortality from synthetic octanoyl sucrose esters was improved by addition of certain humectants (Table 2, columns 5–7). However, the effect of humectants on the efficacy of octanoyl sucrose esters was not as great as it was for *N. gossei* sugar esters. We did not observe a significant increase in aphid mortality from octanoyl sucrose esters and the addition of humectants at a concentration of 0.5% ($F = 0.77$; df = 18, 57; $P > 0.7256$) (Table 2, column 5). However, the toxicity of octanoyl sucrose esters against tobacco aphids was significantly improved with the addition of certain humectants when the concentration of humectants was 1% ($F = 21.35$; df = 18, 57; $P < 0.0001$) (Table 2, column 6). Combinations of octanoyl sucrose esters with Incromectant AQ and Aqua-Tein C resulted in the highest aphid mortality (49%). As the concentration of humectant was raised to 5%, aphid mortality from combinations of octanoyl sucrose esters plus certain humectants increased ($F = 67.42$; df = 18, 57; $P < 0.0001$) (Table 2, column 7). Combinations of octanoyl sucrose esters with either Incromectant AQ or Incromectant LQ resulted in >90% aphid mortality.

Aphid mortality increased significantly as the humectant concentration increased from 0.5 to 5% for 8 of the humectants used in combination with octanoyl sucrose esters at a rate of 1 mg/ml (Table 2). Aphid mortality from synthetic octanoyl sucrose esters and the addition of either Elas-Tein 10 or Collasol was not significantly different at either of the 3 concentrations of humectants ($P > 0.05$) (Table 2, rows 2 and 4). The efficacy of synthetic octanoyl sucrose esters was not different with the addition of Collagen Native Extra 1%, Cromoist O-25, Cropeptide W, Amino Collagen, Cromoist CS, Hydrolactin 2500, Incromectant AMEA 70, or Incromectant AMEA 100 at concentrations of 0.5 and 1%.

Aphid mortality from 7 different sugar esters alone was not significantly different ($F = 1.09$; df = 6, 21; $P = 0.4$) (Table 3, column 2). Overall aphid mortality from each sugar ester with humectants was significantly different ($F = 3.20$; df = 6, 161; $P = 0.005$). Aphid mortality from different sugar esters was significantly different after the addition of Elas-Tein 10 ($F = 8.35$; df = 6, 21; $P < 0.0001$) (Table 3, column 3), Hydrolactin 2500 ($F = 38.3$; df = 6, 21; $P < 0.0001$) (Table 3, column 4), Aqua-Tein C ($F = 44.43$; df = 6, 21; $P < 0.0001$) (Table 3, column 5), Incromectant AMEA 100 ($F = 10.54$; df = 6, 21; $P < 0.0001$) (Table 3, column 6), and Volpo G-31 ($F = 3.09$; df = 6, 21; $P < 0.025$) (Table 3, column 7). Humectant Elas-Tein 10 improved the efficacy of only *N. palmeri* sugar esters ($\alpha = 0.05$) (Table 3). Hydrolactin 2500 significantly improved the efficacy of all sugar esters examined except *N. glutinosa* sugar esters ($\alpha = 0.05$). Volpo G-31 and Incromectant AMEA 100

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mined 12 h after treatment with the aid of a stereo microscope. Aphids were considered dead if no movement was detected when they were probed gently with a camel's-hair brush. All aphids within a petri dish were counted. The number of aphids in each replication varied from 100 to 200.

Field Experiments. Field experiments were conducted in 1995 in a K346 tobacco field located at the Pee Dee Research and Education Center. Tobacco was transplanted 23 May, and rows were 1.22 m apart with plants 55.9 cm apart in the rows. Tillam (6-E, Zeneca, Wilmington, DE) was applied as a preplant broadcast-incorporated treatment at 6.3 liters/ha and Prowl (3.3 EC, American Cyanamid, Wayne, NJ) was applied at layby (last cultivation) at 1.4 liters/ha for weed control. Granular fertilizer (6:6:18 [N:P:K]) and sodium nitrate (16% N) were sidedressed after transplanting at 756 and 336 kg/ha, respectively.

Experimental plots were marked off in the field in a randomized complete block design using 4 replicates. Plots (7.6 m long, 1.22 m wide) consisted of 10 plants. Plots were separated by 5 untreated plants. Each plot was 1 row wide and was considered a replication. Three independent field experiments were conducted. The number of treatments in each field experiment varied (Tables 4, 5, and 6). All treatments in each field experiment were applied within 2 h on the same day. All 3 field experiments were conducted at ≈1900 hours EST on 20 August, 24 August, and 9 September 1995, respectively. A CO_2 hand sprayer or a high-clearance sprayer (Hagie, Clarion, IA) with a compressed air system was used to apply treatment solutions. An average of 0.06 liter of spray solution was applied to both sides of 4 upper leaves of each plant (878 liters/ha) by using the CO_2 hand sprayer with 1 full cone nozzle (TG-2) at a working pressure of 2.8 kg/cm^2 (40 psi). A spray volume of 1,869 liters/ha (200 gal/acre) solution was applied using a high-clearance sprayer with 5 hollow cone nozzles (D2-25) at a working pressure of 5.6 kg/cm^2 (80 psi). The nozzle arrangement on the high-clearance sprayer consisted of a center nozzle over the top and 2 drop nozzles on each side of each row of plants. The center nozzle sprayed straight down, whereas the upper nozzle on each side was angled downward at 45°, and the lower nozzle on each side was angled upward at 45°. Ambient relative humidity and leaf surface moisture affect the efficacy of *N. gossei* sugar esters and possibly other sugar esters (Xia and Johnson 1997). Temperature affects the evaporation of spray solutions on leaf surfaces and the status of leaf surface moisture; therefore, both temperature and relative humidity were recorded at the time of each spraying with a thermohygrometer (Brooklyn Thermometer, Farmingdale, NY). Twenty-four hours after treatments were applied, 4 upper leaves or leaf sections were randomly collected from each treated plot and 4 untreated plots and taken to the laboratory. The numbers of live and dead aphids were counted on each leaf or leaf section to determine percentage mortality. Only the first 100 aphids on a leaf or leaf section were counted.

Statistical Analysis. Abbott's formula was used if mortality in the control was >5% (Abbott 1925, Busvine 1971). Analysis of variance procedures (ANOVA, MEANS statement, SAS Institute 1990) were used to conduct analysis of variance among the treatments and blocks and to compute the means and standard errors of dependent variables. Waller–Duncan k-ratio t-test (ANOVA, Waller option; SAS Institute 1990) was used to compare means between treatments ($\alpha = 0.05$).

Results

Laboratory Experiments. Humectants used alone did not have any significant effect on tobacco aphids at a concentration of either 0.5% ($F = 0.49$; df = 18, 57; $P > 0.951$), 1% ($F = 0.46$; df = 18, 57; $P > 0.964$), or 5% ($F = 0.55$; df = 18, 57; $P > 0.917$). Aphid mortality from the humectants ranged from 2.7 ± 2.0% to 6.2 ± 1.6%. There were significant differences in aphid mortality among the treatments of *N. gossei* sugar esters plus a humectant at all 3 humectant concentrations (Table 2). The efficacy of *N. gossei* sugar esters was enhanced by adding certain humectants at a concentration of 0.5% ($F = 23.48$; df = 18, 57; $P < 0.0001$) (Table 2, column 2). Aphid mortality from a combination of *N. gossei* sugar esters and Volpo G-31, Incromectant AMEA 100, Incromectant LMEA, Incromectant AMEA 70, Incromectant LQ, or Incromectant LAMEA at a concentration of 0.5% was ≈20% higher than *N. gossei* sugar esters alone. Insecticidal activity increased among most combinations of *N. gossei* sugar esters and humectants when the concentration of humectant was raised to 1% ($F = 14.76$; df = 18, 57; $P < 0.0001$) (Table 2, column 3). The addition of Volpo G-31 and Incromectant AMEA 100 at a concentration of 1% resulted in 63% and 57% more aphid mortality than sugar esters alone, respectively. Fifteen humectants significantly improved the efficacy of *N. gossei* sugar esters when the concentration of humectant was increased to 5% ($F = 169.85$; df = 18, 57; $P < 0.0001$) (Table 2, column 4), and 10 combination treatments resulted in >90% aphid mortality. Combination treatments of 1 mg/ml *N. gossei* sugar esters and 5% Incromectant AMEA 100 or Volpo G-31 resulted in aphid mortality of 96 and 98%, respectively.

Aphid mortality significantly increased as the humectant concentration increased from 0.5 to 5% for 12 of the humectants used in combination with *N. gossei* sugar esters at a concentration of 1 mg/ml (Table 2, rows 8–19). Aphid mortality from *N. gossei* sugar esters and Elas-Tein 10 or Collagen Native Extra 1% was not significantly different at either of the 3 concentrations of humectants ($P > 0.05$) (Table 2, rows 2 and 3). The efficacy of *N.*

Table 6. Field evaluation of Volpo G-31 humectant to improve *N. gossei* sugar esters for controlling the red color form of adult and immature tobacco aphids by using a high-clearance sprayer

Treatment[a]	% aphid mortality after 24 h mean ± SEM
5% Volpo G-31	7 ± 2.1a
0.5 mg/ml sugar esters	24 ± 5.9b
0.5 mg/ml sugar esters + 5% Volpo G-31	67 ± 12.5d
1.0 mg/ml sugar esters	42 ± 9.7c
1.0 mg/ml sugar esters + 1% Volpo G-31	69 ± 14.3d
1.0 mg/ml sugar esters + 5% Volpo G-31	84 ± 8.7e

Values followed by the same letter are not statistically different (α = 0.05, Waller–Duncan k ratio t-test [SAS Institute 1990]). Temperature and relative humidity at the time of application were 31°C and 53%, respectively.

[a] Spray volume = 1,869 liters/ha; working pressure = 5.6 kg/cm^2 (80 psi); and 5 nozzles per row.

were observed in this field test (F = 0.35; df = 3, 18; P = 0.7876) (Table 5).

Volpo G-31 significantly increased *N. gossei* sugar ester toxicity in the field when applied with a high-clearance sprayer (α = 0.05) (Table 6). The combination of 0.5 mg/ml sugar esters and 5% Volpo G-31 resulted in 67% aphid mortality compared with 24% mortality from 0.5 mg/ml sugar esters alone. The addition of Volpo G-31 at a rate of 1% significantly improved the efficacy of sugar esters applied at a rate of 1 mg/ml (α = 0.05). Increasing the concentration of Volpo G-31 from 1 to 5% significantly increased aphid mortality when they were applied with the sugar esters at a rate of 1 mg/ml (α = 0.05) (Table 6). No significant block effects were observed in this field test (F = 1.54; df = 3, 15; P = 0.2449).

Discussion

This study confirmed our previous discovery that leaf surface moisture and ambient relative humidity affected the efficacy of *N. gossei* sugar esters (Xia and Johnson 1997). Humectants enhanced aphid toxicity from the different natural sugar esters from *Nicotiana* spp. and synthetic sucrose esters. The improved efficacy of sugar esters was related to the type of humectant and sugar esters. Certain humectants, such as Incromectant AMEA 100 and Volpo G-31, were highly effective in improving the efficacy of all natural sugar esters evaluated in this study. The efficacy of *N. gossei* sugar esters was enhanced the most by the addition of certain humectants. Although we examined the effects of various humectants on the aphid toxicity of specific synthetic sucrose esters in this study, more research needs to be conducted to determine the best humectant(s) for other synthetic sucrose esters.

One consideration when using humectants as adjuvants for agrochemical application is the cost to growers. We demonstrated that certain humectants improved the efficacy of *N. gossei* sugar esters significantly, even at a concentration of 0.5%. However, a rate of 3–5% probably is needed in field applications to achieve acceptable aphid control based on the results of this research. Thus, using a humectant in the field could cost from $35 to $50/ha if a 3% humectant concentration is applied. Another concern when using humectants for agrochemical field applications is their possible effects on beneficial insects and other organisms in the environment. This study showed that all of the humectants tested were not toxic to tobacco aphids even at a rate of 5%. However, we do not know whether these humectants have any adverse effects on the natural enemies of insect pests or on the environment.

All laboratory tests in this study were conducted at relative humidity levels between 20 and 40%, and spray coverage was consistent and thorough. Aphid mortality resulting from sugar ester treatments without humectants was consistent but low in laboratory tests. In contrast, field applications in this study were made at higher relative humidity conditions, and this may have contributed to the higher aphid mortality from sugar esters alone in field applications. All field applications in this study were made at ≈1900 hours EST. On clear summer days in South Carolina, relative humidity is usually higher after 1900 hours until the following morning. Therefore, relative humidity could be higher after application than at the time of application.

Spray coverage was another important factor affecting the efficacy of sugar esters in this study, in addition to leaf surface moisture and ambient relative humidity. Insecticidal activity of *N. gossei* sugar esters against the tobacco aphid was primarily from contact toxicity (Neal et al. 1994, Puterka and Severson 1995). Therefore, aphid mortality from sugar esters would depend on the thoroughness of droplet coverage if moisture and humidity are sufficient. The hand-held CO_2 sprayer provided better spray coverage than the high-clearance sprayer, especially on the lower surfaces of the tobacco leaves where most aphids are located. This was probably the reason for the lower aphid control when using the high-clearance sprayer (Table 6).

Humectants have been reported to improve the efficacy of herbicides (Reed et al. 1992). This study is the 1st report to use humectants to enhance the efficacy of insecticides in the field. Humectants may be used widely in insecticide formulation and field application if either ambient relative humidity or leaf surface moisture is an important factor affecting insecticidal activity.

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Table 3. Comparison of effects of humectants on the toxicity of 3 natural sugar esters and 4 synthetic sucrose esters against the red color form of adult and immature tobacco aphids in the laboratory using a leaf spray technique

Type of sugar ester[a]	% Aphid mortality after 12 h mean ± SEM					
	Sugar esters	Sugar esters and Elas-Tein 10	Sugar esters and Hydrolactin 2500	Sugar esters and Aqua-Tein C	Sugar esters and IAMEA 100[b]	Sugar esters and Volpo G-31
N. gossei sugar esters	9 ± 3.8a,a	10 ± 2.3a,a	85 ± 7.2e,b	92 ± 4.8c,c	94 ± 4.2e,c	97 ± 2.7c,c
N. palmeri sugar esters	7 ± 1.4a,a	18 ± 4.2b,b	51 ± 13.7d,c	93 ± 5.0c,d	92 ± 4.7e,d	93 ± 2.3c,d
N. glutinosa sugar esters	7 ± 2.0a,a	14 ± 3.5b,a	14 ± 3.4a,a	33 ± 8.9a,b	74 ± 19.0cd,c	70 ± 19.3a,c
Heptanoyl sucrose esters	5 ± 1.8a,a	6 ± 1.9a,a	39 ± 9.1c,b	40 ± 8.7a,b	86 ± 10.3de,c	76 ± 20.9abc,c
Octanoyl sucrose esters	7 ± 1.9a,a	8 ± 2.9a,a	23 ± 4.9ab,b	66 ± 6.4b,d	50 ± 14.4a,c	82 ± 8.4abc,e
Nonanoyl sucrose esters	6 ± 3.3a,a	8 ± 2.6a,a	19 ± 7.2ab,b	41 ± 9.8a,c	67 ± 9.2bc,d	67 ± 12.0a,d
Decanoyl sucrose esters	5 ± 1.6a,a	8 ± 3.6a,a	29 ± 5.7bc,b	68 ± 6.1b,d	54 ± 8.0ab,c	73 ± 10.8ab,d

Values followed by the same letter are not statistically different within a column (letters before comma) or a row (letters after comma) ($\alpha = 0.05$, Waller–Duncan k ratio t-test [SAS Institute 1990]).
[a] Concentrations of sugar esters and humectants are 1 mg/ml (wt:vol) and 5% (vol:vol), respectively.
[b] Incromectant AMEA 100.

were the most effective humectants. Aphid mortality from the combinations of 5% Aqua-Tein C, Incromectant AMEA 100, or Volpo G-31 with *N. gossei* and *N. palmeri* sugar esters at a rate of 1 mg/ml was increased by as much as 80% over the corresponding sugar esters only.

In most cases, the efficacy of *N. gossei* and *N. palmeri* sugar esters was enhanced significantly with the addition of a humectant (Table 3). Aphid mortality from these 2 sugar esters plus Aqua-Tein C, Incromectant 100, or Volpo G-31 was >90%. The enhanced efficacy of the 4 synthetic sucrose esters was related to the type of synthetic sucrose ester and humectant (Table 3).

Field Experiments. Humectants enhanced the toxicity of *N. gossei* sugar esters applied in the field with a CO_2 hand-held sprayer (Table 4). Addition of 5% humectants significantly improved the efficacy of *N. gossei* sugar esters at concentrations of 0.5 mg/ml ($F = 8.30$; df = 5, 18; $P = 0.0003$) and 1 mg/ml ($F = 14.11$; df = 5, 18; $P < 0.0001$). Aphid mortality from combinations of 5% humectants and sugar esters at a rate of 0.5 mg/ml was from 38% (sugar esters and Incromectant LQ) to 66% (sugar esters and Volpo G-31) higher than sugar esters alone (Table 4). Incromectant LQ and Incromectant LMEA did not improve the efficacy of *N. gossei* sugar esters when the sugar esters were applied at a rate of 1 mg/ml ($\alpha = 0.05$). Increasing the rate of the *N. gossei* sugar esters from 0.5 to 1 mg/ml did not increase the efficacy of the sugar esters against aphids except for the addition of Volpo G-31 (Table 4).

The addition of Volpo G-31 at a 5% concentration to 3 concentrations of *N. gossei* sugar esters significantly enhanced the efficacy of the sugar esters applied with a CO_2 hand-held sprayer in the field ($\alpha = 0.05$) (Table 5). It appeared that the effect of Volpo G-31 on the efficacy of *N. gossei* sugar esters was especially prominent at the low rate of sugar esters. No significant block effects

Table 4. Field evaluation of humectants (5%) on the efficacy of *N. gossei* sugar esters against the red color form of adult and immature tobacco aphids by using a single nozzle CO_2 hand-held sprayer

Treatment[a]	% aphid mortality mean ± SEM after 24 h Concn sugar ester	
	0.5 mg/ml[b]	1 mg/ml[c]
Sugar esters	26 ± 13.5a,a	54 ± 9.4a,b
Sugar esters + Incromectant LQ	64 ± 7.7b,a	60 ± 5.5ab,a
Sugar esters + Incromectant LMEA	65 ± 18.7b,a	67 ± 11.6b,a
Sugar esters + Crosilk 10,000	61 ± 20.8b,a	69 ± 11.3b,a
Sugar esters + Cromoist CS	75 ± 17.8bc,a	82 ± 7.3c,a
Sugar esters + Volpo G-31	91 ± 4.4c,a	98 ± 2.4d,b

Values followed by the same letter are not statistically different within a column (letters before comma) or a row (letters after comma) ($\alpha = 0.05$, Waller–Duncan k ratio t-test [SAS Institute 1990]).
[a] A 0.06-liter solution was applied to both sides of the 4 top leaves per plant (878 liters/ha) at a working pressure of 2.8 kg/cm^2 (40 psi).
[b] Temperature and relative humidity at the time of application were 29.2°C and 73%, respectively.
[c] Temperature and relative humidity at the time of application were 31°C and 66%, respectively.

Table 5. Field evaluation of Volpo G-31 humectant and *N. gossei* sugar esters for controlling the red color form of adult and immature tobacco aphids by using a single nozzle CO_2 hand-held sprayer

Treatment[a]	% Aphid mortality after 24 h, mean ± SEM
5% Volpo G-31	6 ± 1.9a
0.5 mg/ml sugar esters	15 ± 5.2b
0.5 mg/ml sugar esters + 5% Volpo G-31	83 ± 9.8d
1.0 mg/ml sugar esters	70 ± 10.0c
1.0 mg/ml sugar esters + 5% Volpo G-31	91 ± 5.8de
2.0 mg/ml sugar esters	86 ± 6.4d
2.0 mg/ml sugar esters + 5% Volpo G-31	99 ± 1.6e

Values followed by the same letter are not statistically different ($\alpha = 0.05$, Waller–Duncan k ratio t-test [SAS Institute 1990]). Temperature and relative humidity at the time of application were 28°C and 71%, respectively.
[a] A 0.06-liter solution was applied to both sides of the 4 top leaves per plant (878 liters/ha) using a working pressure of 2.8 kg/cm^2 (40 psi).

tial funding for this study was supplied by the Wade Stackhouse Fellowship from the College of Agriculture, Forestry and Life Sciences, Clemson University. This is Technical Contribution No. 4233 of the South Carolina Agricultural Experiment Station.

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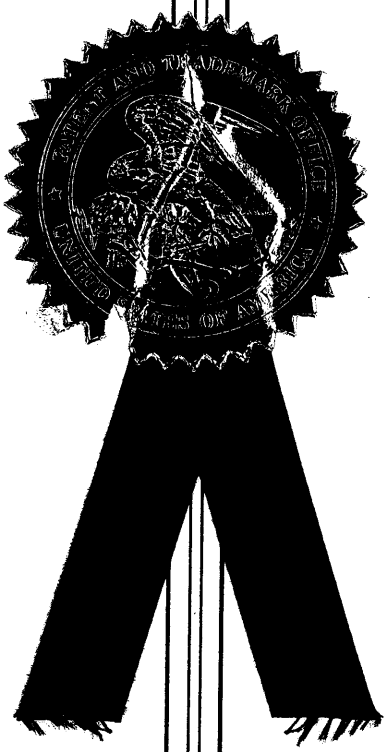
The United States of America



The Commissioner of Patents and Trademarks

Has received an application for a patent for a new and useful invention. The title and description of the invention are enclosed. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law.

Therefore, this

United States Patent

Grants to the person(s) having title to this patent the right to exclude others from making, using, offering for sale, or selling the invention throughout the United States of America or importing the invention into the United States of America for the term set forth below, subject to the payment of maintenance fees as provided by law.

If this application was filed prior to June 8, 1995, the term of this patent is the longer of seventeen years from the date of grant of this patent or twenty years from the earliest effective U.S. filing date of the application, subject to any statutory extension.

If this application was filed on or after June 8, 1995, the term of this patent is twenty years from the U.S. filing date, subject to any statutory extension. If the application contains a specific reference to an earlier filed application or applications under 35 U.S.C. 120, 121 or 365(c), the term of the patent is twenty years from the date on which the earliest application was filed, subject to any statutory extension.

Bruce Lehman

Commissioner of Patents and Trademarks

Arnitra Manley

Attest

US005756716A

United States Patent [19]

Farone et al.

[11] Patent Number: 5,756,716

[45] Date of Patent: May 26, 1998

[54] **SUGAR-ESTER MANUFACTURING PROCESS**

[75] Inventors: **William A. Farone**, Orange County, Calif.; **Robert W. Serfass**, York, Me.

[73] Assignee: **Kimball Chase Tech. Ltd.**, Portsmouth, N.H.

[21] Appl. No.: **481,647**

[22] Filed: **Jun. 7, 1995**

[51] **Int. Cl.**[6] **C07H 1/00**

[52] **U.S. Cl.** **536/120**; 536/18.5; 536/115; 536/119; 536/127

[58] **Field of Search** 536/115, 119, 536/127, 120, 18.5

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Primary Examiner—Elli Peselev

Attorney, Agent, or Firm—John J. Connors; Connors & Assoc.

[57] **ABSTRACT**

Disclosed is a process for manufacturing an sugar ester product of a sugar and a fatty acid. First, a fatty acid and methyl or ethyl alcohol is reacted in the presence of sulfuric acid catalyst to produce a fatty acid ester and water. The sulfuric acid catalyst is neutralized with a metal carbonate to make a metal sulfate, with the fatty acid ester being separated from the metal sulfate, the alcohol and the water. The recovered fatty acid ester is reacted in the presence of a metal carbonate catalyst with sugar dissolved in dimethyl sulfoxide to produce the sugar ester product and alcohol. The dimethyl sulfoxide is separated from the reaction mixture by vacuum distillation, and then water is added to emulsify the sugar ester product and unreacted fatty acid ester. The unreacted sugar and the metal carbonate is dissolved in the water. Next, the emulsified sugar ester product and unreacted fatty acid ester is separated from the water containing dissolved unreacted sugar and metal carbonate by breaking the emulsion of the sugar ester product and unreacted fatty acid ester. The sugar ester product is purified by dissolving the unreacted fatty acid ester in ethyl acetate, and substantially all the dimethyl sulfoxide, alcohol, and ethyl acetate is recovered for reuse in the process. Finally, substantially all the unreacted sugar in a concentrated useful form is recovered.

27 Claims, 7 Drawing Sheets

NOTICE

If the application for this patent was filed on or after December 12, 1980, maintenance fees are due three years and six months, seven years and six months, and eleven years and six months after the date of this grant, or within a grace period of six months thereafter upon payment of a surcharge as provided by law. The amount, number and timing of the maintenance fees required may be changed by law or regulation. Unless payment of the applicable maintenance fee is received in the Patent and Trademark Office on or before the date the fee is due or within a grace period of six months thereafter, the patent will expire as of the end of such grace period.

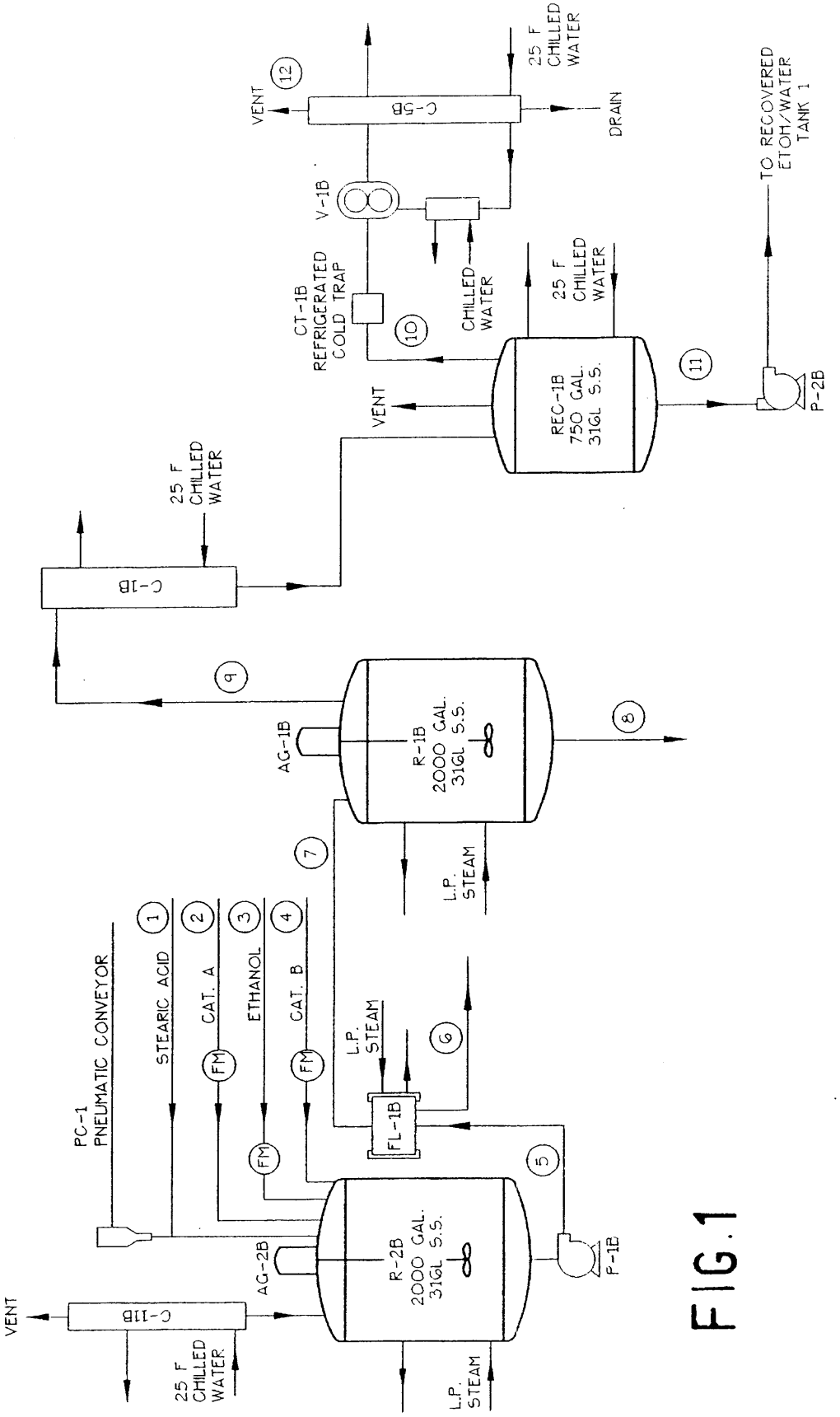
VENT
12
C-1B
25 F CHILLED WATER
DRAIN
V-1B
CT-1B REFRIGERATED COLD TRAP
10
CHILLED WATER
25 F CHILLED WATER
REC-1B 750 GAL. 316L S.S.
11
TO RECOVERED ETOH/WATER TANK 1
P-2B
VENT
25 F CHILLED WATER
C-1B
9
AG-1B
R-1B 2000 GAL. 316L S.S.
8
7
L.P. STEAM
1
2
3
4
STEARIC ACID
CAT. A
ETHANOL
CAT. B
PC-1 PNEUMATIC CONVEYOR
FM
L.P. STEAM
6
FL-1B
5
AG-2B
R-2B 2000 GAL. 316L S.S.
P-1B
VENT
C-2B
25 F CHILLED WATER
L.P. STEAM

FIG. 1

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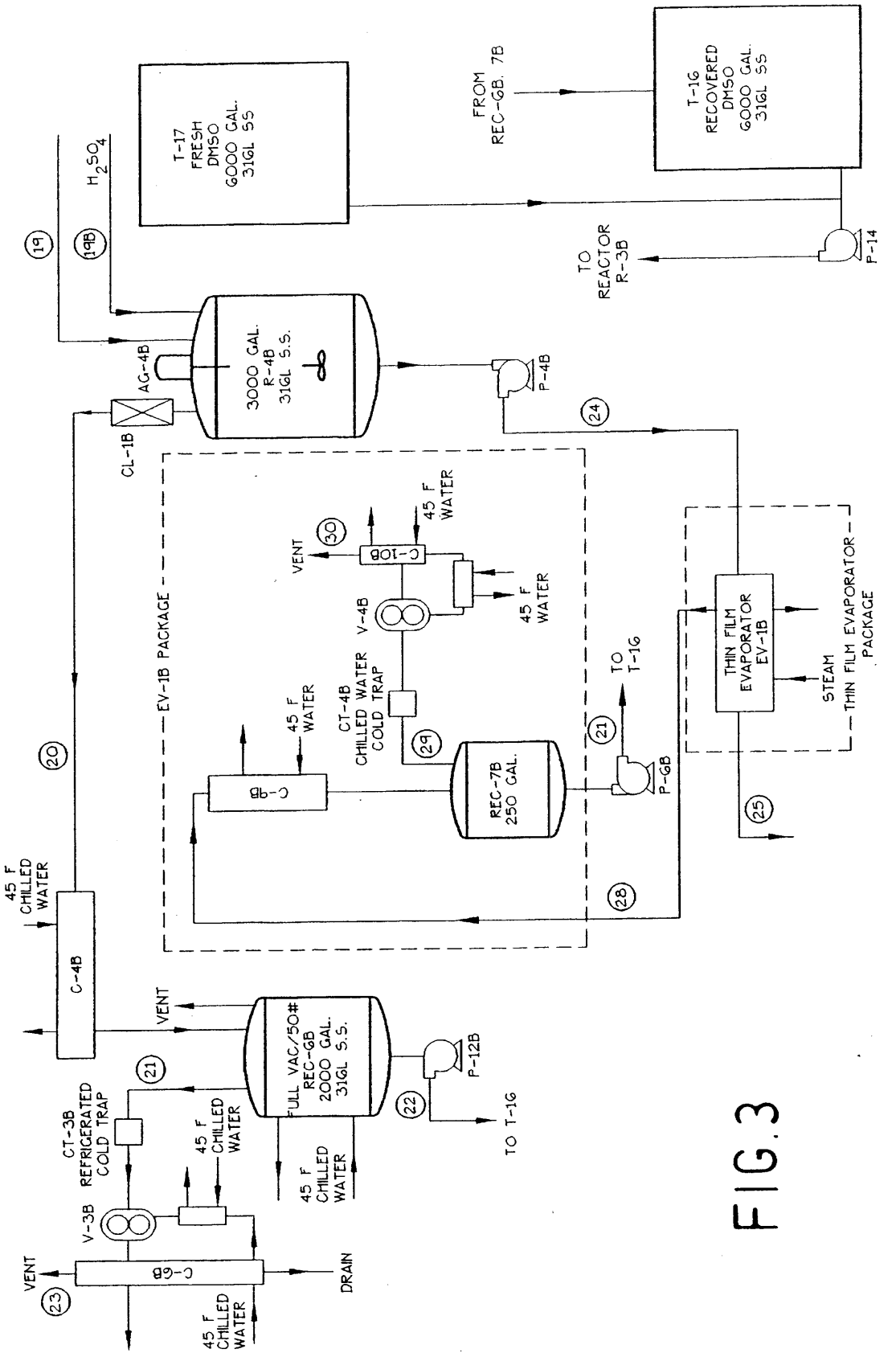
T-17 FRESH DMSO 6000 GAL. 316L SS
FROM REC-GB. 7B
T-1G RECOVERED DMSO 6000 GAL. 316L SS
TO REACTOR R-3B
P-14
19
19B
H_2SO_4
3000 GAL. R-4B 316L S.S.
AG-4B
CL-1B
P-4B
24
EV-1B PACKAGE
30
VENT
C-10B
45 F WATER
V-4B
CT-4B CHILLED WATER COLD TRAP
29
REC-7B 250 GAL.
C-9B
P-9B
21
TO T-1G
THIN FILM EVAPORATOR EV-1B
STEAM
THIN FILM EVAPORATOR PACKAGE
25
28
20
45 F CHILLED WATER
C-4B
FULL VAC/50# REC-6B 2000 GAL. 316L S.S.
P-12B
22
TO T-1G
CT-3B REFRIGERATED COLD TRAP
V-3B
C-6B
DRAIN
23

FIG. 3

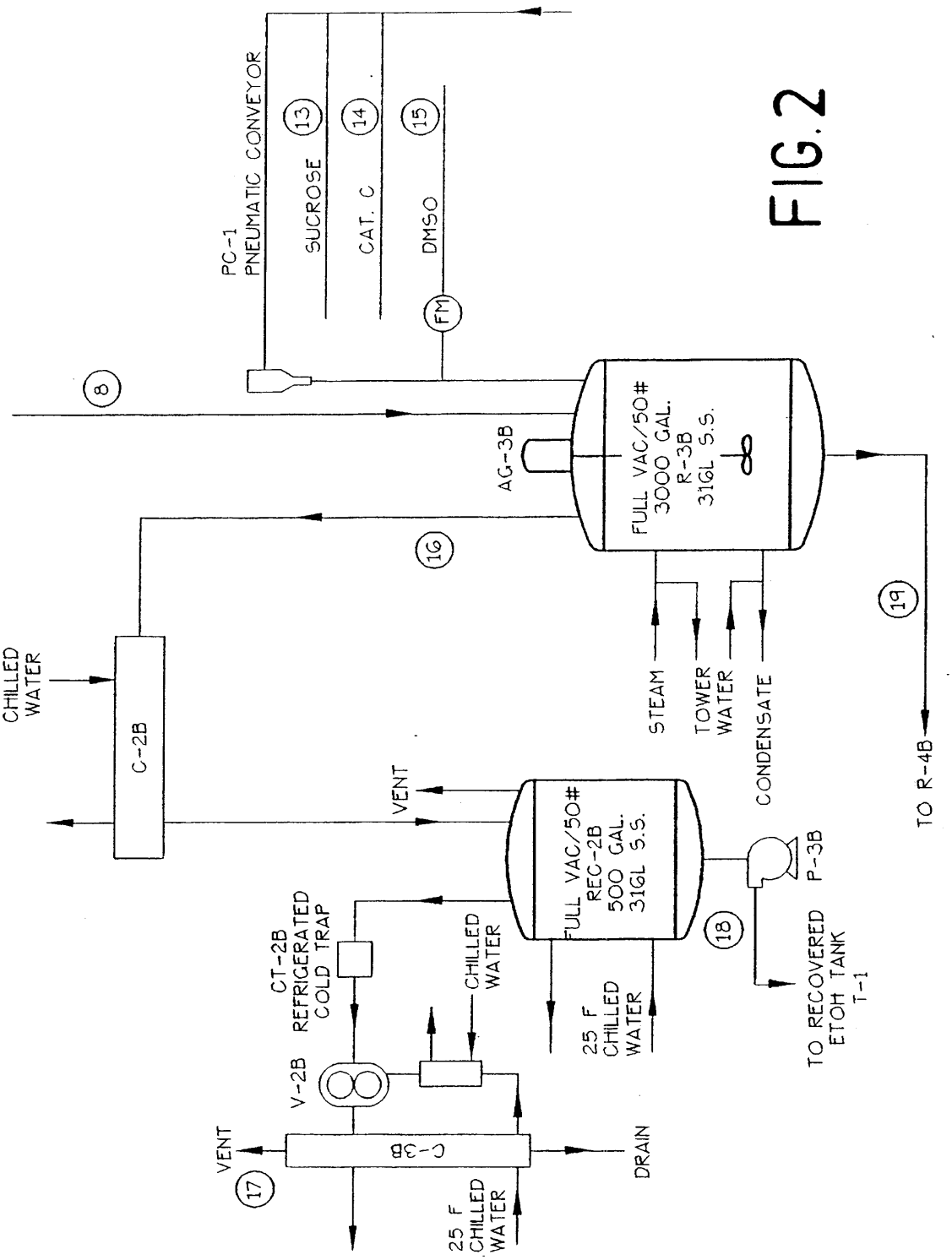
PC-1
PNEUMATIC CONVEYOR
SUCROSE
CAT. C
DMSO
13
14
15
FM
8
AG-3B
FULL VAC/50#
3000 GAL.
R-3B
31GL S.S.
16
19
TO R-4B
STEAM
TOWER
WATER
CONDENSATE
CHILLED
WATER
C-2B
VENT
FULL VAC/50#
REC-2B
500 GAL.
31GL S.S.
P-3B
18
TO RECOVERED
ETOH TANK
T-1
CT-2B
REFRIGERATED
COLD TRAP
CHILLED
WATER
25 F
CHILLED
WATER
V-2B
C-3B
VENT
17
DRAIN
25 F
CHILLED
WATER

FIG. 2

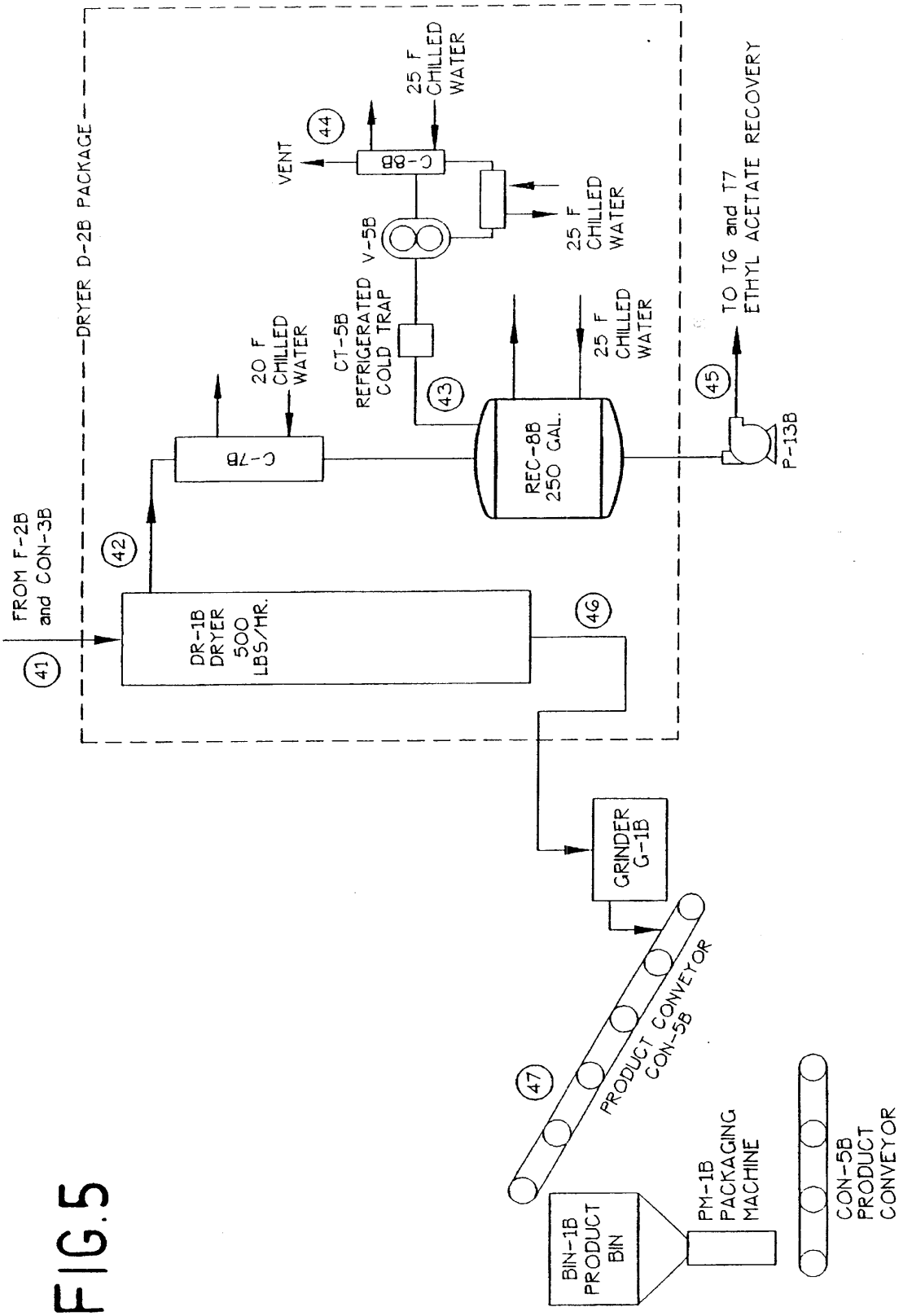
DRYER D-2B PACKAGE
FROM F-2B and CON-3B
41
DR-1B DRYER 500 LBS/HR.
42
C-7B
20 F CHILLED WATER
CT-5B REFRIGERATED COLD TRAP
V-5B
C-8B
VENT
44
25 F CHILLED WATER
25 F CHILLED WATER
25 F CHILLED WATER
43
REC-8B 250 GAL.
45
TO TG and T7 ETHYL ACETATE RECOVERY
P-13B
46
GRINDER G-1B
47
PRODUCT CONVEYOR CON-5B
BIN-1B PRODUCT BIN
PM-1B PACKAGING MACHINE
CON-5B PRODUCT CONVEYOR

FIG.5

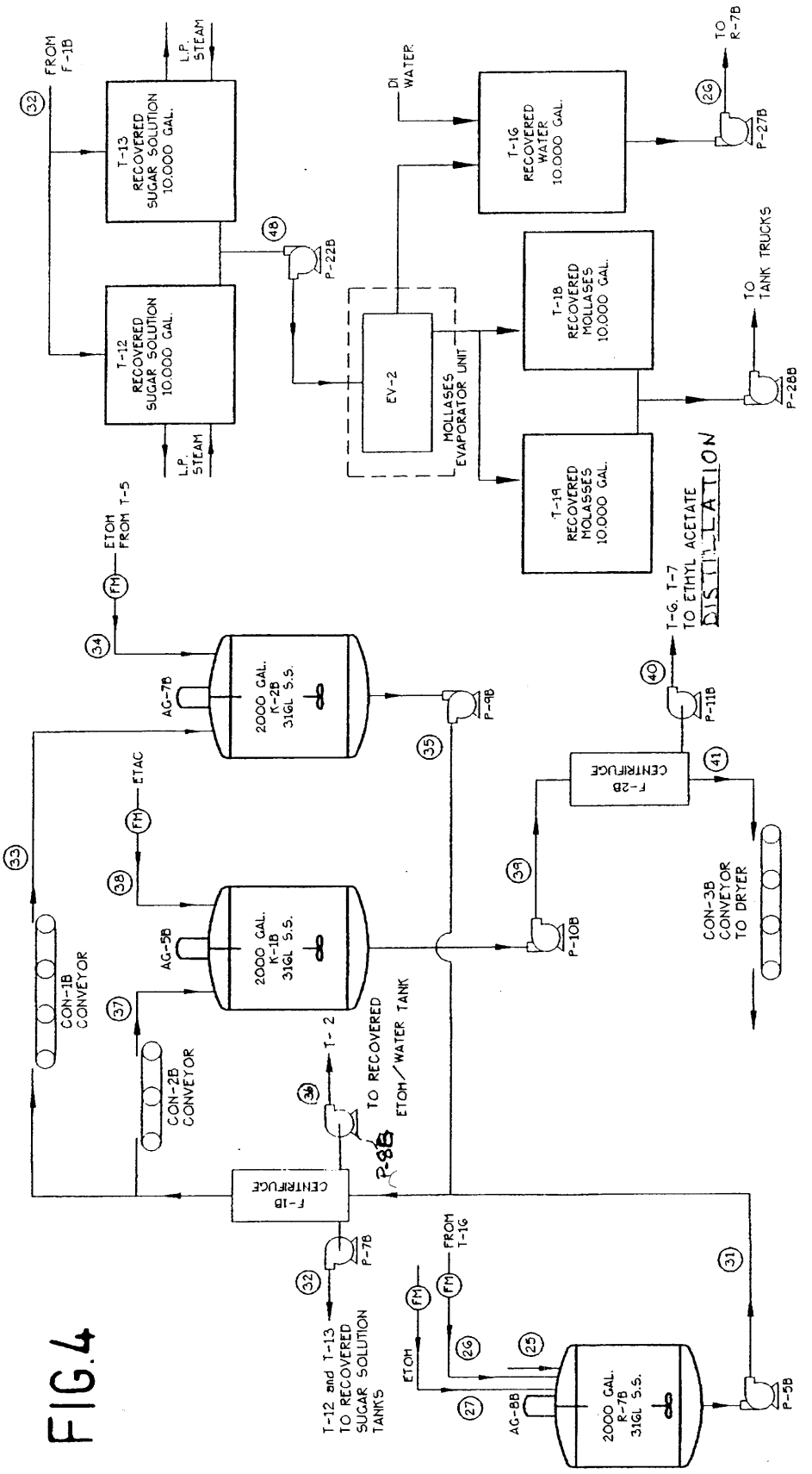
FROM F-1B
L.P. STEAM
T-13 RECOVERED SUGAR SOLUTION 10,000 GAL.
T-12 RECOVERED SUGAR SOLUTION 10,000 GAL.
L.P. STEAM
P-22B
DI WATER
T-16 RECOVERED WATER 10,000 GAL.
TO R-7B
P-27B
EV-2
MOLASSES EVAPORATOR UNIT
T-18 RECOVERED MOLASSES 10,000 GAL.
T-19 RECOVERED MOLASSES 10,000 GAL.
TO TANK TRUCKS
P-28B
ETOH FROM T-5
AG-7B
2000 GAL. K-2B 316L S.S.
P-9B
T-6, T-7 TO ETHYL ACETATE DISTILLATION
P-11B
ETAC
AG-5B
2000 GAL. K-1B 316L S.S.
F-2B CENTRIFUGE
P-10B
CON-1B CONVEYOR
CON-2B CONVEYOR
T-2
TO RECOVERED ETOH/WATER TANK
P-8B
CON-3B CONVEYOR TO DRYER
F-1B CENTRIFUGE
P-7B
T-12 and T-13 TO RECOVERED SUGAR SOLUTION TANKS
FROM T-16
ETOH
AG-8B
2000 GAL. R-7B 316L S.S.
P-5B

FIG.4

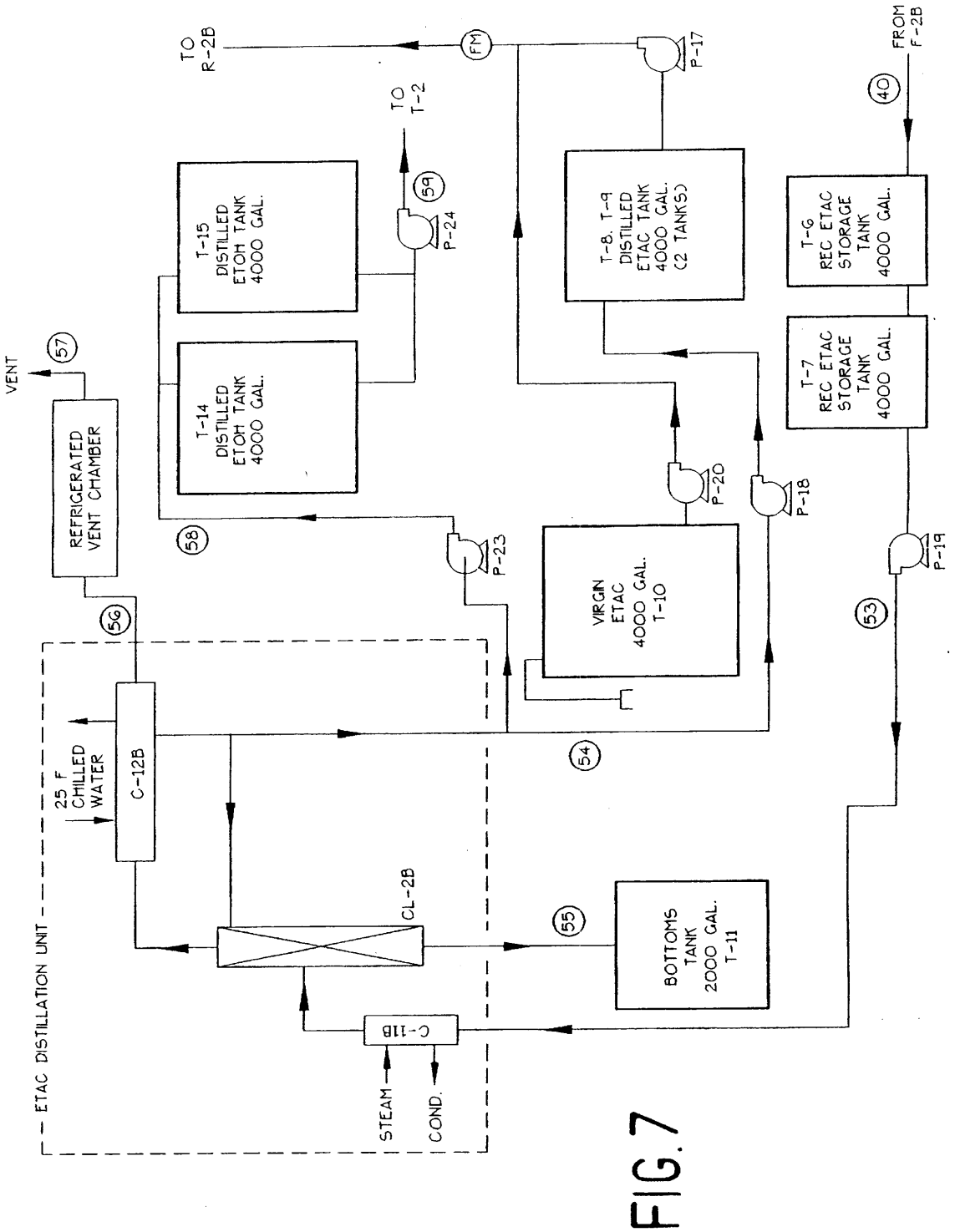
TO R-2B
FM
P-17
TO T-2
59
P-24
T-15 DISTILLED ETOH TANK 4000 GAL.
T-14 DISTILLED ETOH TANK 4000 GAL.
T-8, T-9 DISTILLED ETAC TANK 4000 GAL. (2 TANKS)
FROM F-2B
40
T-6 REC ETAC STORAGE TANK 4000 GAL.
T-7 REC ETAC STORAGE TANK 4000 GAL.
VENT
57
REFRIGERATED VENT CHAMBER
58
P-23
P-20
P-18
P-19
53
VIRGIN ETAC 4000 GAL. T-10
56
54
25 F CHILLED WATER
C-12B
CL-2B
55
BOTTOMS TANK 2000 GAL. T-11
C-11B
STEAM
COND.
ETAC DISTILLATION UNIT

FIG. 7

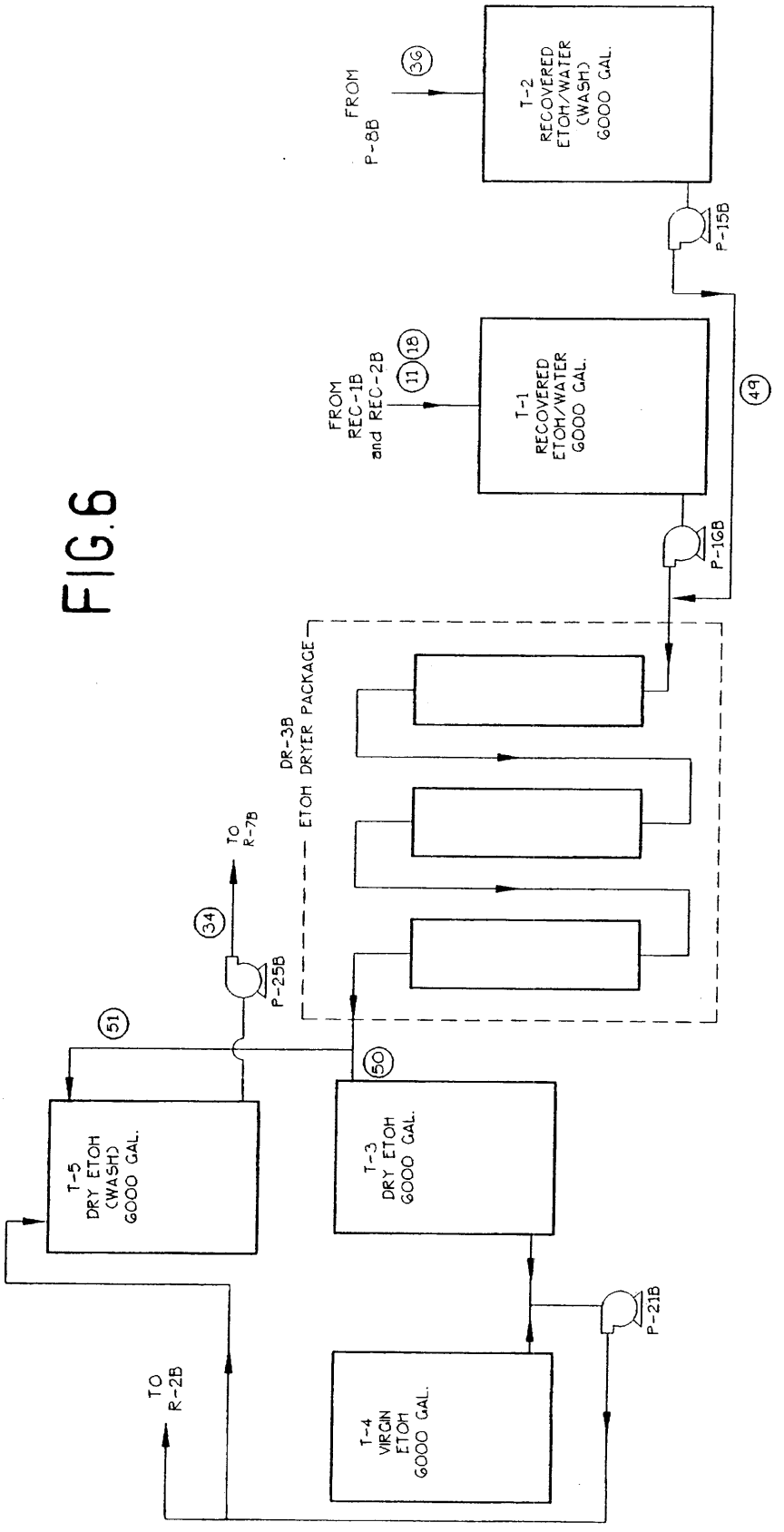
T-2
RECOVERED
ETOH/WATER
(WASH)
6000 GAL.
FROM
P-8B
36
P-15B
T-1
RECOVERED
ETOH/WATER
6000 GAL.
FROM
REC-1B
and REC-2B
11
18
49
P-16B
DR-3B
ETOH DRYER PACKAGE
TO
R-7B
34
P-25B
51
50
T-5
DRY ETOH
(WASH)
6000 GAL.
T-3
DRY ETOH
6000 GAL.
P-21B
TO
R-2B
T-4
VIRGIN
ETOH
6000 GAL.

FIG.6

ester product and unreacted fatty acid ester, and the other phase including the water, the unreacted sugar and the metal carbonate. The preferred solvent is n-butanol to form an n-butanol phase and a water phase. Then the two phases are physically separated, for example, by filtration, decanting or centrifuging. For fatty acid having an average carbon chain length of less than 16, the sugar ester product and the unreacted fatty acid ester are recovered by vacuum distillation of the solvent, for example, the n-butanol. Other suitable solvents depending on the sugar ester product being made include t-butyl alcohol, 2-butanol, hexane, heptane, methyl isobutyl ketone, and isomers of amyl alcohol.

For higher chain sugar ester where the average carbon chain length of the fatty acid is 16 or greater, adding ethyl alcohol or methyl ethyl ketone to the emulsion to break the emulsion of the ester product and unreacted fatty acid ester.

The metal carbonate in the water phase is recovered when unreacted sugar is recovered as molasses. It serves as a mineral supplement to the molasses when used as animal feed.

Step 6

In this step 6, the ester product from step 5 is purified by dissolving the unreacted fatty acid ester in a suitable solvent, preferably in ethyl acetate. The ester product and the unreacted fatty acid ester remain together as a mixture after the separation of step 5. This mixture is washed with the ethyl acetate which dissolves the unreacted fatty acid ester. When no more than 5 percent by weight of the unreacted fatty acid ester remains in the sugar ester product, washing is discontinued. Most of the time, less than 1 percent by weight of the unreacted fatty acid ester remains in the sugar ester product after washing. When the average carbon chain length of the fatty acid is 16 or greater, the mixture of the sugar ester product and the unreacted fatty acid ester is washed with dry ethyl alcohol to remove any trace water in the mixture prior to washing with the solvent for the unreacted fatty acid ester. When ethyl acetate solvent is used, the sugar ester product is completely purified by removal of traces of ethyl acetate so that no more than 350 part per million of ethyl acetate remain in the final ester product.

Step 7

In step 7, substantially all the dimethyl sulfoxide, alcohol, and ethyl acetate is recovered for reuse in the process by fractional distillation and drying of the non-aqueous solvents.

Step 8

Finally, substantially all the unreacted sugar in a concentrated useful form is recovered.

Steps 1, 2 and 3 are by batch and the other steps are either batch or continuous. In this process, all liquid, solid, and solution streams from the process are reused or recovered as useful by-products. As required, an on-line methods of monitoring and controlling process are employed, preferably using FT-IR methods described in U.S. Pat. No. 5,262,961. Where the sugar used is sucrose, the sugar ester product meets FDA standards.

DESCRIPTION OF THE DRAWING

The preferred embodiment of this invention, illustrating all its features, will now be discussed in detail. This embodiment depicts the novel and non-obvious process of this invention making, for example, sucrose stearate as shown in the accompanying drawing, which is for illustrative purposes only. This drawing includes the following figures (Figures), with like numerals indicating like parts:

FIG. **1** is a process flow diagram showing the way the ethyl stearate is manufactured from stearic acid and ethanol.

FIG. **2** is a process flow diagram showing the way the sucrose stearate product is made by the transesterification of sucrose and the ethyl stearate.

FIG. **3** is a process flow diagram showing the way dimethyl sulfoxide is recovered for reuse in the process.

FIG. **4** is a process flow diagram showing the way the sucrose stearate product is purified.

FIG. **5** is a process flow diagram showing the way the sucrose stearate product is dried.

FIG. **6** is a process flow diagram showing the way the ethanol is recovered and reused.

FIG. **7** is a process flow diagram showing the way the ethyl acetate is recovered and reused.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The process of this invention, which is best explained in terms of eight steps, has been tested in a pilot plant with a 50 gallon reactor and sufficiently large batches made to understand the details of the process as described.

In Step 1, the ethyl or methyl ester is made. We prefer the ethyl ester since traces left in the product will not produce methanol when ingested. An important part of the process is the degree of completion of this reaction. Sulfuric acid is used as the catalyst with plenty of excess ethanol to insure reaction completion. This portion of the process is monitored by Fourier Transform Infrared Spectroscopy, or similar fast reliable method (herein FT-IR methods), to insure that there is very little free fatty acid left, typically less than 0.5%.

U.S. Pat. No. 5,262,961, entitled Method For Monitoring and Controlling A Chemical Process describe a preferred way to monitor the various streams utilized in the present invention. According to this method, samples of a stream are analyzed on-line in real time, with the results being used essentially immediately to make adjustments in process conditions to achieve optimal results, for example, highest yields. This method of monitoring and controlling the process of the present invention comprises

(a) measuring the concentration of process components in samples from the process using a spectrometric instrument to obtain spectral data characteristic of the process components,

(b) analyzing the spectral data using a chi-squared mathematical technique to determine the unknown concentration of process components in said samples, and

(c) monitoring the physical parameters of the process and altering said physical parameters based on the determination of concentration of process components in step (b) as required to optimize the process.

In Step 2, the acid in Step 1 is neutralized with potassium carbonate. This makes potassium sulfate, but it should be noted that potassium carbonate will be also used in the next step as the catalyst and no problems occur if too much potassium carbonate is added. Thus, we limit the chemicals and the by-products. The potassium sulfate is separated for the fatty acid ester by filtration or centrifugation. Any ethanol associated with it in the cake is recovered. The recovered potassium sulfate is a useful soil amendment by-product. Next the ethanol is separated from the fatty acid by vacuum distillation, leaving the desired ethyl stearate. Note, that some water was produced in the esterification which goes off with the ethanol. The ethanol is dried using molecular sieves periodically to keep it nearly anhydrous.

In Step 3, the ethyl stearate is dissolved in dimethyl sulfoxide (DMSO) in a reaction vessel adding sugar and

SUGAR-ESTER MANUFACTURING PROCESS

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a process for making sugar esters where substantially all unreacted ingredients are recovered as valuable products or reused in the process, thereby eliminating discharging pollutants into the environment.

2. Background Discussion

In the late 1950s and early 1960s interest developed in "natural" alternatives to surface active agents, also known as surfactants, used in detergent formulations, drugs, cosmetics, toiletries and foods. The soaps (sodium and potassium salts of fatty acids) were replaced by the petroleum derived surfactants slightly before this time. Nevertheless, there was continued interest in using the fatty acids derived from beef tallow and vegetable oils in better surfactants. One class of surfactants that aroused great interest was the esters of sugars and fatty acids.

While there has been much work done to find various methods for synthesizing and purifying sucrose esters, there are only a few commercial source of these materials that meet the Food and Drug Administration requirements, one being Ryoto Sugar Esters made by the Mitsubishi-Kasai Food Company. The history of the sucrose esters, and their specifications, are discussed in the Ryoto Sugar Ester Technical Information Bulletin and refer back to the work originally sponsored by the Sugar Research Foundation in 1954.

When sucrose and fatty acid are reacted to form the ester there are eight positions on the sucrose molecule where the ester can form. Typically, monoesters, diesters and triesters are formed with a small amount of higher esters. When the reaction is with a single fatty acid, for example, stearic acid or lauric acid, thin layer chromatographic analysis and liquid chromatographic analysis shows that there are many different esters that form, typically 2–4 monoesters and up to 6–8 diesters. If a mixture of acids is used, such as when a commercial fatty acid mixture is used, the number of isomers is even higher. Each of the fractions have slightly different properties. For example, the lauric acid esters are liquid at room temperature while the stearic acid esters are solid. Other products and mixtures make products that are semi-solids. They have differing solubilities in water and organic solvents, but for the most part, as with most surfactants, the longer chain length esters, for example, sucrose stearates, are very insoluble in water and low chain alcohols. Although desirable, these differing solubilities and physical properties make an integrated production facility a difficult goal.

In order for a sucrose ester manufacturing process to be commercially useful it must be able to produce esters which meet the following FDA requirements:

(a) at least 80% by weight mono-, di- and tri-esters,

(b) less than 5% by weight free sucrose,

(c) has an acid value is less than 6,

(d) no more than 2% by weight ash,

(e) no more than 350 parts per million ethyl acetate,

(f) no more than 3 parts per million arsenic,

(g) no more than 50 parts per million heavy metals,

(h) no more than 10 parts per million lead,

(i) no more than 10 parts per million methyl ethyl ketone,

(j) no more than 10 parts per million methyl alcohol,

(k) no more than 2 parts per million dimethyl sulfoxide,

(l) no more than 10 parts per million isobutyl alcohol.

Although it is not stated specifically in the FDA requirements, the above requirements allow for a significant amount of starting products to be carried over into the final product. For example, almost all of the previous techniques recommend the use of the methyl esters of the fatty acids to make the final sucrose ester by transesterification. This leaves behind methyl esters in significant quantities, for example, 0.25% by weight residual methyl ester means that methanol will be generated by ingestion of the methyl ester by hydrolysis in the stomach that exceeds the FDA standard of 10 parts per million. The literature does not teach a commercial process to meet all of the FDA requirements.

The classic method for the production of the sucrose esters is best illustrated by the production of sucrose stearate. It is, however, much more difficult to produce other chain lengths. This fact has not been discussed in the prior art. It almost appears that the sucrose stearate was studied and then it was assumed that the methods would apply to sucrose laurate, which is also referred to as sucrose cocoate after the coconut oil source of the lower chain length fatty acids.

In the early processes dimethyl formamide (DMF) was used as the solvent. The methyl ester of the fatty acid was made and this methyl ester was reacted with sucrose in the DMF in the presence of a catalyst such as potassium carbonate for 4–6 hours at 83°–95° C. Typical recipes (all are parts by weight) used 30 to 127 parts sucrose to 30 parts methyl stearate, 2 parts potassium carbonate and 300 parts solvent. These methods achieved high yields of 60–75% by weight. The basic problem was purifying the reaction mixture. The reaction products are highly viscous containing partially decomposed sugars and solvents.

This lead to a series of attempted improvements. For example, U.S. Pat. No. 3,644,333 to Osipow et al based on U.S. Pat. No. 3,480,616, tried to eliminate the use of a solvent by making a "transparent emulsion." Typically, in this process, sucrose was mixed with methyl stearate and sodium stearate and a significant amount of previously prepared sucrose ester. Potassium carbonate was still used as the catalyst, but water was used as the solvent. The water was driven off and then the reaction was completed using the mixture as its own solvent. Although much was made in the patent and the literature about the requirement for the emulsion, it should be noted that Example V in U.S. Pat. No. 3,644,333 does not make the emulsion and the inventors indicate they achieve the same yield of products. The yields are very low, on the order of 30–35% by weight. Further, there was much more waste and degraded products and the desired product was much more difficult to remove from the reaction mixture. A typical recipe used 80.4 parts sucrose, 75 parts methyl stearate, 12.3 parts sodium stearate, 40.5 parts sucrose monostearate, 0.75 parts potassium carbonate, and 166.8 parts water. In addition to the low yields, it was been found that the inclusion of sodium stearate, or significant quantities of any alkali fatty acid salt, results in a product which is much more difficult to purify and, in particular, gives a product which does not meet the 2% maximum ash FDA requirement.

Alternative methods have been described by Feuge. For example, in U.S. Pat. No. 3,714,144, he uses the sodium, potassium or lithium soap of the fatty acid in a molten sugar solution. The reaction proceeds for 2–20 minutes under vacuum at 170°–190° C. The product is made in very low yield and the destroyed sugar and alkali metals are very

ONE HUNDRED AND NINE PAGES (109 PAGES) OF CONFIDENTIAL BUSINESS INFORMATION (CBI), THE ENTIRE CONTENTS OF ATTACHMENT 4, HAVE BEEN REMOVED FROM THIS COPY

lauric, myristic, myristoletic, palmitic, stearic, oleic, ricinoleic, linoleic, linolenic, arachidic, eicosenoic, behenic, and erucic fatty acids.

9. The process of claim **1** where the reaction time and temperature in step (c) increases as the chain length of the fatty acid decreases.

10. The process of claim **9** where the reaction time is from 3 to 6 hours and the reaction temperature is from 90 to 105 degrees Celcius when the average carbon chain length of the fatty acid is 16 or greater, and the reaction time is from 8 to 24 hours and the reaction temperature is from 105 to 125 degrees Celcius when the average carbon chain length of the fatty acid is less than 16.

11. A process for manufacturing a sugar ester product from a sugar and a fatty acid, comprising

(a) reacting a fatty acid and methyl or ethyl alcohol in the presence of sulfuric acid catalyst to produce a fatty acid ester and water, said fatty acid having an average carbon chain length less than 16

(b) neutralizing the sulfuric acid catalyst with a metal carbonate to make a metal sulfate, and separating the fatty acid ester from the metal sulfate, the alcohol and the water,

(c) reacting in the presence of a metal carbonate catalyst the fatty acid ester from step (b) with sugar dissolved in dimethyl sulfoxide to produce the sugar ester product and alcohol,

(d) separating the dimethyl sulfoxide from the reaction mixture by vacuum distillation, and then adding water with the sugar ester product and unreacted fatty acid ester being emulsified and the unreacted sugar and the metal carbonate being dissolved in the water, and then dissolving the sugar ester product and unreacted fatty acid ester in a solvent which is immiscible with the water to thereby form two separate liquid phases, one phase including the solvent, the sugar ester product and unreacted fatty acid ester, and the other phase including the water, the unreacted sugar and the metal carbonate

(e) separating the emulsified sugar ester product and unreacted fatty acid ester from the water containing dissolved unreacted sugar and metal carbonate by breaking the emulsion of the sugar ester product and unreacted fatty acid ester,

(f) purifying the sugar ester product from step (e) by dissolving the unreacted fatty acid ester in ethyl acetate to effect purification,

(g) recovering substantially all the dimethyl sulfoxide, alcohol, and ethyl acetate for reuse in the process, and

(h) recovering substantially all the unreacted sugar in a concentrated useful form.

12. The process of claim **11** where the solvent is selected from the group consisting of n-butyl alcohol, 2-butanol, hexane, heptane, methyl isobutyl ketone, and isomers of amyl alcohol.

13. The process of claim **12** where the two phases are separated by filtration, decanting or centrifuging.

14. The process of claim **1** where for higher chain sugar ester where the average carbon chain length of the fatty acid is 16 or greater, step (e) comprises adding ethyl alcohol or methyl-ethyl ketone to the emulsion to break the emulsion of the sugar ester product and unreacted fatty acid ester.

15. The process of claim **14** where for an average carbon chain length of the fatty acid of 16 or greater, the mixture of of the sugar ester product and the unreacted fatty acid ester is washed between steps (e) and (f) with dry ethyl alcohol to remove water in the mixture.

16. The process of claim **1** where the metal carbonate is recovered from the water phase and used to neutralize the sulfuric acid in step (b).

17. The process of claim **1** where the sugar ester product and the unreacted fatty acid ester are recovered as mixture from step (e), and said mixture of the sugar ester product and the unreacted fatty acid ester is washed with a solvent for the unreacted fatty acid ester until the there is less than 5 percent by weight of the unreacted fatty acid ester remaining in the sugar ester product.

18. The process of claim **17** where the solvent is ethyl acetate, with the sugar ester product being finally purified by removal of traces of ethyl acetate so that no more than 350 part per million of ethyl acetate remain in the final sugar ester product.

19. The process of claim **1** where the yield in step (c) is from 85 to 90 percent.

20. The process of claim **1** where all liquid, solid, and solution streams from the process are reused or recovered.

21. The process of claim **1** where the metal carbonate is identical in both step (b) and (c).

22. The process of claim **1** where the dimethyl sulfoxide is refluxed during step (c) at a temperature above the boiling point of the alcohol to remove the alcohol produce during step (c).

23. The process of claim **1** where the separated dimethyl sulfoxide from step (d) is reused in the process.

24. The process of claim **1** the where separated alcohol and water from step (b) are reused in the process.

25. The process of claim **1** where excess alcohol is used in step (a) to drive the reaction essentially to completion, so that less than 1 percent by weight of the fatty acid remains.

26. The process of claim **1** employing an on-line method of monitoring and controlling said process comprising

(a) measuring the concentration of process components in samples from the process using a spectrometric instrument to obtain spectral data characteristic of the process components,

(b) analyzing the spectral data using a chi-squared mathematical technique to determine the unknown concentration of process components in said samples, and

(c) monitoring the physical parameters of the process and altering said physical parameters based on the determination of concentration of process components in step (b) as required to optimize the process.

27. The process of claim **1** where the sugar used is sucrose and the sugar ester product comprises

(a) at least 80% by weight mono-, di- and tri- esters,

(b) less than 5% by weight free sucrose,

(c) has an acid value is less than 6,

(d) no more than 2% by weight ash,

(e) no more than 350 parts per million ethyl acetate,

(f) no more than 3 parts per million arsenic,

(g) no more than 50 parts per million heavy metals,

(h) no more than 10 parts per million lead,

(i) no more than 10 parts per million methyl ethyl ketone,

(j) no more than 10 parts per million methyl alcohol,

(k) no more than 2 parts per million dimethyl sulfoxide,

(l) no more than 10 parts per million isobutyl alcohol.

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Guidance for Industry

Q3C Impurities: Residual Solvents

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)
December 1997
ICH

Guidance for Industry

Q3C Impurities: Residual Solvents

U.S. Department of Health and Human Services
Food and Drug Administration
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December 1997
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Guidance for Industry[1]

Q3C Impurities: Residual Solvents

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statutes and regulations.

I. INTRODUCTION (1)

The objective of this guidance is to recommend acceptable amounts for residual solvents in pharmaceuticals for the safety of the patient. The guidance recommends use of less toxic solvents and describes levels considered to be toxicologically acceptable for some residual solvents. A complete list of the solvents included in this guidance is provided in a companion document entitled *Q3C — Tables and List.*[2] The list is not exhaustive, and other solvents may be used and later added to the list.

Residual solvents in pharmaceuticals are defined here as organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or in the preparation of drug products. The solvents are not completely removed by practical manufacturing techniques. Appropriate selection of the solvent for the synthesis of drug substance may enhance the yield, or determine characteristics such as crystal form, purity, and solubility. Therefore, the solvent may sometimes be a critical parameter in the synthetic process. This guidance does not address solvents deliberately used as excipients nor does it address solvates. However, the content of solvents in such products should be evaluated and justified.

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[1] This guidance was developed within the Expert Working Group (Quality) of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and has been subject to consultation by the regulatory parties, in accordance with the ICH process. This document was endorsed by the ICH Steering Committee at *Step 4* of the ICH process in July 1997. At *Step 4* of the process, the final draft is recommended for adoption to the regulatory bodies of the European Union, Japan, and the United States.

Arabic numbers in subsections reflect the organizational breakdown in the document endorsed by the ICH Steering Committee at Step 4 of the ICH process.

[2] This guidance was published originally in the *Federal Register* on December 24, 1997 (62 FR67377). At that time the list was included as Appendix 1. In this reformatted version, the list has been removed and made into a companion document, and the remaining appendices have been renumbered.

TABLE OF CONTENTS

A. Classification of Residual Solvents by Risk Assessment (3.1)

The term *tolerable daily intake* (TDI) is used by the International Program on Chemical Safety (IPCS) to describe exposure limits of toxic chemicals and the term *acceptable daily intake* (ADI) is used by the World Health Organization (WHO) and other national and international health authorities and institutes. The new term *permitted daily exposure* (PDE) is defined in the present guidance as a pharmaceutically acceptable intake of residual solvents to avoid confusion of differing values for ADI's of the same substance.

Residual solvents assessed in this guidance are listed in a companion document *entitled Q3C — Tables and List*. Common names and structures are used. They were evaluated for their possible risk to human health and placed into one of three classes as follows:

Class 1 solvents: Solvents to be avoided—

> Known human carcinogens, strongly suspected human carcinogens, and environmental hazards.

Class 2 solvents: Solvents to be limited—

> Nongenotoxic animal carcinogens or possible causative agents of other irreversible toxicity such as neurotoxicity or teratogenicity.
>
> Solvents suspected of other significant but reversible toxicities.

Class 3 solvents: Solvents with low toxic potential—

> Solvents with low toxic potential to man; no health-based exposure limit is needed. Class 3 solvents have PDE's of 50 milligrams (mg) or more per day.

B. Methods for Establishing Exposure Limits (3.2)

The method used to establish permitted daily exposures for residual solvents is presented in Appendix 2. Summaries of the toxicity data that were used to establish limits are published in *Pharmeuropa*, Vol. 9, No. 1, Supplement, April 1997.

C. Options for Describing Limits of Class 2 Solvents (3.3)

Two options are available when setting limits for Class 2 solvents.

Option 1: The concentration limits in parts per million (ppm) stated in Table 2 (see companion document) can be used. They were calculated using equation (1) below by assuming a product mass of 10 mass of 10 grams (g) mass of 10 grams administered daily.

Since there is no therapeutic benefit from residual solvents, all residual solvents should be removed to the extent possible to meet product specifications, good manufacturing practices, or other quality-based requirements. Drug products should contain no higher levels of residual solvents than can be supported by safety data. Some solvents that are known to cause unacceptable toxicities (Class 1, see Table 1 in the companion document *Q3C — Tables and List*) should be avoided in the production of drug substances, excipients, or drug products unless their use can be strongly justified in a risk-benefit assessment. Some solvents associated with less severe toxicity (Class 2, see Table 2 in the campanion document) should be limited in order to protect patients from potential adverse effects. Ideally, less toxic solvents (Class 3, see Table 3 in the companion document) should be used where practical.

Recommended limits of Class 1 and 2 solvents or classification of solvents may change as new safety data becomes available. Supporting safety data in a marketing application for a new drug product containing a new solvent may be based on concepts in this guidance or the concept of qualification of impurities as expressed in the guidance for drug substance, *Q3A Impurities in New Drug Substances* (January 1996) or drug product, *Q3B Impurities in New Drug Products* (November 1997), or all three guidances.

II. SCOPE OF THE GUIDANCE (2)

Residual solvents in drug substances, excipients, and drug products are within the scope of this guidance. Therefore, testing should be performed for residual solvents when production or purification processes are known to result in the presence of such solvents. It is only necessary to test for solvents that are used or produced in the manufacture or purification of drug substances, excipients, or drug products. Although manufacturers may choose to test the drug product, a cumulative method may be used to calculate the residual solvent levels in the drug product from the levels in the ingredients used to produce the drug product. If the calculation results in a level equal to or below that recommended in this guidance, no testing of the drug product for residual solvents need be considered. If, however, the calculated level is above the recommended level, the drug product should be tested to ascertain whether the formulation process has reduced the relevant solvent level to within the acceptable amount. Drug product should also be tested if a solvent is used during its manufacture.

This guidance does not apply to potential new drug substances, excipients, or drug products used during the clinical research stages of development, nor does it apply to existing marketed drug products.

The guidance applies to all dosage forms and routes of administration. Higher levels of residual solvents may be acceptable in certain cases such as short-term (30 days or less) or topical application. Justification for these levels should be made on a case-by-case basis.

See Appendix 1 for additional background information related to residual solvents.

III. GENERAL PRINCIPLES (3)

Consider another example using acetonitrile as residual solvent. The maximum administered daily mass of a drug product is 5.0 g, and the drug product contains two excipients. The composition of the drug product and the calculated maximum content of residual acetonitrile are given in the following table.

Component	Amount in Formulation	Acetonitrile Content	Daily Exposure
Drug substance	0.3 g	800 ppm	0.24 mg
Excipient 1	0.9 g	2,000 ppm	1.80 mg
Excipient 2	3.8 g	800 ppm	3.04 mg
Drug Product	5.0 g	1,016 ppm	5.08 mg

In this example, the product meets neither the Option 1 nor the Option 2 limit according to this summation. The manufacturer could test the drug product to determine if the formulation process reduced the level of acetonitrile. If the level of acetonitrile was not reduced during formulation to the allowed limit, then the manufacturer of the drug product should take other steps to reduce the amount of acetonitrile in the drug product. If all of these steps fail to reduce the level of residual solvent, in exceptional cases the manufacturer could provide a summary of efforts made to reduce the solvent level to meet the guidance value, and provide a risk-benefit analysis to support allowing the product to be utilized with residual solvent at a higher level.

D. Analytical Procedures (3.4)

Residual solvents are typically determined using chromatographic techniques such as gas chromatography. Any harmonized procedures for determining levels of residual solvents as described in the pharmacopoeias should be used, if feasible. Otherwise, manufacturers would be free to select the most appropriate validated analytical procedure for a particular application. If only Class 3 solvents are present, a nonspecific method such as loss on drying may be used.

Validation of methods for residual solvents should conform to ICH guidances, *Q2A Text on Validation of Analytical Procedures* (March 1995) and *Q2B Validation of Analytical Procedures: Methodology* (November 1996).

E. Reporting Levels of Residual Solvents (3.5)

Manufacturers of pharmaceutical products need certain information about the content of residual solvents in excipients or drug substances in order to meet the criteria of this guidance. The following statements are given as acceptable examples of the information that could be provided from a supplier of excipients or drug substances to a pharmaceutical manufacturer. The supplier might choose one of the following as appropriate:

- Only Class 3 solvents are likely to be present. Loss on drying is less than 0.5 percent.

(1) $$\text{Concentration (ppm)} = \frac{1000 \times \text{PDE}}{\text{dose}}$$

Here, PDE is given in terms of mg/day and dose is given in g/day.

These limits are considered acceptable for all substances, excipients, or products. Therefore, this option may be applied if the daily dose is not known or fixed. If all excipients and drug substances in a formulation meet the limits given in Option 1, then these components may be used in any proportion. No further calculation is necessary provided the daily dose does not exceed 10 g. Products that are administered in doses greater than 10 g per day should be considered under Option 2.

Option 2: It is not considered necessary for each component of the drug product to comply with the limits given in Option 1. The PDE in terms of mg/day as stated in Table 2 (see companion document) can be used with the known maximum daily dose and equation (1) above to determine the concentration of residual solvent allowed in drug product. Such limits are considered acceptable provided that it has been demonstrated that the residual solvent has been reduced to the practical minimum. The limits should be realistic in relation to analytical precision, manufacturing capability, and reasonable variation in the manufacturing process and the limits should reflect contemporary manufacturing standards.

Option 2 may be applied by adding the amounts of a residual solvent present in each of the components of the drug product. The sum of the amounts of solvent per day should be less than that given by the PDE.

Consider an example of the use of Option 1 and Option 2 applied to acetonitrile in a drug product. The permitted daily exposure to acetonitrile is 4.1 mg per day; thus, the Option 1 limit is 410 ppm. The maximum administered daily mass of a drug product is 5.0 g, and the drug product contains two excipients. The composition of the drug product and the calculated maximum content of residual acetonitrile are given in the following table.

Component	**Amount in Formulation**	**Acetonitrile Content**	**Daily Exposure**
Drug substance	0.3 g	800 ppm	0.24 mg
Excipient 1	0.9 g	400 ppm	0.36 mg
Excipient 2	3.8 g	800 ppm	3.04 mg
Drug product	5.0 g	728 ppm	3.64 mg

Excipient 1 meets the Option 1 limit, but the drug substance, excipient 2, and drug product do not meet the Option 1 limit. Nevertheless, the product meets the Option 2 limit of 4.1 mg per day and thus conforms to the recommendations in this guidance.

acceptable provided they are realistic in relation to manufacturing capability and good manufacturing practice (GMP).

D. Solvents for Which No Adequate Toxicological Data Were Found (4.4)

The solvents listed in Table 4 (see companion document) may also be of interest to manufacturers of excipients, drug substances, or drug products. However, no adequate toxicological data on which to base a PDE were found. Manufacturers should supply justification for residual levels of these solvents in pharmaceutical products.

- Only Class 2 solvents X, Y, . . . are likely to be present. All are below the Option 1 limit. (Here the supplier would name the Class 2 solvents represented by X, Y, ...)

- Only Class 2 solvents X, Y, . . . and Class 3 solvents are likely to be present. Residual Class 2 solvents are below the Option 1 limit and residual Class 3 solvents are below 0.5 percent.

If Class 1 solvents are likely to be present, they should be identified and quantified.

"Likely to be present" refers to the solvent used in the final manufacturing step and to solvents that are used in earlier manufacturing steps and not removed consistently by a validated process.

If solvents of Class 2 or Class 3 are present at greater than their Option 1 limits or 0.5 percent, respectively, they should be identified and quantified.

IV. LIMITS OF RESIDUAL SOLVENTS (4)

A. Solvents to Be Avoided (4.1)

Solvents in Class 1 (Table 1; see companion document) should not be employed in the manufacture of drug substances, excipients, and drug products because of their unacceptable toxicity or their deleterious environmental effect. However, if their use is unavoidable in order to produce a drug product with a significant therapeutic advance, then their levels should be restricted as shown in Table 1, unless otherwise justified. The solvent 1,1,1-Trichloroethane is included in Table 1 (see companion document) because it is an environmental hazard. The stated limit of 1,500 ppm is based on a review of the safety data.

B. Solvents to Be Limited (4.2)

Solvents in Class 2 (Table 2; see companion document) should be limited in pharmaceutical products because of their inherent toxicity. PDEs are given to the nearest 0.1 mg/day, and concentrations are given to the nearest 10 ppm. The stated values do not reflect the necessary analytical precision of determination. Precision should be determined as part of the validation of the method.

C. Solvents with Low Toxic Potential (4.3)

Solvents in Class 3 (Table 3; see companion document) may be regarded as less toxic and of lower risk to human health. Class 3 includes no solvent known as a human health hazard at levels normally accepted in pharmaceuticals. However, there are no long-term toxicity or carcinogenicity studies for many of the solvents in Class 3. Available data indicate that they are less toxic in acute or short-term studies and negative in genotoxicity studies. It is considered that amounts of these residual solvents of 50 mg per day or less (corresponding to 5,000 ppm or 0.5 percent under Option 1) would be acceptable without justification. Higher amounts may also be

APPENDIX 1: ADDITIONAL BACKGROUND

Environmental Regulation of Organic Volatile Solvents (A2.1)

Several of the residual solvents frequently used in the production of pharmaceuticals are listed as toxic chemicals in Environmental Health Criteria (EHC) monographs and the Integrated Risk Information System (IRIS). The objectives of such groups as the International Programme on Chemical Safety (IPCS), the U.S. Environmental Protection Agency (EPA), and the U.S. Food and Drug Administration (FDA) include the determination of acceptable exposure levels. The goal is protection of human health and maintenance of environmental integrity against the possible deleterious effects of chemicals resulting from long-term environmental exposure. The methods involved in the estimation of maximum safe exposure limits are usually based on long-term studies. When long-term study data are unavailable, shorter term study data can be used with modification of the approach such as use of larger safety factors. The approach described therein relates primarily to long-term or *lifetime exposure of the general population* in the ambient environment (i.e., ambient air, food, drinking water, and other media).

Residual Solvents in Pharmaceuticals (A2.2)

Exposure limits in this guidance are established by referring to methodologies and toxicity data described in EHC and IRIS monographs. However, some specific assumptions about residual solvents to be used in the synthesis and formulation of pharmaceutical products should be taken into account in establishing exposure limits. They are as follows:

- Patients (not the general population) use pharmaceuticals to treat their diseases or for prophylaxis to prevent infection or disease.
- The assumption of lifetime patient exposure is not necessary for most pharmaceutical products but may be appropriate as a working hypothesis to reduce risk to human health.
- Residual solvents are unavoidable components in pharmaceutical production and will often be a part of drug products.
- Residual solvents should not exceed recommended levels except in exceptional circumstances.
- Data from toxicological studies that are used to determine acceptable levels for residual solvents should have been generated using appropriate protocols such as those described, for example, by the Organization for Economic Cooperation and Development, EPA, and the FDA Red Book.

GLOSSARY

Genotoxic carcinogens: Carcinogens that produce cancer by affecting genes or chromosomes.

LOEL: Abbreviation for lowest-observed effect level.

Lowest-observed effect level: The lowest dose of substance in a study or group of studies that produces biologically significant increases in frequency or severity of any effects in the exposed humans or animals.

Modifying factor: A factor determined by professional judgment of a toxicologist and applied to bioassay data to relate that data safely to humans.

Neurotoxicity: The ability of a substance to cause adverse effects on the nervous system.

NOEL: Abbreviation for no-observed-effect level.

No-observed-effect level: The highest dose of substance at which there are no biologically significant increases in frequency or severity of any effects in the exposed humans or animals.

PDE: Abbreviation for permitted daily exposure.

Permitted daily exposure: The maximum acceptable intake per day of residual solvent in pharmaceutical products.

Reversible toxicity: The occurrence of harmful effects that are caused by a substance and which disappear after exposure to the substance ends.

Strongly suspected human carcinogen: A substance for which there is no epidemiological evidence of carcinogenesis but there are positive genotoxicity data and clear evidence of carcinogenesis in rodents.

Teratogenicity: The occurrence of structural malformations in a developing fetus when a substance is administered during pregnancy.

F1 takes into account the comparative surface area:body weight ratios for the species concerned and for man. Surface area (S) is calculated as:

$$S = kM^{0.67} \quad (2)$$

in which M = body mass, and the constant k has been taken to be 10. The body weights used in the equation are those shown below in Table A3.1.

F2 = A factor of 10 to account for variability between individuals.

A factor of 10 is generally given for all organic solvents, and 10 is used consistently in this guidance.

F3 = A variable factor to account for toxicity studies of short-term exposure.

F3 = 1 for studies that last at least one half-lifetime (1 year for rodents or rabbits; 7 years for cats, dogs and monkeys).

F3 = 1 for reproductive studies in which the whole period of organogenesis is covered.

F3 = 2 for a 6-month study in rodents, or a 3.5-year study in nonrodents.

F3 = 5 for a 3-month study in rodents, or a 2-year study in nonrodents.

F3 = 10 for studies of a shorter duration.

In all cases, the higher factor has been used for study durations between the time points (e.g., a factor of 2 for a 9-month rodent study).

F4 = A factor that may be applied in cases of severe toxicity (e.g., nongenotoxic carcinogenicity, neurotoxicity or teratogenicity). In studies of reproductive toxicity, the following factors are used:

F4 = 1 for fetal toxicity associated with maternal toxicity.

F4 = 5 for fetal toxicity without maternal toxicity.

F4 = 5 for a teratogenic effect with maternal toxicity.

F4 = 10 for a teratogenic effect without maternal toxicity.

F5 = A variable factor that may be applied if the NOEL was not established.

When only an LOEL is available, a factor of up to 10 could be used depending on the severity of the toxicity.

APPENDIX 2: METHODS FOR ESTABLISHING EXPOSURE LIMITS

The Gaylor-Kodell method of risk assessment (Gaylor, D. W., and R. L. Kodell, "Linear Interpolation Algorithm for Low Dose Assessment of Toxic Substance," *Journal of Environmental Pathology and Toxicology*, 4:305, 1980) is appropriate for Class 1 carcinogenic solvents. Only in cases where reliable carcinogenicity data are available should extrapolation by the use of mathematical models be applied to setting exposure limits. Exposure limits for Class 1 solvents could be determined with the use of a large safety factor (i.e., 10,000 to 100,000) with respect to the NOEL. Detection and quantitation of these solvents should be by state-of-the-art analytical techniques.

Acceptable exposure levels in this guidance for Class 2 solvents were established by calculation of PDE values according to the procedures for setting exposure limits in pharmaceuticals (*Pharmacopeial Forum*, Nov-Dec 1989), and the method adopted by IPCS for Assessing Human Health Risk of Chemicals (EHC 170, WHO, 1994). These methods are similar to those used by the U.S. EPA (IRIS) and the U.S. FDA (Red Book) and others. The method is outlined here to give a better understanding of the origin of the PDE values. It is not necessary to perform these calculations in order to use the PDE values tabulated in Section 4 of this document.

PDE is derived from the NOEL or the LOEL in the most relevant animal study as follows:

$$PDE = \frac{NOEL \times Weight\ Adjustment}{F1 \times F2 \times F3 \times F4 \times F5} \qquad (1)$$

The PDE is derived preferably from a NOEL. If no NOEL is obtained, the LOEL may be used. Modifying factors proposed here, for relating the data to humans, are the same kind of *uncertainty factors* used in EHC (EHC 170, WHO, Geneva, 1994), and *modifying factors* or *safety factors* in *Pharmacopeial Forum*. The assumption of 100 percent systemic exposure is used in all calculations regardless of route of administration.

The modifying factors are as follows:

F1 = A factor to account for extrapolation between species.
F1 = 5 for extrapolation from rats to humans.
F1 = 12 for extrapolation from mice to humans.
F1 = 2 for extrapolation from dogs to humans.
F1 = 2.5 for extrapolation from rabbits to humans.
F1 = 3 for extrapolation from monkeys to humans.
F1 = 10 for extrapolation from other animals to humans.

Table A3.1 – Values Used in the Calculations in This Document

Rat body weight	425 g	Mouse respiratory volume	43 liter (L)/day
Pregnant rat body weight	330 g	Rabbit respiratory volume	1,440 L/day
Mouse body weight	28 g	Guinea pig respiratory volume	430 L/day
Pregnant mouse body weight	30 g	Human respiratory volume	28,800 L/day
Guinea pig body weight	500 g	Dog respiratory volume	9,000 L/day
Rhesus monkey body weight	2.5 kg	Monkey respiratory volume	1,150 L/day
Rabbit body weight (pregnant or not)	4 kg	Mouse water consumption	5 milliliter (mL)/day
Beagle dog body weight	11.5 kg	Rat water consumption	30 mL/day
Rat respiratory volume	290 L/day	Rat food consumption	30 g/day

The equation for an ideal gas, PV = nRT, is used to convert concentrations of gases used in inhalation studies from units of ppm to units of mg/L or mg/cubic meter (m^3). Consider as an example the rat reproductive toxicity study by inhalation of carbon tetrachloride (molecular weight 153.84) summarized in *Pharmeuropa*, Vol. 9, No. 1, Supplement, April 1997, page S9.

$$\frac{n}{V} = \frac{P}{RT} = \frac{300 \times 10^{-6}\ \text{atm} \times 153840\ \text{mg mol}^{-1}}{0.082\ \text{L atm K}^{-1}\ \text{mol}^{-1} \times 298\ \text{K}} = \frac{46.15\ \text{mg}}{24.45\ \text{L}} = 1.89\ \text{mg/L}$$

The relationship 1000 L = 1 m^3 is used to convert to mg/m^3.

The weight adjustment assumes an arbitrary adult human body weight for either sex of 50 kilograms (kg). This relatively low weight provides an additional safety factor against the standard weights of 60 kg or 70 kg that are often used in this type of calculation. It is recognized that some adult patients weigh less than 50 kg; these patients are considered to be accommodated by the built-in safety factors used to determine a PDE. If the solvent was present in a formulation specifically intended for pediatric use, an adjustment for a lower body weight would be appropriate.

As an example of the application of this equation, consider a toxicity study of acetonitrile in mice that is summarized in *Pharmeuropa*, Vol. 9, No. 1, Supplement, April 1997, page S24. The NOEL is calculated to be 50.7 mg kg^{-1} day^{-1}. The PDE for acetonitrile in this study is calculated as follows:

$$PDE = \frac{50.7 \text{ mg kg}^{-1} \text{ day}^{-1} \times 50 \text{ kg}}{12 \times 10 \times 5 \times 1 \times 1} = 4.22 \text{ mg day}^{-1}$$

In this example,

F1 = 12 to account for the extrapolation from mice to humans.

F2 = 10 to account for differences between individual humans.

F3 = 5 because the duration of the study was only 13 weeks.

F4 = 1 because no severe toxicity was encountered.

F5 = 1 because the NOEL was determined.

Guidance for Industry

Q3C — Tables and List

Additional copies are available from:
Center for Drug Evaluation and Research (CDER),
Division of Drug Information (HFD-240),
5600 Fishers Lane, Rockville, MD 20857
(Tel) 301-827-4573
http://www.fda.gov/cder/guidance/index.htm

or

Office of Communication, Training, and
Manufacturers Assistance (HFM-40),
Center for Biologics Evaluation and Research (CBER)
1401 Rockville Pike, Rockville, MD 20852-1448,
http://www.fda.gov/cber/guidelines.htm;
(Fax) 888-CBERFAX or 301-827-3844
(Voice Information) 800-835-4709 or 301-827-1800

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)
December 1997
ICH

Guidance for Industry

Q3C — Tables and List

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)
December 1997
ICH

II. LIST OF SOLVENTS INCLUDED IN THE Q3C GUIDANCE

Solvent	Other Names	Structure	Class
Acetic acid	Ethanoic acid	CH_3COOH	Class 3
Acetone	2-Propanone Propan-2-one	CH_3COCH_3	Class 3
Acetonitrile		CH_3CN	Class 2
Anisole	Methoxybenzene		Class 3
Benzene	Benzol		Class 1
1-Butanol	n-Butyl alcohol Butan-1-ol	$CH_3(CH_2)_3OH$	Class 3
2-Butanol	*sec*-Butyl alcohol Butan-2-ol	$CH_3CH_2CH(OH)CH_3$	Class 3
Butyl acetate	Acetic acid butyl ester	$CH_3COO(CH_2)_3CH_3$	Class 3
tert-Butylmethyl ether	2-Methoxy-2-methyl-propane	$(CH_3)_3COCH_3$	Class 3
Carbon tetrachloride	Tetrachloromethane	CCl_4	Class 1
Chlorobenzene			Class 2
Chloroform	Trichloromethane	$CHCl_3$	Class 2
Cumene	Isopropylbenzene (1-Methyl)ethylbenzene	C_6H_5-$CH(CH_3)_2$	Class 3
Cyclohexane	Hexamethylene		Class 2
1,2-Dichloroethane	*sym*-Dichloroethane Ethylene dichloride Ethylene chloride	CH_2ClCH_2Cl	Class 1
1,1-Dichloroethene	1,1-Dichloroethylene Vinylidene chloride	$H_2C=CCl_2$	Class 1

Guidance for Industry[1]

Q3C — Tables and List

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statutes and regulations.

I. INTRODUCTION

This is the companion document for the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidance for industry *Q3C Impurities: Residual Solvents* (1997), which makes recommendations as to what amounts of residual solvents are considered safe in pharmaceuticals.

This document may be updated if proposals for change are submitted to the International Conference on Harmonisation (ICH) Steering Committee. Proposals for change and the ICH Steering Committee final decision on any proposed changes will be announced through a notice in the *Federal Register* prior to the updating of this document.

[1] This document was developed within the Expert Working Group (Quality) of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and has been subject to consultation by the regulatory parties, in accordance with the ICH process. This document was endorsed by the ICH Steering Committee at *Step 4* of the ICH process in July 1997. At *Step 4* of the process, the final draft is recommended for adoption to the regulatory bodies of the European Union, Japan, and the United States. This guidance was published in the *Federal Register* on December 24, 1997 (62 FR67377), and is applicable to drug and biological products.

3-Methyl-1-butanol	Isoamyl alcohol Isopentyl alcohol 3-Methylbutan-1-ol	$(CH_3)_2CHCH_2CH_2OH$	Class 3
Methylbutyl ketone	2-Hexanone Hexan-2-one	$CH_3(CH_2)_3COCH_3$	Class 2
Methylcyclohexane	Cyclohexylmethane		Class 2
Methylethyl ketone	2-Butanone MEK Butan-2-one	$CH_3CH_2COCH_3$	Class 3
Methylisobutyl ketone	4-Methylpentan-2-one 4-Methyl-2-pentanone MIBK	$CH_3COCH_2CH(CH_3)_2$	Class 3
2-Methyl-1-propanol	Isobutyl alcohol 2-Methylpropan-1-ol	$(CH_3)_2CHCH_2OH$	Class 3
N-Methylpyrrolidone	1-Methylpyrrolidin-2-one 1-Methyl-2-pyrrolidinone		Class 2
Nitromethane		CH_3NO_2	Class 2
Pentane	n-Pentane	$CH_3(CH_2)_3CH_3$	Class 3
1-Pentanol	Amyl alcohol Pentan-1-ol Pentyl alcohol	$CH_3(CH_2)_3CH_2OH$	Class 3
1-Propanol	Propan-1-ol Propyl alcohol	$CH_3CH_2CH_2OH$	Class 3
2-Propanol	Propan-2-ol Isopropyl alcohol	$(CH_3)_2CHOH$	Class 3
Propyl acetate	Acetic acid propyl ester	$CH_3COOCH_2CH_2CH_3$	Class 3
Pyridine			Class 2
Sulfolane	Tetrahydrothiophene 1,1-dioxide		Class 2
Tetrahydrofuran	Tetramethylene oxide Oxacyclopentane		Class 3
Tetralin	1,2,3,4-Tetrahydro-naphthalene		Class 2
Toluene	Methylbenzene		Class 2
1,1,1-Trichloroethane	Methylchloroform	CH_3CCl_3	Class 1

1,2-Dichloroethene	1,2-Dichloroethylene Acetylene dichloride	ClHC=CHCl	Class 2
Dichloromethane	Methylene chloride	CH_2Cl_2	Class 2
1,2-Dimethoxyethane	Ethyleneglycol dimethyl ether Monoglyme Dimethyl Cellosolve	$H_3COCH_2CH_2OCH_3$	Class 2
N,N- Dimethylacetamide	DMA	$CH_3CON(CH_3)_2$	Class 2
N,N- Dimethylformamide	DMF	$HCON(CH_3)_2$	Class 2
Dimethyl sulfoxide	Methylsulfinylmethane Methyl sulfoxide DMSO	$(CH_3)_2SO$	Class 3
1,4-Dioxane	p-Dioxane [1,4]Dioxane		Class 2
Ethanol	Ethyl alcohol	CH_3CH_2OH	Class 3
2-Ethoxyethanol	Cellosolve	$CH_3CH_2OCH_2CH_2OH$	Class 2
Ethyl acetate	Acetic acid ethyl ester	$CH_3COOCH_2CH_3$	Class 3
Ethyleneglycol	1,2-Dihydroxyethane 1,2-Ethanediol	$HOCH_2CH_2OH$	Class 2
Ethyl ether	Diethyl ether Ethoxyethane 1,1'-Oxybisethane	$CH_3CH_2OCH_2CH_3$	Class 3
Ethyl formate	Formic acid ethyl ester	$HCOOCH_2CH_3$	Class 3
Formamide	Methanamide	$HCONH_2$	Class 2
Formic acid		HCOOH	Class 3
Heptane	n-Heptane	$CH_3(CH_2)_5CH_3$	Class 3
Hexane	n-Hexane	$CH_3(CH_2)_4CH_3$	Class 2
Isobutyl acetate	Acetic acid isobutyl ester	$CH_3COOCH_2CH(CH_3)_2$	Class 3
Isopropyl acetate	Acetic acid isopropyl ester	$CH_3COOCH(CH_3)_2$	Class 3
Methanol	Methyl alcohol	CH_3OH	Class 2
2-Methoxyethanol	Methyl Cellosolve	$CH_3OCH_2CH_2OH$	Class 2
Methyl acetate	Acetic acid methyl ester	CH_3COOCH_3	Class 3

Solvents in Class 2 (Table 2) should be limited in pharmaceutical products because of their inherent toxicity. PDEs are given to the nearest 0.1 mg/day, and concentrations are given to the nearest 10 ppm. The stated values do not reflect the necessary analytical precision of determination. Precision should be determined as part of the validation of the method.

Table 2. – Class 2 Solvents in Pharmaceutical Products

Solvent	PDE (mg/day)	Concentration Limit (ppm)
Acetonitrile	4.1	410
Chlorobenzene	3.6	360
Chloroform	0.6	60
Cyclohexane	38.8	3,880
1,2-Dichloroethene	18.7	1,870
Dichloromethane	6.0	600
1,2-Dimethoxyethane	1.0	100
N,N-Dimethylacetamide	10.9	1,090
N,N-Dimethylformamide	8.8	880
1,4-Dioxane	3.8	380
2-Ethoxyethanol	1.6	160
Ethyleneglycol	6.2	620
Formamide	2.2	220
Hexane	2.9	290
Methanol	30.0	3,000
2-Methoxyethanol	0.5	50
Methylbutyl ketone	0.5	50
Methylcyclohexane	11.8	1,180
N-Methylpyrrolidone	48.4	4,840
Nitromethane	0.5	50
Pyridine	2.0	200
Sulfolane	1.6	160
Tetralin	1.0	100
Toluene	8.9	890
1,1,2-Trichloroethene	0.8	80
Xylene[1]	21.7	2,170

[1]Usually 60% m-xylene, 14% p-xylene, 9% o-xylene with 17% ethyl benzene.

1,1,2-Trichloroethene	Trichloroethene	$HClC{=}CCl_2$	Class 2
Xylene[1]	Dimethybenzene Xylol		Class 2

[1]Usually 60% m-xylene, 14% p-xylene, 9% o-xylene with 17% ethyl benzene.

III. SOLVENTS GROUPED BY CLASS

Solvents in Class 1 (Table 1) should not be employed in the manufacture of drug substances, excipients, and drug products because of their unacceptable toxicity or their deleterious environmental effect. However, if their use is unavoidable in order to produce a drug product with a significant therapeutic advance, then their levels should be restricted as shown in Table 1, unless otherwise justified. The solvent 1,1,1-Trichloroethane is included in Table 1 because it is an environmental hazard. The stated limit of 1,500 ppm is based on a review of the safety data.

Table 1. – Class 1 Solvents in Pharmaceutical Products (Solvents That Should Be Avoided)

Solvent	Concentration Limit (ppm)	Concern
Benzene	2	Carcinogen
Carbon tetrachloride	4	Toxic and environmental hazard
1,2-Dichloroethane	5	Toxic
1,1-Dichloroethene	8	Toxic
1,1,1-Trichloroethane	1,500	Environmental hazard

The solvents listed in Table 4 may also be of interest to manufacturers of excipients, drug substances, or drug products. However, no adequate toxicological data on which to base a PDE were found. Manufacturers should supply justification for residual levels of these solvents in pharmaceutical products.

Table 4. – Solvents for Which No Adequate Toxicological Data Were Found

1,1-Diethoxypropane	Methylisopropyl ketone
1,1-Dimethoxymethane	Methyltetrahydrofuran
2,2-Dimethoxypropane	Petroleum ether
Isooctane	Trichloroacetic acid
Isopropyl ether	Trifluoroacetic acid

Solvents in Class 3 (Table 3) may be regarded as less toxic and of lower risk to human health. Class 3 includes no solvent known as a human health hazard at levels normally accepted in pharmaceuticals. However, there are no long-term toxicity or carcinogenicity studies for many of the solvents in Class 3. Available data indicate that they are less toxic in acute or short-term studies and negative in genotoxicity studies. It is considered that amounts of these residual solvents of 50 mg per day or less (corresponding to 5,000 ppm or 0.5 percent under Option 1) would be acceptable without justification. Higher amounts may also be acceptable provided they are realistic in relation to manufacturing capability and good manufacturing practice (GMP).

Table 3. – Class 3 Solvents Which Should Be Limited by GMP or Other Quality-Based Requirements

Acetic acid	Heptane
Acetone	Isobutyl acetate
Anisole	Isopropyl acetate
1-Butanol	Methyl acetate
2-Butanol	3-Methyl-1-butanol
Butyl acetate	Methylethyl ketone
tert-Butylmethyl ether	Methylisobutyl ketone
Cumene	2-Methyl-1-propanol
Dimethyl sulfoxide	Pentane
Ethanol	1-Pentanol
Ethyl acetate	1-Propanol
Ethyl ether	2-Propanol
Ethyl formate	Propyl acetate
Formic acid	Tetrahydrofuran

DIMETHYL SULFOXIDE HEALTH EFFECTS INFORMATION

SUMMARY

A great number of toxicological, environmental and medical studies have been performed with DMSO to determine the safety of this chemical. Many of these studies have been published as referenced at the end of this bulletin. This summary only lists some of the results found, but in depth details are reported in the original publications. In addition, Gaylord Chemical's extensive database of over 19,000 articles on applications and safe process use with DMSO is available for use by those who request it.

DMSO is a low vapor pressure dipolar aprotic chemical which is used extensively because of its excellent solvent properties. Although an industrial solvent, it is also a naturally occurring substance, which is apparently a part of earth's complex sulfur cycle. DMSO is created in the atmosphere at a rate of 20-60 billion pounds per year from dimethyl sulfide, which is produced by metabolic processes in soil and sediments. DMSO is also found in natural waters and soil. Metabolism of DMSO in soil by microorganisms results in the formation of sulfur and dimethyl sulfide. DMSO is also reported to be present at low concentrations (<0.05-3.7 ppm) in food products such as sauerkraut, tomato paste, milk, beer, coffee, tea and in forage crops such as alfalfa and corn silage.

DMSO has low acute and chronic toxicity for animal, plant and aquatic life. Exposure to test organisms at high concentrations by contact, ingestion or inhalation consistently show low toxicity. DMSO is not listed as a carcinogen by regulatory authorities and is actually used as a neutral solvent in the Ames mutagencitity tests. DMSO is not a teratogen in mice, rats or rabbits. Because of this low potential for toxicity, the EPA has approved DMSO as a solvent or a cosolvent, in pesticides which are applied before crop emergence or prior to the formation of edible parts of food plants. Based on more recent studies, the EPA is considering extending the use of DMSO in pesticide formulations applied directly to edible parts of food or feed crops.

In 1978 the FDA approved the use of DMSO in a 50/50 mixture with water as an effective treatment for the symptoms of interstitial cystitis. Since then, a large number of people have received this treatment. The product is marketed today by Baxter Research Medical under the trade name of Rimso-50. In addition, in 1998, the FDA endorsed the recommendation of the expert working group of the International Conference on harmonization relative to the residual solvents in pharmaceuticals. DMSO was placed in the safest category, class 3 solvents with low toxic potential. Class 3 means it has low toxic potential to humans and no health based exposure level is needed.

DMSO

BULLETIN #106

GAYLORD CHEMICAL CORPORATION

DIMETHYL SULFOXIDE (DMSO) HEALTH EFFECTS INFORMATION

INTRODUCTION

Thousands of tons of Dimethyl Sulfoxide (DMSO) have been used in hundreds of industrial plants, laboratories, universities and medical research establishments since 1960. Applications have included pharmaceutical production, solvent cleaning, hydrocarbon refining and agricultural formulations. We know of no instances in which routine or accidental exposure to DMSO in such settings has led to harm to individuals. This bulletin is intended to answer questions about safety of working with DMSO and procedures for minimizing any perceived or potential hazards. It is aimed specifically at workers in laboratories, transportation systems or factories. In these places, the kinds of worker contact can be limited and controlled. When the amount and nature of these exposures are known, protective equipment can be built or worn, or other appropriate action can be taken.

TABLE OF CONTENTS

Rev. 11/09/98

Properties of DMSO

Some of the physical properties of DMSO are:

Physical Property		Metric Units	English Units
Freezing Point		18.55° C	65.4° F
Boiling Point	- 760 mm	189 ° C	372 ° F
Vapor Pressure	- 0.6 in Hg	25 ° C	77 ° F
	- 13 in Hg	100 ° C	212 ° F
	- 310 in Hg	150 ° C	302 ° F
Heat of Vaporization @ 70°C		11.3 Kcal/mol	260 BTU/lb
Flash Point (open cup)		95°C	203° F
Flash Point (closed cup)		89°C	192° F
Auto-ignition Temperature in Air		300-302 ° C	572-575 ° F
Flammability Limits in Air –			
	Lower (100°C)	3-3.5% by volume	
	Upper	42-63%	
Solubility Parameter, Total		13 $(cal/cm^3)^{1/2}$	
Solubility	– Not miscible	Aliphatic hydrocarbons, some fatty acids, and waxes.	
	- Miscible	Water, most aromatic and halogenated hydrocarbons, alcohols, ketones, esters, most sulfur- and nitrogen-containing compounds.	
	- Dissolves or softens	Many resins or polymers, some salts, many complex high-molecular weight compounds.	

When handling or using DMSO a potential for exposure exists. Therefore the following information should be considered regarding possible exposure routes. Skin contacts, the most likely exposure, has been extensively studied with humans and animals. Large dosages over prolonged periods showed only minor toxic effects such as minor skin irritation, itching and burning. Although DMSO is absorbed rapidly through the skin, it has low toxicity and does not carry with it other compounds that do not absorb by themselves. No effects have been found in human or primate eyes from high dosage, long lasting exposure. Some refractive changes were noted in dogs, swine and rabbits. Inhalation of vapors of DMSO at high concentrations (200-2900mg/m^3) for up to 210 hours gave little outward toxicity signs. Although the low vapor pressure of DMSO normally limits exposure to very low levels, we recommend avoiding more than brief exposure to DMSO sprays, mists or high vapor concentrations.

Regarding the chemical reactivity of DMSO, the user should note that this chemical reacts very rapidly with oxidizing agents and some other chemical compounds. As a solvent, it can catalyze some reactions which can become very rapid and out of control. These factors should be considered in setting up experimental or industrial use conditions.

Although DMSO has been show to have low toxicity potential, current good industrial hygiene practice requires avoiding exposure to all chemicals in the workplace and we recommend the use of protective equipment to prevent exposure.

Single-Dose Toxicity of DMSO as LD-50[1] (g/kg)

Species	Applied to Skin	Taken by Mouth	Into Blood Stream	Beneath Skin	Into Body Cavity
Mouse	50	16.5-24.6	3.8-8.9	13.9-20.5	14.7-17.0
Rat	40	17.4-28.3	5.2-8.1	12.0-20.5	13.0
Guinea Pig	-	11.0	-	-	5.5
Chicken	-	14.0	-	-	-
Cat	-	-	4.0	-	-
Dog	>11	10.0	2.5	-	-
Monkey	>11	4.0	4.0	-	-

Using the monkey as an example, it would take more than 1.6 pounds applied to the skin or 0.6 pounds taken by mouth or injected directly into the blood stream, to have a 50% mortality rate in a group weighing 150 pounds each.

Other studies have shown that DMSO has low acute toxicity and is practically non-toxic (LD50>5 g/kg) by ingestion or dermal application. Rat oral LD50s are reported from 17.4 to 28.3 g/kg, whereas LD50s for mice have been reported from 16.5 to 24.6 g/kg. The acute dermal LD50 is 40 g/kg for the rat and 50 g/kg for the mouse, while dermal LD50s > 11 g/kg are reported for both dogs (beagles) and primates (rhesus monkeys). Although DMSO can cause skin and eye irritation, it is not a skin sensitizer.

In addition to LD-50 explained above, another unit LC-50 is used to evaluate the hazard from inhalation. LC-50 is the Lethal Concentration that kills 50% of the test animals. The acute rat inhalation LC-50 is greater than 1.6 mg/l, the only dose level tested, and which is also a No-Observed-Effect-Level (NOEL).[10]

Subchronic Toxicity

The subchronic rat inhalation NOEL of 200 mg/m^3 (0.2 mg/l) was determined from a single concentration study. Extensive monitoring of human patients have shown that DMSO does not affect human renal function. DMSO is a diuretic but no sign of kidney damage has been found in humans or laboratory animals after repeated DMSO treatment. Hemalysis has been reported in animals that received DMSO intravenously.

Reactivity

DMSO reacts very rapidly and vigorously with a number of materials, particularly with those which also react rapidly with water. The reactions are highly exothermic, with rapid steam or gas evolution. In most cases these reactions can be controlled by rate or order of addition or by arranging adequate heat removal. The following types of compounds require care to prevent extremely rapid reactions.

1. Strong oxidizing agents such as perchlorates, permanganates, iodine pentafluoride, silver fluoride and others react very rapidly.
2. Acid chlorides react with DMSO at about the same rate as with ethyl alcohol.
3. Carboxylic acid anhydrides react rapidly.
4. Alkali hydrides used for making DMSO anion require adequate heat removal. (A technical bulletin on reactions of the dimsyl anion is available.)
5. DMSO cannot be used with Ziegler-Natta catalysts or in Friedel-Crafts reactions.
6. Methyl bromide can react to form HBr and Br_2. Uncontrolled reactions have resulted.

Additional information is available from Gaylord.

Acute Toxicity

Evaluation of the degree of hazard due to contact with a chemical is usually by its single-dose LD-50. The LD-50 is the Lethal Dose in number of grams of DMSO per kilogram of body weight which results in 50% mortality of the test animals under standardized conditions. Dozens of test data reports are available from many laboratories throughout the world. The reported LD-50 may vary, but the data confirm a low level of toxicity.
One published summary is the following.

related effects beyond occasional skin irritation, garlicky breath and body odor. Additionally, (Hull et al. 1969)[7] found no DMSO-related effects in any of the 38 human males, age 21-55, who received a topical application of an 80% DMSO gel in a single daily dose of 1 g/kg for 12 weeks.

Continuing research has demonstrated that the ocular effects reported from DMSO treatment of dogs, rabbits, guinea pigs and swine are species-specific and not reproducible in primates, including humans. Even though ocular toxicity, specifically lenticular refractive changes, have been reported in some animal studies with dogs, rabbits and swine (Rubin and Barnett, 1967; Smith et al. 1969)[3] and in guinea pigs (Rengstarff et al., 1972)[4], it was subsequently demonstrated that the ocular effect was species-specific and was not reproducible in primates, including humans (Smith et al., 1969)[3] (de la Torre et al. 1981)[5]. Furthermore, full ophthalmologic examinations revealed no DMSO-related lenticular changes in any of 84 patients treated three times daily for three months with topical 70% DMSO, topical 2% DMSO or 0.85% normal saline (maximum theoretical dosage of 2.6 g DMSO/kg/day), which is comparable to dosages used in the animal studies (Shirley et al., 1988)[6].

Human and Animal Metabolism

DMSO is metabolized in humans by oxidation to dimethyl sulfone, $DMSO_2$ or by reduction to dimethyl sulfide, DMS. DMSO and $DMSO_2$ are excreted in the urine and feces. DMS is eliminated through the breath and skin with a characteristic "garlic" or "oyster-like" odor. Human excretion of orally administered DMSO is complete within 120 hours, with urinary excretion being the primary pathway. The rate of renal clearance has been shown to be similar for chronic and singly administered doses regardless of dose concentration. No residual accumulation of DMSO has been reported in humans or lower animals who have received DMSO treatment for protracted periods of time, regardless of route of dose administration.

Metabolite Toxicity

The metabolites of DMSO are $DMSO_2$, which naturally occurs at low levels in human urine (PDR, 1994)[8], and DMS, which naturally occurs in plants, the atmosphere, and lakes and oceans (Pearson et al., 1981)[9]. Both of these metabolites are readily excreted from the body. Based on their widespread natural occurrence and ready degradation and/or excretion, the production of these metabolites from the proposed use of DMSO on food producing plants is not expected to pose any toxicological concern.

Skin Exposure

DMSO easily penetrates the skin (176 ± 42 g/M^2/hr) compared to, for example, water (14.8 ± 0.1 g/M^2/hr), but because of DMSO's low toxicity (see previous section) and the fact that this same permeability test showed DMSO does not carry less-permeable substances with it through the skin, it can be concluded that DMSO does not pose a significant threat by skin absorption. The penetration rate of DMSO in solutions is a direct function of the mole fraction of DMSO. (Ursin, et. al. 1995)[14]. Although DMSO readily penetrates human skin in concentrations of 70-100%, at concentrations of 67% or less, DMSO molecules are hydrated, which greatly reduces dermal penetration (Sulzberger et. al., 1966; Brayton, 1986: Woodford and Barry, 1986).

No significant abnormalities were found in extensive physical examinations or analyses of blood and urine during repeated applications of large amounts of DMSO to the skin of humans over a long period of time. This was reported by Dr. Richard Brobyn [(2)] to the New York Academy of Sciences.

DMSO was used in two human studies lasting 14 and 90 days. In each case, one gram of DMSO per kilogram of body weight was applied each day by each subject to his own skin. In an (80 kg = 176 lb) individual, it was 80 grams or 2.7 fl. oz. This amount required up to 2 hours for complete absorption from the 90% DMSO gel.

No frank evidence of intolerance resulted from dermal application of 9 grams/kilogram of 90% DMSO to Rhesus monkeys daily for 18 months. In a small (50 kg = 110 lb) individual, this would amount to daily applications of 15.2 fluid ounces or nearly a pint of 90% DMSO.

Observation has indicated that skin application, particularly if frequent with large amounts of DMSO, may result in reddening, itching and burning at the application site. Exposure to large amounts of DMSO by skin or elsewhere may result in sedation, headache, nausea or dizziness.

Chronic Toxicity

DMSO is not listed as a carcinogen by regulatory agencies such as IARC, NTP, OSHA or ACGIH, based on reviews of numerous studies. **In fact, a study supported by the US Public Health Services concluded that DMSO was not a carcinogen and is a safe carrying agent for ingestion studies analogous to mineral oil.** An 18-month study with rhesus monkeys established an oral NOEL of 3 g/kg/day. No tumors were observed and bone marrow smears from the monkeys that received oral or topical doses of DMSO at up to 9 g/kg/day is comparable to an average human (70 kg or 154 lbs) consuming approximately 210 g (or nearly ½ pound) DMSO per day, i.e., 3g/kg/day. In fact, 84 humans that have received daily topical treatment of 2.8 g DMSO/kg/day (equivalent to nearly ½ pound/day/person) for up to three months showed no DMSO-

5. Natural Occurrences in Food. The occurrence of DMSO and its metabolites, dimethyl sulfide and methyl sulfone ($DMSO_2$), has been widely reported in a variety of foods. Pearson [9] and coworkers reported finding 0.07 to 16 ppm DMSO, along with $DMSO_2$, in 14 fruits, vegetables or beverages. This natural occurrence insures that the body can dispose of DMSO by well-established metabolic processes. Naturally-occurring DMSO has been identified in alfalfa, asparagus, barley, beans, beets, cabbage, corn, cucumbers, oats, onions, Swiss chard, tomatoes, apples, raspberries, spearmint, beer, milk, coffee and tea. DMSO concentrations in fresh fruits, vegetables and grains ranged from undetectable (<0.05 parts per million) to 1.8 ppm.

Genotoxicity

DMSO is not mutagenic to *Salmonella, Drosophila*, and fish cell cultures. Because DMSO so non-reactive as a mutagen, it is widely used as a solvent in mutagenicity testing. Although DMSO is bacteriostatic or bactericidal at concentrations of 5-50%, there is no evidence that DMSO causes chromosonal aberrations at levels that are not directly toxic to cells. Bone marrow smears from primates (rhesus monkeys) that received oral or topical doses of DMSO for 18 months showed no DMSO effects (Vogin et al., 1970)[12]. An *in vivo* cytogenetics study of DMSO administered by intraperitoneal injection to male rats found a significant increase in aberrant femoral bone marrow cells when compared to controls (Kapp and Eventoff, 1980)[13]. However, evidence from the *Salmonella* studies and other toxicology data, especially the teratology data, suggests that the increase in aberrant femoral cells likely resulted from direct toxicity of DMSO injected into an animal instead of a classic "mutagenic" response.

According to Brayton (1986), there are no documented adverse genetic effects reported as a result of medicinal DMSO uses (including quasi-medicinal uses for treatment of arthritis or sprains and strains). Additionally, no adverse genetic effects have been reported from occupational exposure to DMSO in over 40 years of industrial use (Brayton, 1986). There is no evidence that DMSO causes chromosomal aberrations at levels that are not directly toxic to cells.

Reproductive and Developmental Toxicity

A mouse teratology NOEL of 12 g/kg/day has been established based on research with a 50% DMSO solution administered orally. Additional teratogenicity studies of orally administered DMSO to pregnant mice, rats, rabbits and guinea pigs have demonstrated that DMSO is not a teratogen in mammals except at high levels that cause overt maternal toxicity and are coincident with the maximum tolerated dose. The data suggest that DMSO is not teratogenic at low levels regardless of the route of

Inhalation

Fishman and coworkers at the Naval Medical Center [10] performed many toxicological measurements on the exposure of rats to DMSO vapors. The following single and repeated exposures were made:

DMSO Concentration	Length of Exposure
1600 milligrams per m^3	4 hours
2900 milligrams per m^3	24 hours
2000 milligrams per m^3	40 hours
200 milligrams per m^3	210 hours (7 hrs/day, 5 days/week for 30 exposures)

Extensive blood and tissue samples were examined. No outward toxic signs were shown. No significant changes were noted during or following repeated exposure.

We suggest, as a good hygiene practice, avoiding exposure to DMSO sprays or mists and very high doses of DMSO vapors.

Environmental Effects

1. Effects on Animals have been described.

2. Effects on Plants. DMSO by itself and DMSO with antibiotics, minerals, nutrients, pesticides and other materials have been sprayed on, injected into, painted on, and fed to a variety of plants. It has a low order of phyto-toxicity in these applications.

3. Effects on Fish. Wilford[11] investigated the toxicity of DMSO in water to 9 species of fish. At 96 hours, the LC-50 was 32,000 to 43,000 ppm DMSO (3-4%). This is far less toxic than acetone (fingernail polish remover) and other widely used solvents.

4. Effects on Sewage Plants. DMSO is biodegradable. In biological systems, it is converted in part to methyl sulfone and to dimethyl sulfide. The sulfone is stable and inert and degraded only slowly by microorganisms or physical factors. At high concentrations, some DMS may escape, producing its characteristic odor.

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administration. Finally, the teratogenic potential of DMSO is dependent on the route of administration, the dose level and gestation stage at exposure.

The one study (Robens, 1968) that did show evidence of teratogenic effects (In hampsters, one of three animal species tested) from oral administration of DMSO is inappropriate to use for a teratologic evaluation of DMSO for the following reasons:

- DMSO was not the compound of interest but was used only as a solvent control at two very high dose levels which precluded establishing a NOEL.
- One of the DMSO levels tested resulted in maternal death and was clearly beyond the maximum tolerated dose (MTD).

DMSO is not considered to be directly embryotoxic and has been shown to be a successful cryoprotectant for mammalian semen and embryos (Brayton, 1986).

In summary, the evidence of the above teratology data suggests that:

1. DMSO is not a teratogen to mammals when administered via oral and dermal routes at dose levels that do not produce overt maternal toxicity.
2. DMSO is not a teratogen at low dose levels regardless of the route of administration.
3. The teratogenic potential of DMSO is dependent on route of administration, the dose level and the gestational time of exposure.

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BIODEGRADATION OF SYNTHETIC DETERGENTS

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Tony Barrington

From: <libref@mines.edu>
To: <libref@mines.edu>
Cc: <avachem@gsinet.net>
Sent: Tuesday, March 25, 2003 7:15 PM
Subject: Re: Ask A Librarian Form/Wayman paper

Hi Tony - sorry for the delay in response. From Chemical Abstracts online, your citation is from a book series, Progress in Industrial Microbiology, 1971, v. 10, p. 219-271. It is described as "a review with 138 refs"

Unfortunately, we don't have v. 10 in our library, though we have others in the series. Do you have access to an academic library, or an Interlibrary Loan service at any library? I can check libraries near you if I know where you are, or you should be able to request a copy via ILL.

- Heather at CSM Ref Desk

Quoting libref@Mines.EDU:

> Ask A Librarian Form
>
> From URL: http://www.mines.edu/library/reference/forms/qform.html
>
> question: Hi:
>
> I have a copy of a paper titled, "Biodegradation of Synthetic Detergents" by
> Cooper H. Wayman, Ph.D., Dept. of Chemistry, Colorado School of Mines. It is
> undated. It appears to be a chapter from a book.
>
> The paper cites a number of sources, which I suspect appear at the end of the
> book and are not a part of the document I have.
>
> It would be most helpful if you could identify the date and source of the
> document I have; even better if you could provide the list of references
> cited.
> expires:
> purpose: We have developed a non-toxic pesticide that is chemically
> similar to the sugar ester substances described in the Wayman paper. We are
> attempting to demonstrate to the USEPA that sugar esters degrade rapidly
> after application and do not present a health risk to farm workers who
> re-enter the treated crop shortly after application. We believe the
> references cited in the Wayman paper would be helpful in this regard.
> course:
> consulted:
> fname: Tony

potentially subjected to environmental hazards, or that it be subjected to the process of biodegradation.

It is the later facet of the above statement that we are concerned with here. Any organic compound which might serve as a carbon source and supply energy to bacteria in their metabolic systems, is potentially susceptible to removal from the environment by the process of biodegradation.

There is no singular or concise definition of biodegradation. The word biodegradation in its simplest context implies the ability of bacteria to utilize organic compounds either to promote cellular growth or to sustain growth at some saturation value during respiration. The biodegradation process depends upon the enzymes generated by bacteria and other micro-organisms. Enzymes function in this process as organic catalysts and determine or control the rate of chemical interaction between the organism and organic compounds, such as a surfactant in water. The actual buildup of enzymes at a bacterial cell wall depends, among other things, upon both temperature and acidity of the solution. The reaction rate between enzymes and chemical compounds increases with increase in temperature, but at temperatures much above 35° C, the enzymatic activity may be substantially impaired. Enzymes are proteins, which have a completely folded and crenellated surface containing numerous grooves and folds. Because of this fact, certain proteins are particularly suited for the adsorption of certain organic molecules (like surfactants) from solutions. After adsorption has occurred at these complementary surfaces, the surfactant molecule may be broken down (degraded) into its component parts. The surfactant thus combines with an enzyme to form a complex molecule which in turn re-forms back into the original enzyme and another product (the degraded surfactant). With respect to surfactants, two generalized enzymatic processes can be envisioned. One process is the aerobic or oxygen-consuming process such as occurs in sewage plants or those treating industrial wastes with an activated sludge system. A second process might well involve anaerobic enzymes with concomitant reduction and hydrogenation.

The best guidelines to date in the formulation of definitions of biodegradation are the following as set forth by a special committee on Standard Methods of the Water Pollution Control Federation:[2]

2.1. Primary Biodegradation

Biodegradation to the minimum extent necessary to change the identity of the compound.

2.2. Environmentally Acceptable Biodegradation

Biodegradation to the minimum extent necessary to remove undesirable properties of the compound such as foaminess and/or toxicity.

2.3. Ultimate Biodegradation

Biodegradation to inorganic end products possibly specified by the reaction of the form:

$$\text{substrate} + O_2 + \text{bacteria} \rightarrow CO_2 + H_2O + \text{nutrients} + \text{cellular mass} \qquad (1)$$

BIODEGRADATION OF SYNTHETIC DETERGENTS

1. INTRODUCTION

The estimate of water to be utilized in the United States by 1980 is 600 billion gallons per day (bgd). Of this amount over 60 bgd has its source in ground water supplies serving about 100 million inhabitants. It is apparent that the quality of surface-water and ground-water should be preserved consistent with good practice and established economic and legal restraints to safeguard the development of this valuable resource.

To date, scientific and engineering prowess has enabled man to produce about 1,000,000 man-made products employed in industry and the household. In order that our affluence not be impaired, we add over 100,000 new products to this vast list annually. A very substantial amount of these compounds are discharged into surface-water and/or ground-water from industrial effluent water, sewage waste water, septic tanks and like sources. Needless to say these compounds, if 'refractory' produce economic, socio-legal, and bulk deterioration of the environment. An excellent example of the type of problem created by discharged wastes is that created by detergents or more appropriately surfactants (surface active agents). Detergent foaming is found in rivers, streams, ground-water, and treatment plants. The specific effects arising from detergents are appearance of foam, lowering of plant treatment efficiency, tastes and odors in water supplies, effects on aquatic organisms by creation of an ecological imbalance, lowering of oxygen contents of natural waters, septic tank problems, and even the so-called apartment-house 'backup'.

Unless these undesirable compounds discharged in waste-water effluents can be chemically degraded (by dissolution, or hydrolysis) or oxidized by aerobic—or facultative anaerobic bacteria, pollution of surface—or ground water is the result.

To some extent the detergent problem has been alleviated with the advent of changes in surfactant formulation producing preparations more conducive to biological oxidation.

It is the purpose of this paper to set forth some principles or guidelines which might be instructive to those engaged or to be engaged in studies on biodegradation. Though surfactants are discussed in this paper, the principles developed are applicable to other systems. More data are presently available on surfactant biodegradability than for other compounds as attested by a very comprehensive article of recent date by Swisher.[1]

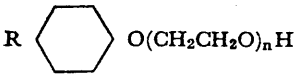
2. SIGNIFICANCE OF BIODEGRADATION

To prevent build-up of any contaminant in an aqueous system, it is essential that the compound be diluted to an innocuous concentration, be adsorbed on a surface of a solid with subsequent transport and removal from the area

3.2. Nonionic Surfactants

Nonionic surfactants do not ionize in water and depend on ethylene oxide polymers to render them soluble in water. An elaborate description of these surfactants can be found in Shick.[5] Major types are:

(a) Polyoxyethylene alkylphenols or alkylphenol ethoxylate

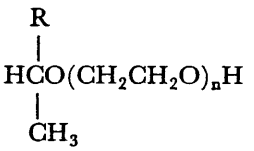


is prepared by the reaction of ethylene oxide (CH_2CH_2O) with an alkylated phenol. Usage of these surfactants is dependent upon the ethylene oxide content.

(b) Polyoxyethylene alcohols

(1) Primary alcohol ethoxylate $RO(CH_2CH_2O)_nH$

(2) Secondary alcohol ethoxylate

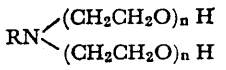


prepared by reacting an alcohol, usually a straight chain type with ethylene oxide. These types of surfactants behave as excellent emulsifiers as a result of their stability in hard water and at varying pH.

(c) Polyoxyethylene esters of fatty acids $RCCO(CH_2CH_2O)_nH$

prepared by reacting a C_{12} to C_{18} fatty acid with ethylene oxide. These types are very inexpensive, produce little foam and are employed in substantial amounts in household and industrial cleaners.

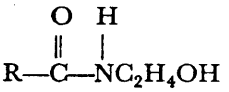
(d) Polyoxyethylene alkylamines



prepared by the reaction of amines with ethylene oxide. Short-chained varieties tend to be cationic, whereas increase in chain length achieves the nonionic property. The short-chained varieties act as good corrosion inhibitors because of their reaction (cationic + charge) with anionic (−) surfaces in the formation of a protective film.

(e) Polyoxyethylene alkylamides

(1) Alkyl monoethanolamide



Because of the current public interest in the environment, it seems that the Law and Society will dictate that primary biodegradation is insufficient and because ultimate biodegradation is never completely attainable, an environmentally acceptable definition will be accepted.

The reason a concise definition on biodegradability is wanting is that the process is dependent upon a very complex set of variables, to wit:

(1) Concentration and chemical structure of the substrate.
(2) Type of biological system under consideration in terms of aerobic or anaerobic organisms, their viability, and acclimitization characteristics of viable species to substrate.
(3) Concentration and composition of the organisms.
(4) Dispersion and/or coagulation aspects of the system under study including sorption characteristics.
(5) Other physical and chemical factors such as agitation rate, pH, temperature, growth promoters or inhibitors or lytic agents, and previous history of the biodegrading organism.

Therefore, the significance attached to the results obtained in studies on biodegradation is dependent upon the intended application. If one purports to represent an enforcement agency or is an environmentalist, one applies the most stringent application of criteria to his definition. Contrariwise, if one is in the role of a devil's advocate, another definition is to be sought. Only experience and history will control our eventual action.

3. TYPES OF SYNTHETIC SURFACTANTS

Synthetic detergents are most frequently composed of two ingredients, organic surfactants and inorganic phosphate builders. These formulations also contain minor amounts of perfumes, optical bleaches, inorganic sulfate, silicate and carboxymethylcellulose. Surfactants are essential to detergency because of the property of lowering of surface tension, so necessary in wetting, dispersion, emulsification, and hardness stability. Builders are usually added to compliment the properties of surfactants or equally play the role of a diluent. The water-soluble surfactants employed in synthetic detergents comprise four different types,[3] amphoteric, nonionic, anionic, and cationic.

3.1. Amphoteric Surfactants

According to Osipow,[4] amphoteric surfactants ionize in solution with either a positive or negative charge on the long-chain ion dependent upon pH. An example of this surfactant is the long chain amino acid which is cationic in acid solution and anionic in basic solution:

$$RNHCH_2COOH \xrightarrow{HCl} (RNH_2CH_2COOH)^+Cl^-$$

where R is the long-chain alkyl group and

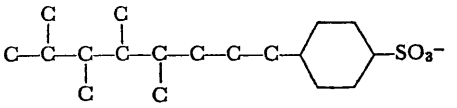
$$RNHCH_2COOH \xrightarrow{NaOH} (RNHCH_2COO)^-Na^+$$

3.3. Cationic Surfactants

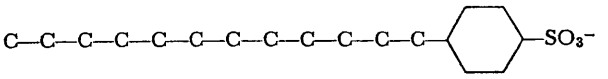
These types ionize in solution with a positive charge. The two major groups are the amines and quaternary ammonium compounds. Because of their germicidal nature in aqueous systems they are not generally biodegradable.

3.4. Anionic Surfactants

(a) Anionic surfactants comprise the bulk of the present day market. These compounds ionize with a negative charge on the long-chain ion. Prior to 1965, the ABS compound was employed in detergent preparations with a molecular structure of the type:



Because of its refractory nature, i.e. resistance to biodegradation, this compound was replaced by the present variety of alkyl benzene sulfonate (LAS) or linear dodecyl benzene sulfonate with a typical structure of the type



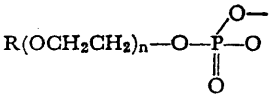
which is believed to be less resistive to biodegradation. Typical compositions of the commercially available packaged variety are:

	%
Organic surfactant	10–40
Sodium tripolyphosphate	25–50
Sodium sulfate	5–10
CMC	1
Sodium silicate	2–10
Optical bleach	Trace

Laundering concentrations of LAS usually contain 200 to 600 parts per million (ppm) of surfactant. Optimum detergency occurs at about pH 10 to 11 and can be controlled by the buffering capacity of phosphate, carbonate, or silicate. Phosphates and silicates also function as water softeners and suspending agents. CMC (carboxymethylcellulose) is present to prevent soil redeposition.

(b) Phosphated esters

(1) Monoester



(2) Diester $[R(OCH_2CH_2)_nO]_2—P—O^-$

(c) Soap $C_{17}H_{35}COONa$ (sodium stearate)

(2) Alkyl diethanolamide $R—\overset{\overset{O}{\|}}{C}N(C_2H_4OH)_2$

usage is dependent upon both the number of oxyethylene groups and other structural attributes of the molecule such as emulsifiers in mineral oils and petroleum fractions.

(f) Alkyl dimethyl amine oxide $R—\overset{\overset{CH_3}{|}}{\underset{\underset{CH_3}{|}}{N}} \rightarrow O$

In (a) through (f), R = C_8–C_{18} Alkyl Chain.

(g) Polyoxyethylene mercaptans $C_{12}H_{25}S(CH_2CH_2O)_nH$

prepared by addition of alkyl mercaptans to ethylene oxide with major use only in shampoos as a result of door and perhaps an unbeknownst health hazard.

(h) Polyol surfactants

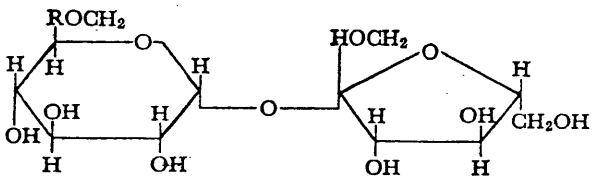
(1) Monoester of ethylene glycol $C_{15}H_{31}COO(CH_2CH_2O)OCC_{15}H_{31}$

essentially complex mixtures because of esterification problems, poor quality control of fatty acid composition, uncertainty in extent of ether linkage, and presence of oxyalkylene chain lengths. They may be used as emulsifiers, solubilizers, wetting agents, detergents, lubricants, and plasticizers.

(i) Block polymers $HO(CH_2CH_2O)_x(CH_3CH_2CH_2O)_y(CH_2CH_2O)^2H$

prepared by addition of polyoxyethylene to both ends of a polyoxypropylene chain and with both ends of this resulting molecule terminating in hydroxide groups.

(j) Sucrose esters[6]



prepared from an alcoholysis reaction of a methyl ester of fatty acid with sucrose employing potassium carbonate as a catalyst. These surfactants could be a good 'bet' as our replacement if third-generation surfactants come into being.

Sucrose:

$$C_{12}H_{22}O_{11} + 2{\cdot}98\ O_2 + 1{\cdot}72\ NH_3 \rightarrow 1{\cdot}72\ C_5H_{11}O_4N + 3{\cdot}40\ CO_2 + 4{\cdot}15\ H_2O \quad (5)$$

Amino Acids

Alanine:

$$C_2H_5O_2N + 1{\cdot}44\ O_2 \rightarrow 0{\cdot}32\ C_5H_9O_3N + 1{\cdot}40\ CO_2 + 1{\cdot}04\ H_2O + 0{\cdot}68\ NH_3 \quad (6)$$

Glutamic Acid:

$$C_5H_9O_4N + 2{\cdot}02\ O_2 \rightarrow 0{\cdot}48\ C_5H_7O_2N + 2{\cdot}60\ CO_2 + 2{\cdot}04\ H_2O + 0{\cdot}52\ NH_3 \quad (7)$$

Fatty Acids

Acetic Acid:

$$C_2H_4O_2 + 0{\cdot}84\ O_2 + 0{\cdot}21\ NH_3 \rightarrow 0{\cdot}21\ C_5H_7O_2N + 0{\cdot}95\ CO_2 + 1{\cdot}58\ H_2O \quad (8)$$

Propionic Acid:

$$C_3H_6O_2 + 1{\cdot}47\ O_2 + 0{\cdot}38\ NH_3 \rightarrow 0{\cdot}38\ C_5H_9O_3N + 1{\cdot}10\ CO_2 + 1{\cdot}86\ H_2O \quad (9)$$

Each of the equations (4–9) indicate that about one or more moles of CO_2 is generated per mole of substrate. However, there is the tacit assumption that the organic substrates do not create metabolic blocks and that the elemental oxygen is the terminal oxidant with complete assimilation.

It is known that substrate oxidation assimilation processes are incomplete. It was early discovered that washed suspensions of *Escherichia coli* and *E. alcaligenes* oxidized lactate, pyruvate, and acetate to 2/3, 3/5, and 3/4 of completion respectively; whereas, *E. coli* oxidizes glucose to the same extent as lactate and takes formate to an unexpected completion.[14] Giesberger[15] has advanced the theory that incomplete oxidation is associated with assimilation in that the CO_2 output + O_2 uptake of various compounds studied are in close agreement with his interpretation of the respiratory quotient. Clifton[16] recognized that *B. calco-acetica* without inhibitors oxidized acetate and butyrate to about 2/3 completion, whereas the cells poisoned with $1{\cdot}6 \times 10^{-3}$ M sodium azide or 2,4-dinitrophenol at a concentration of 4×10^{-3} M effected complete oxidation, concluding that these poisons inhibit the assimilatory process without decreasing the rate of oxidation. Stumm-Zollinger[17] has recently shown that catabolite repression and the resulting substrate utilization are observed in heterogeneous bacterial populations. The report is most exhaustive and presents:

(1) concurrent substrate utilization and growth on both substrates simultaneously (glucose plus benzoate),
(2) sequential substrate elimination accompanied by diauxic growth as a result of inhibition of enzymatic activity (glucose plus galactose), and
(3) sequential substrate utilization accompanied by diauxic growth caused by repression of enzyme formation (glucose plus L-phenylalanine, benzoate plus L-phenylalanine).

Porges[18a,b] indicates that endogenous respiration must be evaluated in terms of availability or non-availability of food, and that synthesis of cellular mass lessens CO_2 formation. In one study, sludge plus 200 parts per million (ppm) of glucose gave 190 ppm of CO_2 in 5 hours, but only 130 ppm in the absence of glucose. However, the theoretical amount of CO_2 from 200 ppm of glucose is

prepared by reacting an alkali metal, amine, or ammonia with a fatty acid. Soaps lost their attractiveness as surfactants because of their precipitation in hard water (greater than 100 ppm as $CaCO_3$) and because of their rapid hydrolysis in unbuffered solutions at high temperatures.

4. THEORETICAL CONSIDERATIONS

Biodegradation has been defined in Section 2. The real significance of this term is related to some form of utilization of energy and the rate at which this energy is expended in terms of growth. That micro-organisms play a substantial role in the regulation of substrate degradation has been shown in excellent accounts of Kluyver and van Niel,[7] and Salton,[8] and Kluyver.[9]

The specific manner through which a substrate is utilized either in a sewage treatment plant or via any degradation route in nature is termed oxidative assimilation.[10] The whole process can best be thought of as two parts; one being the energy or oxidation process and the other the synthesis or assimilation process. In synthesis, a rearrangement occurs wherein there is direct use of the substrate components to produce new cellular material. To effect synthesis, oxidation is required. The energy or fuel is derived from oxidation of the substrate(s). Both reactions occur within the organism at the same time and are linked. Details of the process have been considered elsewhere.[11] The net result of the oxidative assimilation process is to increase cellular mass due to continued reproduction. The mass continues to increase until the food supply becomes limited. Excellent discussions of the relationships among substrate concentration, growth rate and respiration rate are available.[12, 13] Upon exhaustion of the source of energy, endogenous respiration takes place with a decrease in cellular mass, due to oxidative assimilation or 'cannibalism.' During growth the substrate acts as if of internal (endogenous) origin and during decay of external (exogenous) origin. Endogenous respiration may occur during the exogenous removal process wherein both internal and external oxidative assimilation occurs. The significance of these relationships has a bearing on engineering design standards for aerobic biological treatment and demands a knowledge of growth rates, cell yields, oxygen demand, and substrate utilization.

The generalized equations employed to represent the oxidative assimilative reactions are similar to equation (1) in the form of:

$$\text{substrate formula} + O_2 + NH_3 \rightarrow \text{protoplasm} + CO_2 + H_2O \qquad (2)$$

where protoplasm composition to best balance equation is used or

$$\text{Organic matter} + O_2 + NH_3 + \text{other salts} \xrightarrow{\text{bacteria}} \text{bacteria} + CO_2 + H_2O \qquad (3)$$

Typical equations for specific substrate types are:

Carbohydrates

Glucose:

$$C_6H_{12}O_6 + 1{\cdot}26\ O_2 + 0{\cdot}96\ NH_3 \rightarrow 0{\cdot}96\ C_5H_{11}O_4N + 1{\cdot}20\ CO_2 + 2{\cdot}16\ H_2O \qquad (4)$$

where:

A = COD of substrate converted into energy per net COD of cells synthesized

B = total COD of active cells requiring maintenance energy per COD of cells synthesized

ΔF_r = free energy per COD of substrate converted to energy

ΔF_s = ATP free energy required for net synthesis of cells as COD

ΔF_m = ATP free energy required for maintenance of active cells as COD

k_1 = transfer efficiency of substrate energy to ATP energy

Equation (13) shows that the decrease in available energy of the substrate times the efficiency of conversion of substrate energy to ATP energy is equal to the ATP energy required for synthesis and maintenance. This approach shows that the ATP energy for synthesis, ΔF_s, combines two energy relationships. In the first step energy is required or liberated depending upon the original energy state of the substrate, to bring the cell carbon source to an intermediate level which is common to all organisms. In this model, pyruvate was selected as the synthesis intermediate. The second step comprises conversion of the pyruvate intermediate into cellular mass. The total available energy in the synthesis becomes:

$$\Delta F_s = \frac{\Delta F_P}{k_2^n} + \Delta F_c \tag{14}$$

where

ΔF_P = ATP free energy for conversion of COD of cell carbon source into COD of pyruvate

ΔF_c = ATP free energy of conversion of COD of pyruvate into COD of cells

k_2 = transfer efficiency of ATP energy for conversion of cell carbon source to pyruvate

n = constant

The endogenous or maintenance energy term, ΔF_m, was considered insignificant and neglected and ΔF_c was evaluated as 932 to produce a final expression of:

$$-k_1 A \Delta F_r = \frac{\Delta F_P}{k_2^n} + 932 \tag{15}$$

Though the relationship between energy usage in synthesis by the route of mass transfer is quantified, there is an air of hesitancy with respect to the validity of these rigorous approaches. Criticism of the various schools of thought is epitomized in a recent paper.[24] These authors conclude that the application of thermodynamics to bacterial synthesis is without merit. Specifically, it is shown that biological systems are 'open systems' but the thermodynamic systems are 'closed systems'. Hence, the boundaries in an open system lack 'definition' for application of thermodynamics. A further criticism is that the

284 ppm showing that the net CO_2 production was only about 20% of theoretical. The above observations are consistent with some very recent studies by Wayman[19] on the aerobic biodegradation of sucrose esters and ethoxylated alkanes.

4.1. Energy Considerations

Relating certain thermodynamic concepts of mass transfer to establish conservation of energy has been attempted though with little conspicuous success. The most lucid accounts are those of McKinney,[20a,b] Servizi and Bogan,[21a,b] and McCarty.[22a,b]

Most of the concepts of these three schools of thought have been summarized.[11] It has been proposed[20a] that the energy available to the microorganisms is proportional to the change in heat energy liberated during metabolism. This quantity of energy is defined as the biological energy as opposed to any established thermodynamic relationships as might exist. Certain mathematical treatments purport to show that a fixed relationship exists between energy and synthesis irrespective of the type of substrate. However this hypothesis was modified as energy controlled synthesis reactions are substrate dependent.[23] In another paper[21a] it was suggested that cell yield was proportional to the amount of adenosine phosphate (ATP) formed per amount of substrate, ATP being a compound yielding or consuming high energy and proportional to the free energy released during oxidation or the available Gibbs free energy. A cell yield equation was devised in terms of substrate oxidation as follows:

$$Y = k_1 N_{ATP} \tag{10}$$

$$N_{ATP} = -k_2 \Delta F^{\circ}_{OX} \tag{11}$$

$$Y = k_1 k_2 k_3 Z \tag{12}$$

where:

Y = synthesis, in g of cell tissue per mole substrate utilized

N_{ATP} = moles ATP per mole substrate

ΔF°_{OX} = standard free energy of oxidation

k_1 = proportionality const. in g. cells per mole ATP

k_2 = proportionality const. in moles ATP per kcal

k_3 = proportionality const. in kcal. per mole O_2.

These authors[21b] extended their arguments to multicomponent substrates by summation of the individual contribution of a compound in the complex mixture.

A very elegant approach based upon a constant amount of bacterial mass per mole of ATP formed is described in terms of the equation:[22a]

$$-k_1 A \Delta F_r = \Delta F_s + B \Delta F_m \tag{13}$$

on a synthetic medium is linearly related to substrate concentration if that concentration is limiting. Using the above with the assumption that cellular yield in a culture limited by the concentration of one nutrient is not a function of the growth rates yields the equation:[43]

$$\frac{dX}{dt} = Y\,dS/dt \tag{16}$$

where

X = cell conc.

S = substrate conc. (limiting)

t = time

Y = yield coeff. $\left(\frac{\text{mass cells}}{\text{mass substrate}}\right)$

If cells are maintained without growth, a portion of the substrate limitation is destroyed (utilized) as

$$\frac{dX}{dt} + aX = Y\frac{dS}{dt} = F \tag{17}$$

where

aX = amount of substrate for respiration

a = dimensions of reciprocal time or specific maintenance rate

$Y\frac{dS}{dt}$ = feeding rate

F = yield coeff. × rate of consumption of carbon source

After integrating (17) and solving for X;

$$X = \frac{F}{a} - \frac{F}{a}e^{-at} + X_0 e^{-at} \tag{18}$$

where a can be determined by measurement of the maximum population attained at fixed feeding rate, F:

$$X_t \to \infty = F/a \tag{19}$$

or equation 16 can be solved simultaneously in X_0, X_1, and X_2 corresponding to t_0, t_1, and t_2 such that $t_2 - t_0 = 2/(t_1 - t_0)$ and:

$$a = F\left(\frac{2X_1 - X_0 - X_2}{X_1 - X_0 X_2}\right) \tag{20}$$

If a specific dilution rate (D) is defined for continuous culture growth, or $D = \left(\frac{f}{v}\right)\frac{\text{flow rate}}{\text{volume of culture}}$; X_{max} = conc. of bacteria if $a = 0$; then:

$$X = \frac{Y_s}{1 + a/D} = \frac{X_{max}}{1 + a/D} \tag{21}$$

assumption for growth as a function only of the amount of energy potentially available from the substrate is erroneous. Calculations are given for the change in free energy through electron transport for two possible hydrogen acceptors, both yielding excess 'free energy' for the actual amount of ATP production. The writer believes the criticism levelled at this rigorous approach is valid and application of equations (10–15) should be done with caution.

4.2. Kinetic Considerations

Concepts responsible for behavior and growth are well known and have been adequately discussed by Monod.[25] It is indicated that growth is dependent upon the size of a bacterial population in terms of three constants, the growth lag, growth rate, and ultimate population supportable by some growth medium.[26] In both batch systems and continuous culture systems controlling growth factors depend on temperature[27, 28] and concentration[29] among more limiting factors such as amino acids (tryptophan,[30] arginine, proline, histidine[31]), an energy source,[32, 33, 34] a nitrogen source such as ammonia,[33] and phosphate.[33]

Several authors believe that microbial growth rate is subject to stimulatory, or inhibitory, effects based upon nutritional or vitamin requirements.[35] Thirty-four varieties of bacteria isolated from sea water required only inorganic ions common to sea water and carbon sources such as lactic, citric, or succinic acids.[36] Other studies indicate that bacteria isolated from brewery solutions depend upon the vitamins pantothenic acid, nicotinic acid, riboflavin, and thiamine.[37] More specific details on microbial growth and metabolism is found in an excellent review paper in terms of enzymes, carbohydrates, organic acids, nitrogen, vitamins, coenzymes, and inorganic ions by Delwiche.[38]

4.3. Rate Expressions for Bacterial Growth

Some reactions consume carbon and an energy source and effect a coupling of oxidation to growth. Sherris[39] indicates that motility requires an expenditure of ATP, and the accumulation of solutes to a higher concentration in the cell than in the growth medium also requires an initial expenditure of energy.[40, 41] When the cell is permeable to the solute, the cell expends metabolic energy continuously to maintain a higher intracellular than extracellular concentration. The hydrolytic activity of proteins and nucleic acid in the production and their monomers and resynthesis of the large molecules from monomers requires depletion of ATP. McGrew and Mallette[42] have generalized various opinions of microbiologists to account for the necessity of metabolic energy to meet the demand of chemical and physical wear and tear and define this condition to maintain status quo as maintenance energy. This definition was later modified[43] to coincide with an experimentally measurable quantity, the specific maintenance or the respiration coefficient. This maintenance requirement or energy consumption entails removal of substrate without growth. The efforts of Monod[44] and Hinshelwood[45] show that cellular growth

In another study,[17] substrate utilization rate was evaluated by two types of experiments: (1) substrate elimination measured as a function of time in cultures of constant bacterial and enzymatic concentration (enzyme activity); (2) growth and substrate elimination measured in terms of time in cultures of logarithmically increasing bacterial and enzymatic concentration (enzyme formation). A combination of the exponential growth equation, $dB/dt = \mu B$, and the equation describing the relationship between bacterial growth and substrate utilization, $-dB/dS = Y$, gives an expression for the rate of substrate utilization in terms of growth:

$$-dS/dt = (\mu/Y)B \tag{26}$$

where

B = conc. of organisms (dry weight of cells/unit vol.)

Y = yield const.; fraction of substrate converted to bacterial mass

μ = spec. rate const. (time^{-1})

S = substrate conc.

The specific growth rate constant, μ, can be kept constant, usually at a maximum, if during the experiment the substrate is maintained at concentrations higher than the growth-rate limiting concentration. If Y is also assumed to be constant (e.g., $Y \approx 0{\cdot}5$), equation 26 predicts that the rate of substrate elimination is proportional to B. The substrate utilization rate is constant, if B is kept constant experimentally by making it large in comparison to the substrate. In such systems, substrate elimination is measured during a fraction of the generation time only. When the initial bacterial concentration, B_0, is small in comparison with the substrate concentration, the rate of substrate elimination increases logarithmically with time with logarithmic increase in B according to:

$$-dS/dt = (\mu/Y)B_0 e^{\mu t} \tag{27}$$

Integration of equation 27 yields:

$$-\Delta S = (B_0/Y)(1 + e^{\mu t}) \tag{28}$$

where $-\Delta S$ is the substrate utilized at any time. A plot of $\log(-\Delta S)$ versus time gives a linear relationship for $t > 0$. The slope is related to the specific growth rate constant ($2{\cdot}3\mu$). The plot of these data shows the rate of bacterial growth as an approximate measure of the rate of enzyme formation, when determined over a period of several generation times.

It has also been shown[12] that in batch studies it is very difficult to establish a relationship between substrate concentration and growth rate for a given type of micro-organism. As long as the substrate is in ample supply, growth will proceed at a constant maximum rate according to the equation:

$$X = X_0 e^{k_1 t} \tag{29}$$

or

$$\ln(X/X_0) = k_1 t \tag{30}$$

or by taking the reciprocal of equation (21):

$$1/X = \frac{a}{X_{max}} \frac{1}{D} + \frac{1}{X_{max}} \tag{22}$$

which states that if the reciprocal of steady state turbidity (bacterial conc.) is plotted against the reciprocal of the dilution rate, a linear function is obtained with an ordinate intercept of $1/X_{max}$ and a slope of a/X_{max}.

Schulze[46] has defined an equation for continuous flow cultures applied to activated sludge systems cell concentration in a reaction as:

$$\frac{dX}{dt} = k_1 X - DX = X(k_1 - D) \tag{23}$$

where k_1 = spec. growth rate = $\dfrac{\text{cellular mass}}{\text{unit time}}$

$$D = f/v = 1/t_r;\ 1/t_r = \text{mean retention time}$$

The same writer has shown a very interesting relationship between k_1 and the respiration rate. For a series of oxygen consumption rates, K_r, on Warburg measurements for a series of D-values, a plot of k_r versus D indicated that $D = k_1$ for values up to 0·76 showing that the respiration rate was directly proportional to the growth rate, k_1. A similar result has been attained by Herbert[47] where

$$k_r = b + dk_1 \tag{24}$$

where k_r = oxygen consumption rate, mg O_2 per g cell weight per hour

$b = Y$ — intercept, for k_r at $k_1 = 0$

d = slope = mg O_2 consumed per gram cell weight formed

For experimental values obtained from methods of least squares equation 24 becomes:

$$k_r = 16 + 770\, k_1$$

or

$$k_1 = \frac{k_r - 16}{770} \tag{25}$$

In general these data showed that 0·77 g of oxygen are consumed per gram cell weight formed and that respiration rate of bacterial cells can vary over a large range from 16 mg O_2 per gram of cell weight per hour at $k_1 = 0$, i.e. when the cells are not growing, to about 500 mg O_2 per gram cell weight per hour at the maximum growth rate, k_m. Thus, isolated determinations of the respiration rate in bacterial cultures have little significance. The data further show that k_r reached its maximum value at $D = k_m$ and that k_r was independent of D at D-values larger than k_m. This supports the concept that the cells continued to grow at a rate equal to k_m when D exceeded k_m. Equation 25 can be employed to compute growth rates from respiration rates if the endogenous rate and slope are known.

Equation 35 gives decrease in substrate as function of time for bacterial growth for a completely mixed batch system. This equation is distinguished from Monod's in that here the integration is made in terms of variable substrate and not variable bacterial mass. Equation 35 may be employed to predict the decrease in concentration of a substrate due to biodegradation as a function of flow time downstream from a point of waste discharge. M_0 and C_0 are concentration of organisms and substrate at point of discharge, and M and C are similar values at a distance equal to the average stream velocity times the time of flow. Explicit equations can be propounded for substrate concentration and viable organisms as functions of time. The equations cannot be solved analytically because $M = f(C)$, where C is a complex function of time. The equations are solvable by numerical techniques.[51]

Kinetic considerations have been applied to anaerobic fermentation reactions.[52] A rather detailed account is given for the three-step anaerobic process of (1) hydrolysis of complex material, (2) acid production, and (3) methane fermentation.[53] An equation is presented for a continuous flow system similar to equation 32 except dC/dt is replaced by dF/dt or

$$dM/dt = a\left(\frac{dF}{dt}\right) - bM \tag{36}$$

where dF/dt = rate of waste utilization per unit volume of digester,

mass/vol–time

The expression dF/dt, the volumetric waste assimilation is related to the concentration of waste in the digester. The differential is similar to the Monod relation for growth limiting nutrient.[44]

$$dF/dt = \frac{kMS}{K_s + S} \tag{37}$$

where

S = waste concentration in the reactor, mass/volume

k = max. rate of waste utilization per unit weight of micro-organisms occurring at high waste conc., time^{-1}

K_s = half velocity coeff. equal to the waste conc. when dF/dt is equal to one-half of the max. rate, k mass/volume

Equations 36 and 37 can be combined to produce

$$\frac{(dM/dt)}{M} = \frac{akS}{K_s + S} - b \tag{38}$$

where the quantity $(dM/dt)/M$ is equal to the net growth per unit weight of micro-organisms per unit time, and is designated as the net specific growth rate.

In continuous flow systems, the mass of micro-organisms attains a constant value at steady state. Steady state is the condition where the rate at which

and

$$k_1 = 1/X\frac{dX}{dt} = \frac{\ln 2}{g} \tag{31}$$

where all terms are as previously defined except g which represents mean doubling time or generation time. k_1 becomes dependent on substrate concentration only when most of the substrate has been consumed. At this point the culture has practically reached its maximum density and the remaining substrate concentration changes rapidly, leaving no time for the measurement of growth rates at any specific substrate level. The problems associated with batch studies are obviated by studies using continuous culture under steady state conditions enumerated above.

Stratton and McCarty[48] have recently applied bacterial growth kinetics to predict effects of nitrification on the dissolved oxygen balance of streams. This work and several others[49, 50] employ an equation of the form:

$$\frac{dM}{dt} = -a\frac{dC}{dt} - bM \tag{32}$$

where

dM/dt = rate of change of bacterial mass, mg/l-day
dC/dt = rate of change of substrate conc., mg/l-day
b = organism decay parameter, day^{-1}
M = total bacterial mass, mg/l
a = yield const.

If C represents a single nutrient limiting bacterial growth, then

$$dC/dt = -kMC/(K_s + C) \tag{33}$$

where

K_s = half velocity or saturation const., mg/l
k = substrate utiliz. const., mg/day/mg organisms

If the organism decay term, b, is neglected in equation 32, then integration and simplifying produces an equation for the total mass of viable organisms as a function of the quantity of substrate oxidized: (completely mixed batch cultures)

$$M = M_0 + a(C_0 - C) \tag{34}$$

Equations 33 and 34 can be combined to give an equation relating time and substrate concentration in terms of kinetic parameters:

$$-1/K\left\{\left(\frac{-K_s}{M_0 + aC_0} - \frac{1}{a}\right)\log e(M_0 + aC_0 - aC) + \left(\frac{K_s}{M_0 + aC_0}\right)\log e\left(\frac{CM_0}{C_0}\right) + \left(\frac{1}{a}\right)\log e\, M_0\right\} = t \tag{35}$$

but water is in excess and the reaction rate depends only on the concentration of sucrose or

$$-\frac{dC_{sucrose}}{dt} = K_1 C_{sucrose} \tag{47}$$

In the presence of bacterial species, kinetics are very complex. For example, the biological oxidation of glucose

$$C_6H_{12}O_6 \xrightarrow{\text{bacteria}} 6\ CO_2 + 6\ H_2O \tag{48}$$

The rate constant is not a simple function of glucose concentration because of the formation of intermediate products via the Krebs Cycle.

In biochemical oxidations, the rate of depletion of a substrate depends upon the product of substrate concentration and bacterial concentration each raised to some power. Thus,

$$-\frac{dC_{substrate}}{dt} = kC^a_{substrate} \cdot C^b_{bacteria} \tag{49}$$

if $a = b = 1$

Second-order equations like 49 have been applied to BOD and oxygen uptake involved in the activated sludge process and produce equations of the form[54, 55]

$$Y = L - \frac{b + L}{(b/L)\, e^{K(L+b)t} + 1} \tag{50}$$

where

Y = oxygen uptake at time t

L = ultimate oxygen demand

$K = K_1 K_2 K_s$ = proportionality constants

$$b = \frac{B(\text{initial conc. bacteria})}{K_2 K_s}$$

Recently, Wayman and Burt[56] have derived a second order kinetics equation which can be employed to complex conditions of bio-oxidation with the plot of two simple curves measurable experimentally (see Appendix 1).

5. TEST METHODS

Methods to assess biodegradation are arbitrary at best. In order to make an intelligent interpretation of results, some applicable definition within limits is required. A range of definitions on biodegradability has already been presented in another part of this paper.

The most comprehensive discussion of test methods available can be found in Swisher's recent book.[1] That test methods are complex can be found in his consideration of test variables, e.g.

Micro-organisms—nature, acclimation, concentration.
Food—nature, concentration.

micro-organisms are wasted from the system must equal the net microbial growth rate, dM/dt. With time given in days, the daily net specific growth rate $\Delta M/\Delta T/M$, is the reciprocal of the biological solids retention time, SRT:

$$\text{SRT} = \frac{M_T}{(\Delta M/\Delta T)_T} \tag{39}$$

where M_T = total weight of active microbial solids in the system, mass $(\Delta M/\Delta T)_T$ = total quantity of active microbial solids withdrawn daily, including those solids wasted and those lost in the effluent, mass/time. Thus, SRT, is the average retention time of micro-organisms in the waste treatment system and is similar to the sludge age concept of activated sludge. The efficiency of the waste utilization is defined as:

$$E = \frac{S - S_0}{S_0} \times 100 \tag{40}$$

where

E = efficiency of waste treatment, %

S_0 = influent waste conc., mass/vol.

S = effluent waste conc., mass/vol.

4.4. Reaction Rate Constants

There are many types of reactions consistent with studies on biodegradation. This discussion will be limited to first- and second-order rate constants.

A reaction of the type

$$A \xrightarrow{K_1} \text{Products} \tag{41}$$

is expressible by a rate equation of the form

$$\frac{-dC_A}{dt} = K_1 C_A \tag{42}$$

or

$$\frac{dC \text{ products}}{dt} = K_1 C_A \tag{43}$$

where K_1 = first-order rate constant; C_A = conc. of A at time t.

A reaction of the form

$$A + B \xrightarrow{K_2} \text{Products} \tag{44}$$

has a rate equation of the form

$$\frac{-dC_A}{dt} = \frac{-dC_B}{dt} = K_2 C_A C_B \tag{45}$$

where K_2 is a second-order rate constant. At first glance the simple hydrolysis of sucrose appears as second order

$$C_{12}H_{22}O_{11} + H_2O \rightarrow \text{fructose} + \text{glucose} \tag{46}$$

Activated sludge from a sewage treatment plant processing mainly domestic wastes, the surfactant to be tested, and a synthetic sewage of composition

Glucose	13·0 g
Nutrient broth	13·0 g
Beef extract	13·0 g
K_2HPO_4	13·0 g
$(NH_4)_2SO_4$	2·5 g
City tap water	1 litre

are mixed in a specially designed vessel. The mixture is brought to a steady-state and aerated for 23 hours, allowed to settle for 1 hour, supernatant liquid is withdrawn, and an equal amount of synthetic sewage containing 20 mg/l of test surfactant is added to the settled sludge to maintain constancy of volume, such cycle being repeated daily. The test is run for a minimum of 15 days which includes 5 days of acclimatization of organisms, 3 days of equilibration of surfactant at 20 mg/l, and at least 7 days of level (steady-state) operation. Samples are tested by the MBAS method. The disadvantage of this method is that biodegradation is measured over a period of time that exceeds the normal retention time a surfactant is found in an activated sludge system in sewage plants.

A summary of the efficacy of a round-robin test program employing both the presumptive and confirmatory tests is shown in Table 1.

5.2. The River Die-Away Method

This method has been discussed in many recent papers since it was first used.[61] The method involves the inoculation of a source of culture in river water with added surfactant and subsequent measurement of the decay of the surfactant against that in a blank, using the MBAS method. It has been employed in aerobic and anaerobic studies.[62] The method is economical, rapid, simple, but has several disadvantages, e.g., variations from time to time in bacterial count between different rivers, variations in viability and bacterial composition, and variations in nutrients, inhibitors, and growth promoters. Therefore, the attempts of various investigators to compare results from different locales are highly questionable if not impossible.

Table 2 lists results of a screening study for SDA procedures and river die-away tests on nonionic surfactants.[65]

5.3. Standard Method in the United Kingdom

The British Standing Technical Committee[58] has also adopted a standard method similar to the river die-away test, but with some improvements.

Results of this test are expressed as a percent reduction of the initial concentration added. The test is run for a maximum of five days. This test employs a standard seed and medium which overcomes some of the limitations listed for the SDA 'shake flask' procedure. A solution containing 10 mg/l of test surfactant is added to BOD dilution water inoculated with 30 mg of air-dried activated sludge/litre, said solution being stirred gently in the dark at

Toxic or bacteriostatic agents.
Oxygen.
Temperature.
Surfactant concentration.
Analytical method.

The types of generalized tests employed to date are usually classified as River Die-Away, Trickling Filter, Activated Sludge, Oxygen-Uptake or CO_2 Generation, or Field Test Results. Some of these methods have legal significance[57] and others at least are approved by the government.[58]

5.1. SDA Procedure

The Soap and Detergent Association (SDA) has promulgated a test procedure for both ABS and LAS.[59] This procedure involves both a presumptive and a confirmatory test.

In the presumptive test, a compound's biodegradability is tested with respect to a 90 per cent conversion. The advantage of the test is that it is simple and inexpensive. The disadvantage of the test is that it takes 14 days including two, 72-hour adaptive transfers. Micro-organisms are inoculated into a Shake Culture Flask containing a well defined chemical growth medium of the following composition:

NH_4Cl	3·0 g
K_2HPO_4	1·0 g
$MgSO_4 \cdot 7\ H_2O$	0·25 g
KCl	0·25 g
$FeSO_4 \cdot 7\ H_2O$	0·002 g
Yeast extract	0·30 g
Water (distilled or deionized)	1·0 litre

and 30 mg/l test surfactant. One control flask is employed as a blank which contains all test ingredients except the test surfactant. Flasks are stoppered with cotton or plugs to reduce evaporation and contamination, and aerated by a reciprocating shaker at 128 two-four inch strokes/minute at a temperature of 25 ± 3° C. Two 72-hour adaptive transfers are made prior to the 8-day test. One ml of the 72-hour culture is transferred to 100 ml of a fresh medium plus surfactant to effect transfer from blank to blank or test surfactant I to test surfactant I, etc. Samples are taken at zero time (immediately after inoculation and mixing of the flask and on the 7th and 8th days. If biodegradation exceeds 90 per cent as measured by the standardized methylene blue active substance (MBAS) analysis, no further testing is warranted. The obvious limitation of this method is that it does not measure rate per day or per hour, but only total degradation in one week.

If the surfactant used up in the presumptive test is less than 80 per cent, it is considered not to be biodegradable; if the surfactant falls within 80 to 90 per cent, its biodegradability must be confirmed. In the confirmation test, the surfactant must be at least 90 per cent biodegradable to pass. The test employs a modification of the Semi-Continuous Activated Sludge Test.[60]

TABLE 2. FIRST COOPERATIVE SCREENING STUDY (Soap and Detergent Assoc., ref. 65)

Surfactants tested	Semi-continuous actived-sludge degradation†		River die-away median degradations‡						Shake culture median degradations using initially unacclimated seed§					
			Foam loss %		CTAS-MBAS loss %		Surface tension, dynes/cm²		Foam loss %		CTAS-MBAS loss %		Surface tension dynes/cm²	
	Residual effluent foam ml/50 ml	CTAS-MBAS loss %	Week 2	Week 4	Week 2	Week 4	Week 2	Week 4	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14
Linear alkylate sulfonate														
Commercial composite	0·0 (Initially 20 ml)	97	99	100	91	93	70	72	88	91	97	95	60	66
Dodecene-1-derived	0·0 (Initially 20 ml)	100	100	100	99	99	72	71	90	100	96	99	58	70
Tetrapropylene-derived ABS	3·5 (Initially 20 ml)	66	80	93	68	68	59	69	10	7	17	19	42	41
Linear primary alcohol ethoxylate	0·0 (Initially 15 ml)	100	100	100	100	100	70	71	98	100	100	100	70	71
Linear secondary alcohol ethoxylate	1·8 (Initially 15 ml)	94	81	98	93	100	63	71	50	62	88	93	60	61
Random linear nonylphenol ethoxylate	5·0 (Initially 15 ml)	94	58	92	77	100	47	59	0	0	57	68	42	42
Nonrandom linear decylphenol ethoxylate	2·4 (Initially 15 ml)	99	85	99	96	100	63	70	6	6	74	87	50	55
p,t-Octylphenoxynonaethoxy-ethanol	15·0 (Initially 15 ml)	95	43	80	81	100	48	52	0	0	15	44	40	44
Branched tridecyl alcohol ethoxylate	4·0 (Initially 15 ml)	63	10	91	87	100	56	65	0	0	18	31	41	45
Tripropylene-derived nonylphenol ethoxylate	4·8 (Initially 9 ml)	95	92	92	93	98	45	49	0	0	3	19	39	40
Tetrapropylene-derived dodecylphenol ethoxylate	2·0 (Initially 9 ml)	96	55	87	16	98	39	47	8	15	3	0	30	33

† Laboratory activated-sludge units were operated on a 23-hour aeration cycle with degradation measured by cobaltothiocyanate (CTAS) and methylene blue (MBAS) colorimetric procedures and reduction in foaming character of clarified unit effluent. The surfactant and synthetic food were added at the same time in this study. Median data during the fourth operating week are reported. Initial foams were estimated from average of die-away (10 mg/l) and shake-flask (30 mg/1) initial levels.

‡ Initial and weekly samples of the die-away system were analyzed by the colorimetric, foam and surface-tension techniques. The surface tension value reported compares to an initial median value of 43 dynes/cm² Test concentration was 10 mg/l.

§ Seed was obtained from domestic activated-sludge treatment plants and given two adoptive transfers prior to the test period. Surfactant test concentration was 30 mg/l.

TABLE 1. SURFACTANT REMOVED (PERCENT) (Soap and Detergent Assoc., ref. 60)

Sample	Shake flask test					Semicontinuous test				
	Mean	95% Conf. limits	Lower tolerance limit†	Number labs.	Number reps.	Mean	95% Conf. limits	Lower tolerance limit†	Number labs.	Number reps.
1. Dodecene-1 derived LAS	99·5	99·3 to 99·7	98·0	17	113	99·6	99·2 to 99·9	97·1	11	43
2. LAS Composite 1-1	93·5	92·1 to 94·8	86·8	11	52	97·4	95·9 to 98·6	92·3	7	27
3. LAS 3S	95·6	94·5 to 96·5	89·7	15	86	98·3	97·1 to 99·2	93·9	11	43
4. ABS Lot 3	21·5	14·0 to 29·0	<0	13	43	58·2	46·5 to 69·9	9·4	12	12
Unknowns										
5. A	94·5	92·2 to 96·5	88·2	7	23	97·5	95·6 to 98·8	92·5	4	11
6. B	90·0	87·2 to 92·5	82·0	8	25	94·5	92·8 to 96·0	87·8	5	15
7. C	94·0	91·3 to 96·1	87·4	7	25	97·4	95·0 to 99·1	92·4	4	10

† 95% of individual results will fall above this value (95% confidence).

TABLE 3. SUMMARY OF BUNCH-CHAMBERS DIE-AWAY TEST DATA FROM THIRD COOPERATIVE STUDY† (Soap and Detergent Assoc.[65])

Surfactant tested	Median 4th week foam (or MBAS) loss, %	Number of laboratories reporting specified foam (or MBAS) loss level		
		<80%	80%–90%	>90%
Linear alkylate sulfonate (LAS)	94 (91)	0 (0)	0 (2)	4 (3)
Tetrapropylene-derived ABS	<60 (35)	2 (5)	0 (0)	2 (0)
Linear secondary alcohol ethoxylate	85‡	1	3	1
Nonrandom linear C_{10} phenol ethoxylate	75	3	1	0
p,t-Octylphenoxynonaethoxyethanol	<60	4	0	0

† Bunch-Chambers (FWPCA) die-away test system consisted of 20 mg/l surfactant and 50 mg/l yeast extract diluted in a 90/10 mixture of BOD dilution water and settled sewage. After incubating for seven days, the mixture was analyzed and sub-cultures were set up in fresh media and recharged surfactants. The above data were collected at the completion of the fourth die-away period.

‡ Data reported by one laboratory show that higher removals can be obtained by reducing the yeast concentration to 25 mg/l or reducing the surfactant level to 10 mg/l.

5.6. WARBURG OXYGEN UPTAKE METHOD

This method has been used for many years by biochemists. The method involves the inoculation of organisms and surfactant into a system of constant volume with an excess of oxygen. One measures the amount of oxygen depletion in a closed system as a function of time. Any CO_2 evolved in the process is absorbed in a KOH well. The method is subject to a number of drawbacks and is not considered convenient at the present time.[65] Lucid descriptions and reviews of techniques can be found elsewhere.[67, 68, 69] For a very general comprehensive review on respirometry, a recent work by Montgomery[70] should be consulted.

Swisher[1] states that results from oxygen uptake studies may be difficult to interpret because of the need to compare with other parameters during the course of—or at the end of an experiment. Blankenship[71] has also raised substantial questions with respect to the validity of Warburg results; out of 30 runs, 6 did not level off at a definite oxygen value, while the other 24 range in value from 0·1 to 0·9 $\mu l/\mu g$; instability of bacterial species distribution during preliminary propagation of the mixed cultures was mentioned as a possible reason for the variation. In addition to the unreliability of Warburg runs, the equipment is expensive, the length of the runs is of substantial duration, and significant amounts of time are required to reduce the data.

5.7. WAYMAN-YAP PROCEDURE FOR CO_2 PRODUCTION

In recent studies[72] on the rates of ultimate biodegradation of sucrose esters and ethoxylates of alcohols, a method was developed to measure biodegradation in terms of production of $C^{14}O_2$ or untagged CO_2. In this particular procedure (see Fig. 1) to the flask is added surfactant and bacteria in BOD dilution water or other growth media after purging the system with CO_2 free air.

20° ± 1°C for 3 weeks. Samples are removed daily and surfactant concentration remaining is determined by the Longwell and Maniece methylene blue method.

Though the above procedure has been described for anionics, it has also been discussed for application to nonionics using foam measurement and chromatographic techniques.[63, 64] Details of the chromatographic procedure is given elsewhere.[65]

5.4. Official German Test for Anionic Surfactant Biodegradability

This procedure employs the continuous Activated Sludge Procedure and is an official and legal test method under the law.[57] The law requires that the surfactant tested must meet a standard biodegradability of at least 80 per cent. The law specifies the type of equipment to be used in the test.

Sewage containing 20 mg/l of test surfactant is fed into a 3-l aeration chamber with overflow into a 2-l sedimentation chamber. Three hours residence time is allowed to conform to sewage plant practice. Sludge is recirculated from the settler to the aerator by means of an airlift. The clarified overflow from the sedimentation chamber, essentially sewage effluent, is collected daily and the MBAS determined and the percentage reduction recorded. The daily determinations are repeated over a test cycle of 21 days.

The method is disadvantageous because of the length of time for testing and the cost of equipment and labor. However, the test has some real merit over the SDA because it realistically assesses the amount of time activated sludge is in contact with the surfactant, namely 3 hours. Many American plants also have this order of retention time.

5.5. The Bunch-Chambers Method

Recently, an excellent method has been published that permits the amount of biodegradability of any organic compound[66] to be determined.

The purpose of this method is to permit continuous acclimatization of the organism to the test substrate. In effect four consecutive die-away tests are performed, each of 1 week duration. Ninety ml of BOD dilution water containing 5·0 mg of yeast extract and 2·0 mg or other suitable amount of test compound is inoculated with 10 ml of settled sewage. For each test material, three of the above described systems are prepared into three separate 250-ml Erlenmeyer flasks. The flasks are stored loosely capped or with cotton plugs for 7 days. Weekly transfers of 10 ml are made from each flask to another containing 90 ml of BOD dilution water, yeast extract, and surfactant for three weeks. Each subculture is analyzed at the end of 7 days for the amount of test substrate remaining.

The method is simple and inexpensive. However, it is time consuming and measures only the amount of total biodegradation but not the more desirable characteristic, the rate. Table 3 lists results of both anionics and nonionics in a cooperative test program.[65]

disappearance of the original compound. Such measurements do not answer the question of whether or not CO_2 is produced or whether the products formed are more undesirable or toxic than the original substrate.

5.8. Wayman-Burt Method of Bacterial Growth[56]

A complete mathematical model to assess biodegradability using second-order kinetics is given in Appendix I. Essentially the method measures the rate of surfactant degradation in terms of utilization of a substrate by measurement of the rate of bacterial growth (Figs. 2, 4). Bacterial growth of a specific

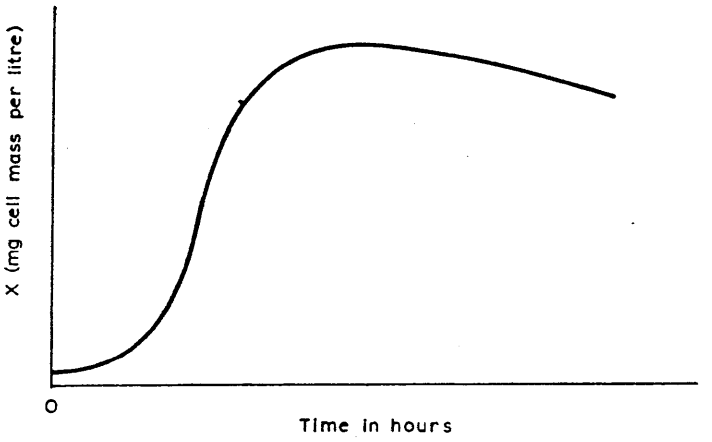


Figure 2

substrate is expressible in terms of an exponential of the form $X_0 e^{\beta T}$ during log growth phase where:

$\beta = AK - R$
X_0 = initial cellular mass
A = substrate concentration
K = rate constant
R = respiration constant

When $A = 0$, die-off occurs at the rate of $-R$. Because growth is not negative, it was decided to use a blank where growth rates are reproducible. The blank selected was water containing 2·0 ppm of bacto yeast extract, 4·0 ppm of urea, and BOD dilution water. Hence, any substrate can be evaluated under similar conditions and compared to the blank and to easily biodegradable or more refractory-type compounds in water. If $K = 0$, the rate of utilization of the substrate by bacteria is independent of the substrate concentration or equivalent to the blank. Positive or negative values are respectively superior

If a tagged compound is employed, complete oxidation will generate $C^{14}O_2$; otherwise only CO_2 is generated in the reaction. The carbon dioxide is purged from the solution with CO_2 free air and conducted to the trap-vial containing ethanolamine and ethanol. The CO_2 produced in the biodegradation reaction is absorbed in ethanolamine-methanol mixture in a counting vial. The amount of $C^{14}O_2$ can be determined by counting its radioactivity by addition of scintillator liquid to the vial. The total amount of CO_2 is determined by reacting amine carbonate with acid, and the CO_2 liberated is absorbed in a

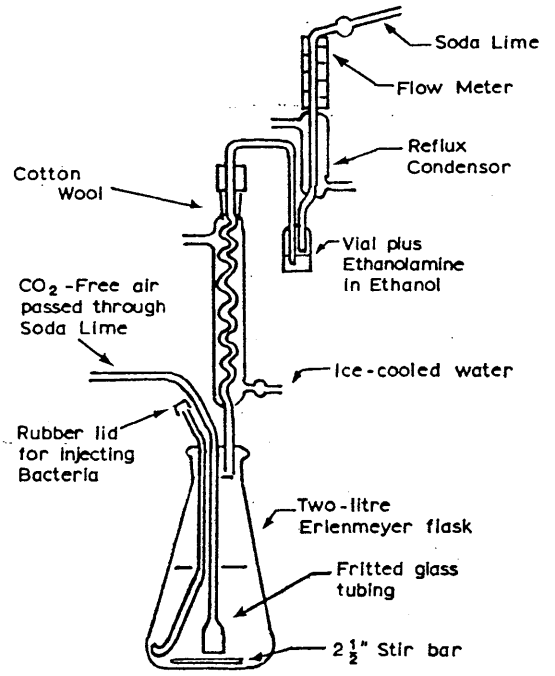


Figure 1

known amount of $Ba(OH)_2$ solution. The excess of $Ba(OH)_2$ is then back titrated with HCl standard.

By sampling the gas stream as a function of time, the rate of production of CO_2 during biological oxidation can be determined for various experimental conditions in the flask. The CO_2 produced can be measured against a blank and contrasted to the amount theoretically possible assuming complete oxidation to CO_2, H_2O, and nutrients.

Results obtained for studies on sucrose ester and ethoxylated alcohols are presented in the next section.

The advantage of this method over others is that it measures whether or not a compound is completely biodegradable to the innocuous substances, CO_2 and H_2O. Other methods for measurement of biodegradation merely measure the

6. RESULTS OF BIODEGRADATION STUDIES

The effects of refractory substances on the impairment of water quality is well documented.[73, 74, 75] The conversion from the so-called 'hard' surfactants to 'biologically soft' types was based upon structural difficulties not amenable to biological oxidation. With the 'hard' types, blockage of beta oxidation was believed to be the result of the quaternary carbon on ABS.[76] Because of the inefficiencies with ABS, the 'big soapers' inaugurated major attempts to improve biological oxidation of commercial surfactants with development of the LAS (linear alkylate sulfonate).[1, 77] Most of the definitive studies on biodegradation of LAS can be found elsewhere.[1] Degradation attains more rapidly when the phenyl group is located nearer the end of a hydrocarbon chain; between alkyl chain lengths C_6 and C_{12}, degradation increases with increase in chain length.[77] It has been indicated that not only the alkyl chain

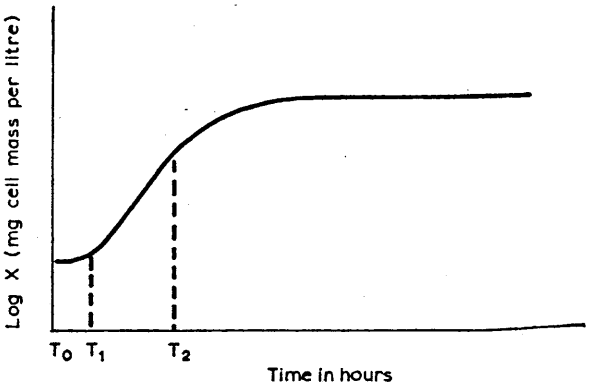


Figure 4

but the benzene ring is degradable in LAS.[78] With a C_{12}-LAS mixture and two of its components in pure form, 3-phenyl- and 6-phenyl-dodecane-*p*-sulfonates, all showed 90 per cent ring degradation under a range of conditions once acclimation occurred. The general scheme for metabolism of surfactants involves a number of oxidative mechanisms: (1) terminal or initial ω-oxidation, the first step in the degradation at the terminus of the hydrophobic group, (2) β-oxidation, the process whereby the aliphatic portion of the hydrophobic group is degraded, and (3) aromatic oxidation, which is applicable when the hydrophobic group contains a benzene ring.[1]

6.1. Anionic Surfactants

LAS. Because of space limitations only selected results on river-die-away tests will be presented.[3] Figure 3 shows results for ABS and LAS (straight chain ABS) under aerobic and anaerobic conditions at 10°C. At this low temperature neither straight-chain nor branched chain ABS can be sub-

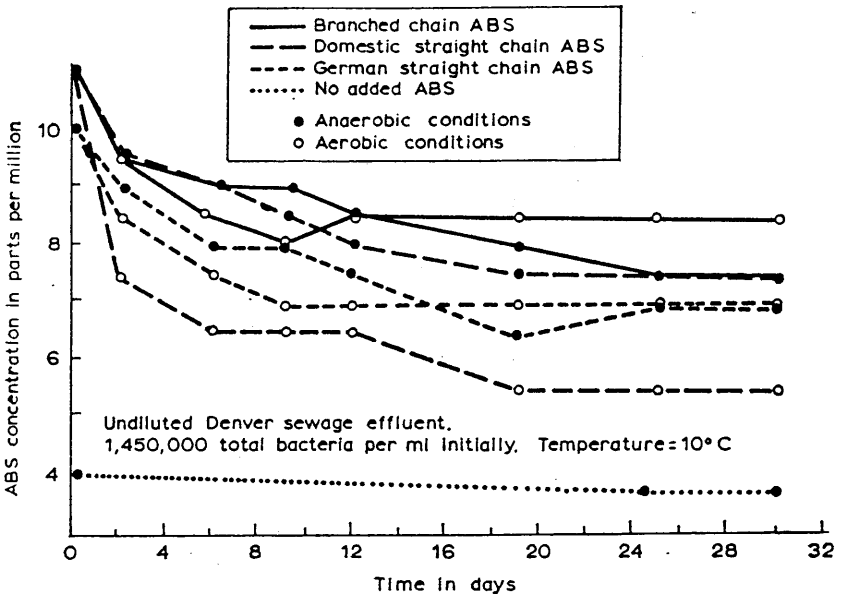


Figure 3

or inferior to the blank. Table 4 lists results for some typical synthetic surfactants. The results show that sucrose ester degrades much faster than either of the commercial surfactants Tergitol 15-S-9 or LAS. A computer program was developed to generate the constants.

TABLE 4. DEGRADATION RATE CONSTANTS FOR SURFACTANTS AT 25° C (Wayman and Burt[56])

Substrate	$K\left(\frac{1}{\text{mg-hour}}\right)$
Glucose	+0·0045
Sucrose Monolaurate	+0·0014
Tergitol 15-S-9	−0·0004
LAS	−0·0018

The advantage of this method is that it quantifies studies on biological oxidation. It removes the uncertainty involved in river die-away type studies, by permitting one to evaluate biodegradation under identical conditions by merely substituting one substrate for another. The method has the disadvantage that one must sample growth in a dilute bacterial solution over one-half hour periods for a total time of 10–18 hours. This involves much labor and dish washing.

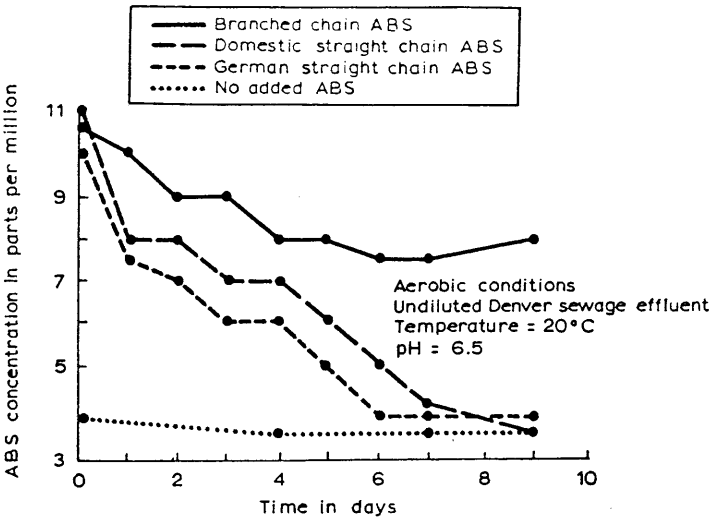


Figure 7

some types of LAS can be degraded to about background under aerobic conditions at 20°C. Figure 9 shows that at increased temperature, say, 35°C, LAS can be rapidly degraded under aerobic conditions only.

Swisher[1] has given a tabular summary of various aspects of degradation of anionic surfactants in terms of individual isomers of ABS, LAS, TBS and related polypropylene products, alkyl aromatic sulfonates of known structure, aliphatic hydrocarbon and hydroxyalkane sulfonates, linear primary

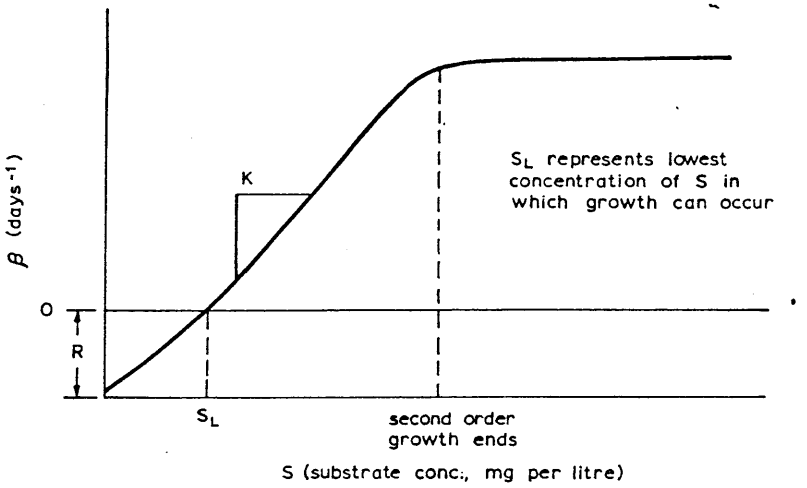


Figure 8

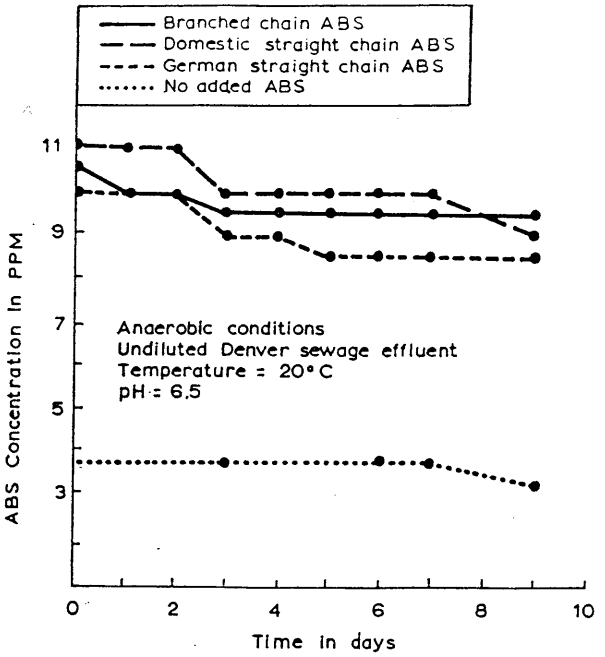


Figure 5

stantially degraded. This indicates that the rate of break-down in surface waters or ground waters during winter months is minimal under either aerobic or anaerobic conditions. Figures 5 and 6 show very little degradation of surfactant under anaerobic conditions at 20° C. Figures 7 and 8 indicate that

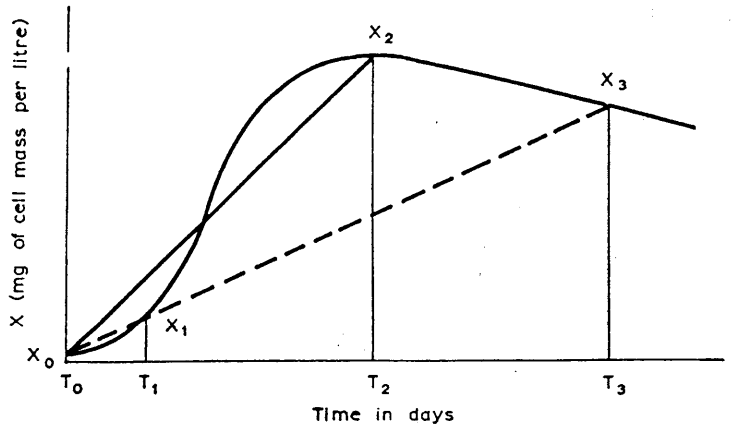


Figure 6

whether TBS is removed by sorption on bacterial surfaces.[87] However, other investigators[88, 89, 90] using radiosulfur techniques indicate that TBS will degrade under conditions of proper acclimatization to about 10–30 per cent.

Sulfonated esters and amides. Though some investigators suggest rapid degradation of sulfonated esters and amides e.g. Igepons A and T by hydrolytic release of the fatty acid,[91, 92] other studies[93] suggest that long periods of time, up to 30 days, may be required to effect even limited degradation to about 10 per cent.

Linear primary alkyl sulfates. Linear primary alkyl sulfates (LPAS) are apparently oxidizable with initial hydrolysis to the alcohol[92] and subsequent

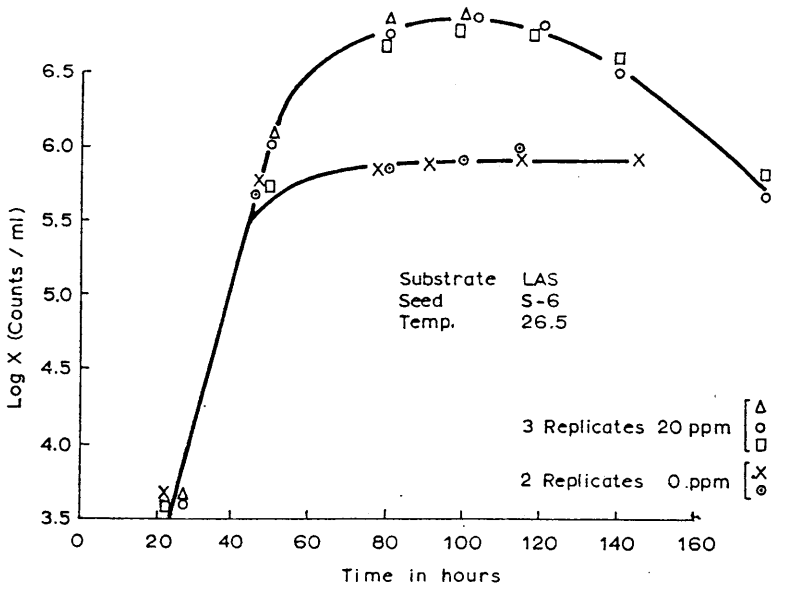


Figure 10

oxidation of the alcohol by dehydrogenation catalyzed by dehydrogenase enzymes at least up to alcohols of C_{11}.[94, 95, 96] Several workers[97, 98] have indicated extensive removal of LPAS in anaerobic systems probably as a result of simple hydrolysis. Oba[99] has shown that under aerobic conditions there is hydrolysis of the sulfate to the alcohol which is rapidly biodegraded, but under anaerobic conditions the alcohol is not biodegraded. There is substantial evidence[100, 101] to support the premise that secondary- and branched-alkyl sulfates are not readily biodegradable. Further support to substantiate this meager degradation of nonlinear alkyl sulfates is found in the 20 day respirometer studies of Pitter[97] showing almost complete biodegradation of glucose and LPAS to carbon dioxide and water and only 30–86 per cent with the nonlinear types. To date insufficient data are available to discuss the biodegradability of ethoxylate sulfates.[1]

alkyl sulfates, secondary alkyl sulfates, phosphates, phosphonates, carboxylates, soaps and alcohol ethoxylate sulfates.

Tallow-base. Cordon[79] has studied the metabolism of several tallow-based detergents. Alcohol sulfates, ether alcohol sulfate, and esters of α-sulfo fatty acid were biodegraded to 99, 94 and 61–87 per cent, respectively when compared to LAS as a reference material only degrading to 80 per cent.

Lignite-tar base. Recently,[80] a study has shown that synthetic detergents prepared from low temperature lignite tar (chloroparaffins) will biodegrade to over 90 per cent after appropriate acclimatization, according to the German test procedure for continuous activated sludge.

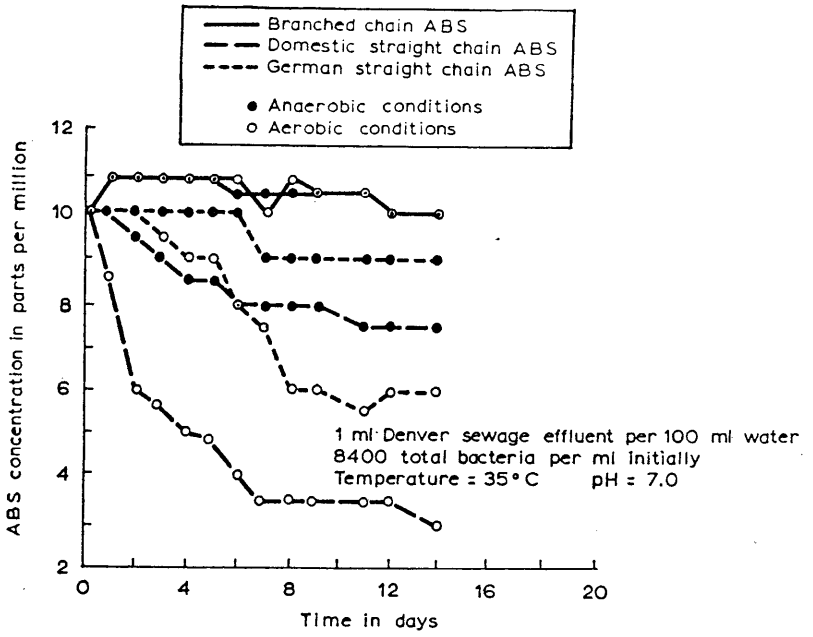


Figure 9

Quaternary alkylbenzenesulfonates. Studies on the breakdown of quaternary alkylbenzenesulfonates (QBS) seem to show that the benzene ring does not degrade substantially, in the presence of *E. coli* up to 27 days.[82] Several investigators[82, 83, 84] have shown that the molecule is attacked at some intermediate point on the chain without ring degradation when a mixed bacterial culture is used. Swisher has also found limited ring degradation (10–20 per cent) of QBS employing a semi-continuous activated sludge process.[85] The mechanism involved is attacked at any point of the molecule including the ring end,[86] intermediate points on the chain,[84] or at the terminal quaternary group itself.[1]

Tetrapropylene benzenesulfonates. With respect to tetrapropylene benzenesulfonate (TBS), it is uncertain whether true biodegradation occurs or

respectively, as compared to the value at 26·5° C. The rate constant for each value can be calculated from

$$K = \frac{\beta}{20} = 0 \cdot 0099 \ (26 \cdot 5^\circ \mathrm{C})$$

where

$$K = \frac{\text{litres}}{\text{mg-hr}}$$

assuming the respiration term is small enough to be ignored. This approach seems to be a most attractive way to evaluate biodegradation of a compound. It not only quantifies studies in this field, but permits investigators to compare results on more compatible and realistic bases.

6.2. Nonionic Surfactants

In contrast to anionic surfactants, nonionic surfactants contain both organic hydrophobic and hydrophilic groups permitting, at least theoretically, an opening at each end of the molecule for biodegradation. The generalized scheme for biodegradation of nonionics is believed to follow two steps,[5] oxidation of the hydrophobic group and hydrolysis of the ethylene oxide group with production in the biodegradation of polyoxyethylene surfactants of four molecular species:

(1) with intact hydrophobe
   (a) intact original molecule
   (b) degraded ethylene oxide chain

(2) with carboxylated hydrophobe
   (a) intact ethylene oxide chain
   (b) degraded ethylene oxide chain

Polyoxyethylene glycols. Thus, Osburn and Benedict[102] have demonstrated a combined hydrolysis and carboxylation mechanism. Their scheme proposes a bacterial and/or enzyme induced hydrolysis with one unit converted to a mole of ethylene glycol with termination at the new hydroxyl group after which the ethylene glycol is further degraded. There is much uncertainty as to the authenticity of ethylene glycol degradation. Lamb[103] found that it took 20 days for ethylene glycol (E_1) to absorb only 78 per cent of its theoretical oxygen demand, but that diethylene glycol (E_2) and triethylene glycol (E_3) attained a value of less than 20 per cent of the theoretical oxygen demand in 20 days. Mills[104] indicates that $E_2 - E_4$ do not biodegrade rapidly confirming Lamb's data, but he did show 50 to 70 per cent degradation of a mixed higher polymer averaging $E_8 - E_9$ in a continuous flow system under anaerobic conditions at a 7 day retention time. Fincher[105] claims to have isolated and cultured a soil organism, TEG-5 (a member of the Pseudomonas-Achromobacter group), capable of degrading $E_2 - E_4$ essentially to completion. Later studies have indicated that higher polymers can be completely metabolized with

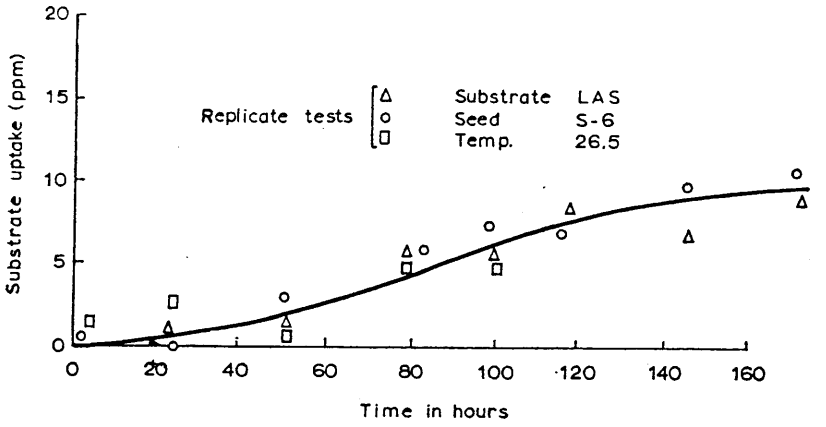


Figure 11

Though most procedures to date have employed river water-, soil-, or sewage bacteria in biodegradation studies, a recent study was undertaken to determine the influence of algae on surfactant biodegradation. Using both ABS and LAS it was determined in mixed algae-bacterial cultures that most of the surfactant degradation was attributable to bacteria but that these types of surfactants have some toxic effect on algae.[81]

In some recently unpublished data of Wayman and Burt, a novel method was employed to assess biodegradation of LAS employing second order kinetics.[56] Figures 10 and 11 show the log growth curve and substrate uptake curve, respectively using river water bacteria at an LAS concentration of 20 ppm for 26·5°C. Figure 12 shows a plot of $\%\beta$ versus temperature. The results show that at 15° and 5°C, the β's are 55·5 and 22·5 per cent,

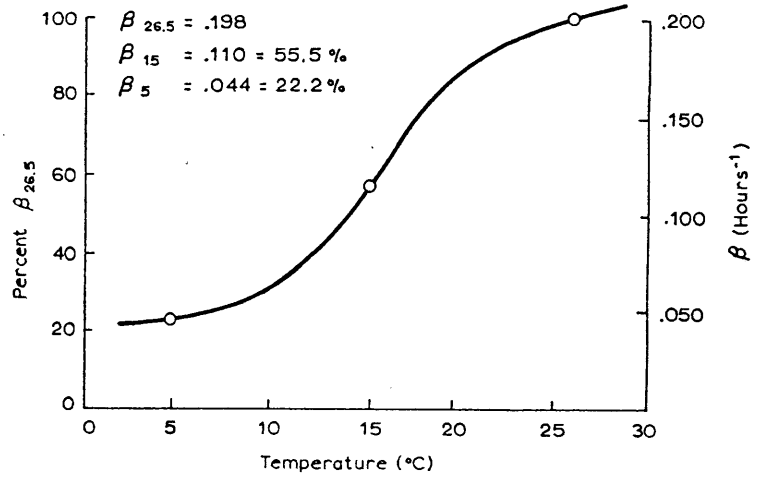


Figure 12

carboxylate can be degraded by β-oxidation down to HO_2CCOE_N or the polyglycol which can be further degraded as already discussed. Results on river die-away studies[109] and the British inoculation-aeration procedure[63] confirm removal of the original LPAE in about one week with subsequent removal of the polyethylene glycol (PEG). Recent work of Patterson[121] reveals that both the hydrolytic or oxidative pathways are possible. Other studies[108] indicate that ethoxylate is rapidly degraded, except that the higher ethoxylates are very refractory being negligibly degraded in the range E_{15-20}. Several investigators[115, 122, 123] have studied the effects of structure on the rate of biological oxidation. The results listed in Table 6 are typical for these compounds.[115]

TABLE 6

Hydrophobe	% EO	Time (Days) 0	1	2	3	4	5	6	8	10	13
		% remaining of 20 ppm									
1-Decanol	65	100	89	11	1	1	0				
1-Hexadecanol	63	100	80	13	1	1	0				
1-Dodecanol	67	100	95	23	0	0	0				
1-Octanol	62	100	91	64		34	17	2	0	0	
1-Octadecanol	63	100	93	56		11	5	2	2	1	0
Branched-chain tridecyl alcohol	76	100	100	64		55		55	51		48

(Reprinted from ref. 5, p. 984, by courtesy of Marcel Dekker, Inc.) Biodegradation of LPAE in River Water.

Straight-chain LPAE degrade very rapidly approaching the rate for soaps and LPAS, but branching does impede the rate as noted for branched-chain tridecyl alcohol (only 50 per cent up to about 6 days).

Linear secondary alcohol ethoxylates. Many investigators have shown that linear secondary alcohol ethoxylates (LSAE) degrade at about the same rate as LPAE[63, 106, 124] though it is shown that the highly branched tetrapropylene-oxo-$C_{13}E_8$ could only be degraded to about 30 per cent in 49 days.[63] Recent unpublished studies[56] on Tergitol 15-S-9 show that this compound will only degrade about 30 per cent in 6 days at 25° C under anaerobic conditions with essentially no degradation at 5° C; because there was no substantial bacterial growth, it seems certain that nonionics may degrade anaerobically by hydrolysis. Figure 13 shows that under aerobic conditions Tergitol 15-S-9 will degrade rapidly at 25° C ($K = 0{\cdot}014$) but to lesser amounts at 15° and 5° C.[56] Recent studies of C^{14} tagged Tergitol 15-S-9 by Wayman and Yap[72] indicate that this compound can be completely degraded to carbon dioxide and water, but the specific type of bacteria and temperature are important. Employing a surfactant concentration of 20 ppm and bacteria isolated from an activated sludge gave results of 55 and 18 per cent of available $C^{14}O_2$ in 180 hours at 27° and 15° C, respectively. However, using the same conditions in Clear Creek River Water near Golden, Colorado, gave only 10 and about 2 per cent of the available $C^{14}O_2$ in 500 hours.

proper acclimatization. Vath[106] found E_9 completely metabolized. Borslap[107] found that E_9 and E_{23} were biodegraded to 98 and 90 per cent respectively; this result is in conflict with the data of Pitter[108] who found complete degradation in the range E_2–E_6, but essentially no degradation for E_{13}, E_{22}, E_{34}, and E_{80}.

Alkyl phenol ethoxylates. A very substantial literature is available with respect to the alkyl-phenol ethoxylates (APE) in terms of length of the ethylene oxide chain, the chain length and degree of branching of the hydrophobe, position of attachment to the hydrophobe, and nature of the connecting link.[109–118] The most detailed study on APE is that on three types of systems using the river die-away, shake-flask, and continuous activated sludge. Results of the river die-away are given in Table 5.[115] Degradation markedly increases the greater the length of the alkyl chain. Degradation decreases with increase in complexity of branching, but moderately branched to straight-chain structures with less than 10 EO units per mole hydrophobe seem to degrade via the

TABLE 5

Hydrophobe	% EO	Time (Days) 0	1	2	4	6	8	12	15	22	26
		% remaining 20 ppm									
Straight-chain tetradecylphenol	70	100	100	76	30	7	7	7	7	7	7
Straight-chain dodecylphenol	74	100	100	69	19	10	9	9	9	9	9
Straight-chain decylphenol	66	100	80	46	24	20	18	11	10	10	4
Straight-chain nonylphenol	65	100	80	55	48	47	43	40	35	35	35
Straight-chain octylphenol	59	100	91	72	70	70	68	65	64	64	46
Branched-chain nonylphenol	65	100	82	80	80	80	80	80	75	72	46

(Reprinted from ref. 5, p. 980, by courtesy of Marcel Dekker, Inc.) Biodegradation of APE in River Water.

carboxylation route. A recent study[119] on octyl phenol ethoxylate (OPE_{10}) showed about 90 per cent degradation under field conditions. Buerger[120] has found that straight-chain nonionic surfactants with 10 moles of EO completely degrade in 3 hours at 20 ppm previously acclimatized in an activated sludge unit for 20 days; the reported mechanism being disappearance of the hydrophobic portions one by one while the hydrophilic (polyethylene glycol and phenol group) with a branched alkyl chain remained intact. With increase in the number of EO groups beyond 10 per mole, degradation was diminished.

Linear primary alcohol ethoxylates. Linear primary alcohol ethoxylates (LPAE) seem to degrade quite readily with removal of the ethoxylate as polyethylene glycol which is subsequently degraded. Swisher[1] indicates that it is uncertain whether the initiation is promoted by a hydrolytic or oxidative pathway, or both. If the alcohol is formed by hydrolysis and oxidized to a fatty acid, it seems clear that subsequent degradation by β-oxidation would substantially occur. If oxidation is the initiating force, then the terminal

acid are easily biodegradable[128, 129] as were those wherein the fatty acid contains at least one OH, NH_2, NHR, NOH, or CO group.[130] Other investigators have also shown high amounts of biodegradation with fatty acyl sucrose derivatives of the laurate,[131, 132, 133] myristate,[133] palmitate,[125, 133] stearate,[133, 134] hydroxystearate,[62] tallowate,[62] hydroxy fatty acyl,[135] and the fatty acyl.[108]

That sucrose esters are the best surfactants to employ to minimize water pollution abatement problems is because they break down in anaerobic environments where other surfactants are quite refractory. Kulovana and Pitter[136] found that sucrose esters of the higher fatty acids degrade more than

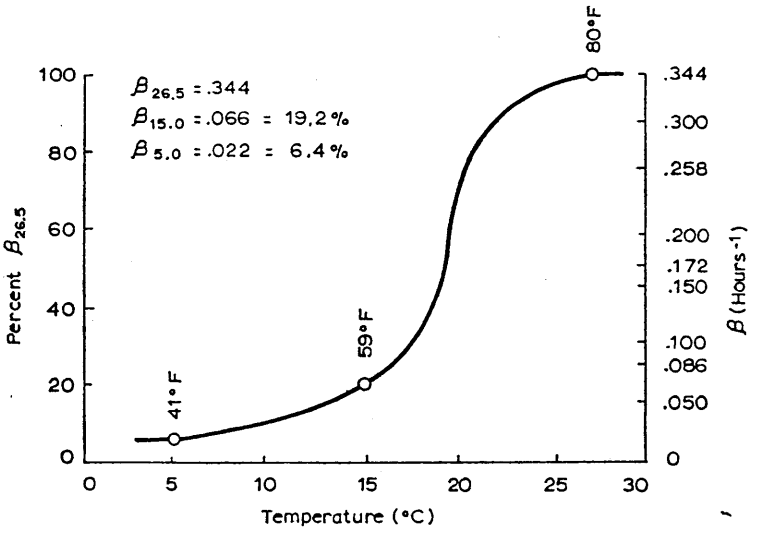


Figure 14

90 per cent after 5 days incubation under both aerobic and anaerobic conditions and have no deleterious effect on the anaerobic fermentation process. Earlier studies[62] show that sucrose esters disappear quite readily under anaerobic conditions. Recent unpublished data of Wayman and Burt[56] indicates that sucrose monolaurate disappears completely under anaerobic conditions from solutions originally containing 20 ppm in about 5 days providing there is proper acclimatization at 20° C; studies without anaerobic bacteria indicate that hydrolysis plays a minor role or that bacterial hydrolysis is controlling; at 5° C essentially no removal of sucrose ester was achieved indicating the importance of biodegradation over hydrolysis.

Ethoxylates of sucroglycerides are also readily degradable. These substances appear to be easily biodegradable as a result of hydrolysis[137] and polyoxyethylene coco sucroglycerides are readily biohydrolyzed into glycerol, sugar, fatty acids, and glycolic chains, all being readily metabolized by micro-organisms.[138]

Polyoxyethylene fatty acid esters. Studies on polyoxyethylene fatty acid esters indicate that these compounds are readily biodegradable. Weil and Stirton[125] show that $C_{16}H_{33}(OC_2H_4)_{10}OH$ and $C_{11}H_{23}CO(OC_2H_4)_{10}OH$ biodegrade in river water almost to completion in less than 40 hours being only slightly less degradable than that of sucrose monopalmitate. A similar result has been reported by Sawyer.[91]

The rates of biodegradation found for polyoxyethylene alkylamides seem spectacular. Using both the river die-away and shake culture procedures, it was determined that polyoxyethylene lauryl-stearyl amide degrades to nearly completion in 1–2 days.[123]

Alkanolamides. The alkanolamides are derived from natural fatty acids and should be easily biodegradable. However, available data seem to be in

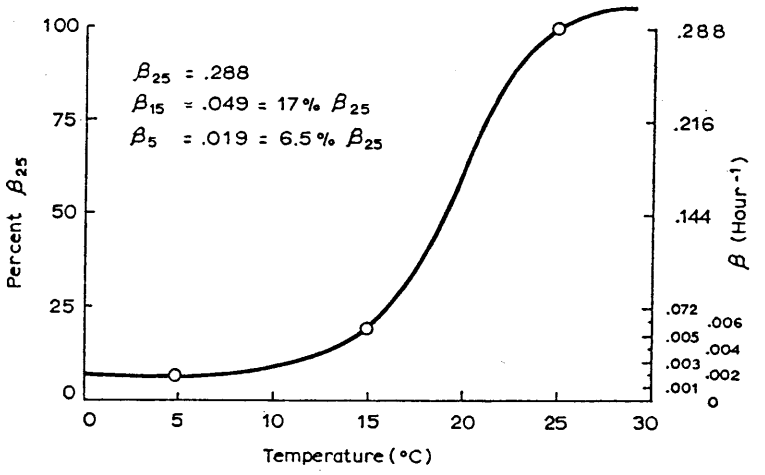


Figure 13

conflict. Knaggs and co-workers[126, 127] indicate that the lauryl diethanolamide and the lauryl monothanolamide were not degraded to completion until about 11 days. Other research studies[123] reveal complete degradation rates on C_{12} diethanolamide in about 2 days with the shake-flask method and about 6 days with the river die-away test. Apparently, the discrepancy lies in the test method because the same investigators obtained different results when resorting to different investigative techniques.

Sucrose esters and sucroglycerides. The biodegradation of sucrose esters has not received the attention it merits. Swisher[1] devotes an entire table to these compounds but does not discuss their behavior. Several investigators have observed that these compounds break down very rapidly under environmental conditions by both hydrolysis and biodegradation. Weil and Stirton[125] showed that sucrose monopalmitate degrades almost to completion within one day, and at a much faster rate than the most easily degradable polyoxyethylene surfactants, followed by a slower rate of degradation of the intermediates formed. Sugar esters of ricinoleic, mono-, di-, and trihydroxystearic

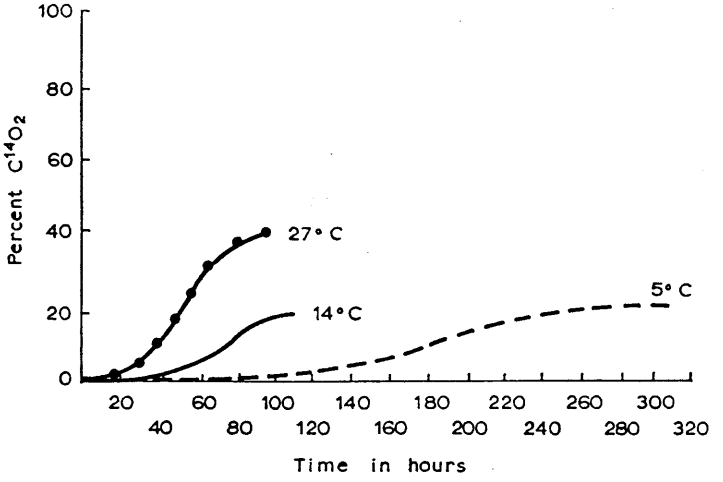


Figure 17

By employing the Wayman-Burt method,[56] it was found that the biodegradation of sucrose esters surpassed the rates of either Tergitol 15-S-9 or LAS. At 26·5°C, $K = 0{\cdot}0172$ l/mg-hour, while the values at 15° and 5°C were 19·2 and 6·4 per cent of the value at 26·5°C (Fig. 14). These data indicate that bacterial species can easily grow on sucrose esters and that the compound is rapidly degraded in contrast to anionics and other nonionics.

Additional attention should be directed toward sucrose esters as a possible third-generation surfactant because these compounds degrade to carbon di-

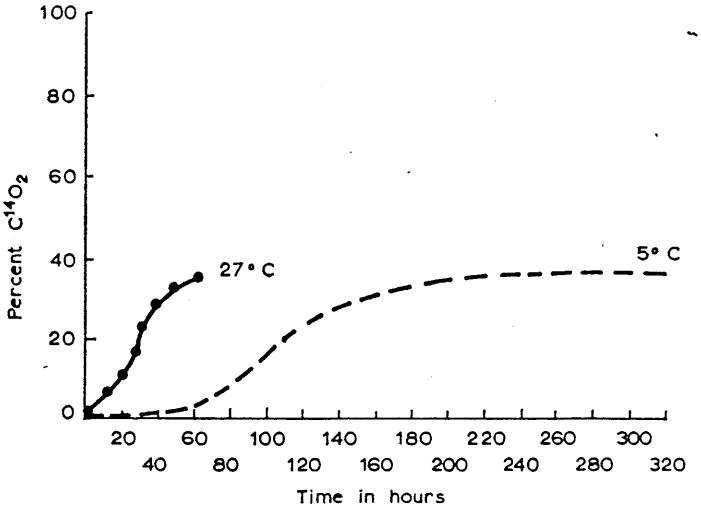


Figure 18

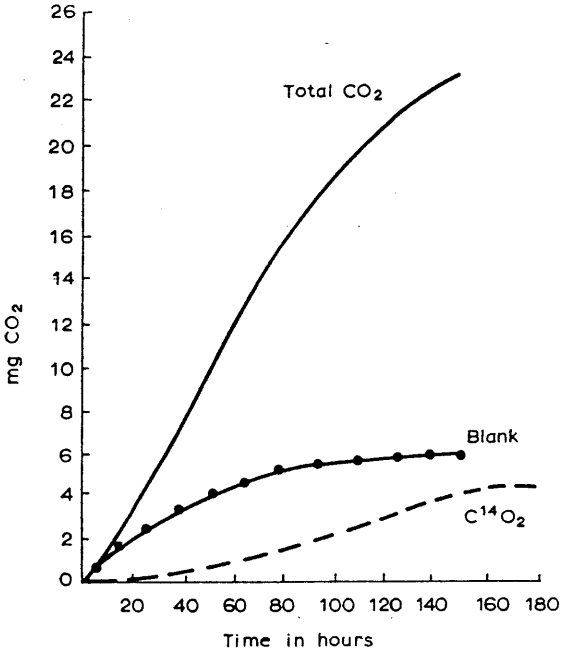


Figure 15

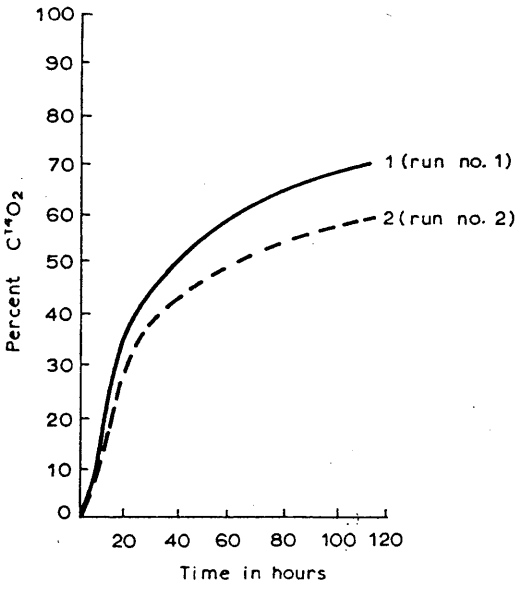


Figure 16

and

$$\lim_{t\to\infty} \frac{S_0 - X_0}{Y} (e^{K_t} - 1) \rightarrow -\infty$$

Figure 1a is a normal bacterial growth curve and shows that equation 2 holds for only limited conditions.

$$\lim_{t\to\infty} f(t) = 0 \text{ for } X = f(t)$$

$$\lim_{t\to\infty} f(t) = 0 \text{ for } s = f(t)$$

When Fig. 2 is replotted in terms of Fig. 4 it is obvious that equation 2 will be at least approximately valid for some small incremental time range, say T_1 to T_2, between the end of log phase and the initiation of growth limitation. For this reason it has been employed extensively for the region known as 'log growth'. It must be acknowledged that a real system based upon a model, wherein substrate is not in excess of some growth limitation is restricted, and growth limitation is present.

Monod[42] considered the growth to be limited according to a hyperbolic equation of the form

$$\mu = 1/X \frac{dx}{dt} = \mu_m \frac{S}{K_s + S} \tag{6}$$

where

μ_m = max. growth rate

K_s = conc. at which $\mu = \mu_m/2$

There are several objections to the Monod equation. It holds for some systems, but does not describe growth in terms of primary kinetic parameters. It will not account for bacterial die-off unless s is negative. The equation is extremely difficult to integrate $X = f(t)$. A second order equation in terms of measurable experimental constants is most desirable.

Consider a reaction of the type

$$\underset{(S)}{\text{Substrate}} + \underset{(X)}{\text{Bacteria}} \rightarrow \underset{(X)}{\text{Products (bacterial protoplasm)}}$$

or

$$\frac{dX}{dt} = K(X)(S) \tag{7}$$

and this gives

$$\frac{dX}{dt} = -Y \frac{dS}{dt} \tag{3}$$

This consideration permits the derivation of a second order equation in terms of Y and K, but still suffers from a portion of the disadvantage of the Monod equation, in that, it will be asymtotic to $X = YS_0 + X_0$ and die-off can occur only when S is less than zero. It overcomes all other restraints, however.

oxide and water in very short periods of time. The unpublished studies of Wayman and Yap[72] support this proposal. Figure 15 shows that C^{14} sucrose monolaurate begins to generate $C^{14}O_2$ in about 60 hours in river water at 25° C and 20 ppm surfactant concentration. By employing activated sludge in place of river water, about 55–65 per cent of the available $C^{14}O_2$ is produced in 120 hours (Fig. 16). Figures 17 and 18 show that both the sucrose portion and fatty acid portion are readily biodegraded at 27° C and to lesser extents as the temperature is lowered. These results strongly suggest that detergent manufacturers should divert their attention to these compounds as replacements for LAS and other nonionic surfactants because of their excellent ultimate degradation propensities. In view of the fact[18b] that glucose only degrades to give about 20 per cent of the theoretical CO_2, the advantages of sucrose ester surfactants are obvious.

APPENDIX 1

Derivation of second order rate equation applicable to biodegradation[56] for substrate limited systems

The simplest model to assess bacterial growth is the first order so-called log growth. The model shows that the rate of growth of cells dX/dt, in mass units, is proportional to cellular mass only:

$$\frac{dX}{dt} = KX \tag{1}$$

which integrates to

$$X = X_0 e^{Kt} \tag{2}$$

Substrate uptake concentration in this type of system is proportional to growth,

$$\frac{dX}{dt} = -Y\frac{dS}{dt} \tag{3}$$

The minus sign indicates that ΔS is in the negative direction, i.e. decreasing with time such that

$$X - X_0 = Y(S_0 - S) \tag{4}$$

which when substituted into (2) yields

$$X - X_0 = X_0(e^{Kt} - 1)$$

$$YS_0 - YS = X_0(e^{Kt} - 1)$$

$$S = S_0 - \frac{X_0}{Y}(e^{Kt} - 1) \tag{5}$$

This representation can only hold for small periods of time because

$$\lim_{t \to \infty} X_0 e^{Kt} \to \infty$$

Figure 3a can be employed to illustrate the approximation. For some value t_2, $t_2 \approx T_{max}$, and

$$\int_0^T X\,dt = \frac{XT}{2} \tag{10A}$$

Then for all

$$t : t < t_2, \int_0^T X\,dt < \frac{XT}{2}$$

and for

$$t > t_2, \int_0^T X\,dt > \frac{XT}{2}.$$

For $t \ll t_2$, the respiration is small, but for $t \gg t_2$, respiration is the primary factor in s utilization. Based upon Fig. 3a, it can be seen that the developed value of respiration becomes smaller than the actual respiration, and, if the culture growth is followed far into the die-off region, e.g. t_3, the error propagates itself and is serious. This restriction is not so limiting, because in the die-off phase, growth is sensitive to many factors, and the shape of the die-off curve may vary with essentially no change in the environment. Hence, it is only necessary to predict behavior of the respiration term near T_{max}, and this is exactly where the approximation is well-behaved.

Thus,

$$X - X_0 + Ys - Ys_0 \overset{0}{=} -RXT$$

or

$$S = \frac{X_0}{Y} + S_0 - X\left(1/Y + \frac{RT}{2Y}\right) \tag{11}$$

Hence, the total differential becomes

$$dX/dt + RX = KX\frac{X_0}{Y} + S_0 - X\left(\frac{1}{Y} + \frac{RT}{2Y}\right) \tag{12}$$

or if A is substituted for $S_0 + X_0/Y$

$$dX/dt = KXA - RX - X^2\left(\frac{K}{Y} + \frac{KRT}{2Y}\right)$$

$$= (KA - R)X - K/Y\left(1 + \frac{RT}{2}\right)X^2 \tag{12}$$

Equation 12 is in the form of Bernoulli's equation

$$dX/dt + \Phi X = \Psi X^n \tag{13}$$

where

$$\Phi(Z) = -(AK - R) \text{ and } \Psi(t) = -\frac{K}{Y}\left(1 + \frac{RT}{2}\right)$$

soluble by the substitution

$$Z = X^{1-n} = 1/X$$

The derivation will include die-off phenomena and also loss of diversion of s to respiration. Hence, equation 3 can be modified to

$$\frac{dX}{dt} + RX = -Y\frac{dS}{dt} \tag{8}$$

where R is a specific respiration constant defined as

$$R = -1/X\frac{dX}{dt} \text{ for } \frac{dS}{dt} = 0 \tag{9}$$

This definition of R is convenient and practical because R is a function only of the culture, and independent of the substrate used. R can be visualized as the amount of material diverted from synthesis for respiration (i.e. maintenance) when $dS/dt \neq 0$. R cannot represent the total respiration in a growing culture, and is defined only for die-off. Equations in differential form (7 and 8) adequately define the system, but give rise to two problems:

(1) At the maximum, $dX/dt = 0$. From (7), $dX/dt = 0$, only if $(S) = 0$, (when $X \neq 0$, a trivial condition). But if $(S) = 0$, $dX/dt = 0$ for all T greater than T_{max} and die-off cannot occur, and

(2) Because R was defined as $-1/X\ dX/dt$ when $dS/dt = 0$, the limit as $T \to \infty$ must be $-dX/R\,dt = X$. In the integrated form it can be seen that this is incorrect.

This system nonetheless produces a curve of $X = f(t)$ that is remarkably similar to the normal growth curve. Both of the disadvantages enumerated disappear if (7) is modified to

$$dX/dt + RX = KXs \tag{9A}$$

so when $S = 0$, $dS/dt = 0$, and $R = -1/X\,dX/dt$ in both (8 and 9A). Now $dX/dt = 0$ even if $KXs \neq 0$, when $KXs = RX$. Thus $Ks = R$ when $dX/dt = 0$ is a good definition of a substrate limited system. This indicates that growth ceases when uptake (KXs) is equal to respiration (RX), which leads to the equation

$$-Y\,dS/dt = KXs \tag{10}$$

Equation 10 indicates that substrate uptake is second order, with s being proportioned between respiration and synthesis. The two important equations are 8 and 9A.

Equation 8 may be integrated over its limits and the resultant equation for s substituted in equation 9A or

$$\int_{x_0}^{x} dX/dt + Y\int_{s_0}^{s} dS = -R\int_0^T X\,dt$$

The real problem arising is an approximation for

$$\int_0^T X\,dt.$$

but if

$$KR\beta T \gg 2\beta R - KR \text{ and } 2Y\beta^2 \gg X_0(RK - 2\beta K)$$

$$X = \frac{2Y\beta^2 X_0}{KX_0[R(\beta T - 1) + 2\beta] + 2Y\beta^2 e^{-\beta T}} \tag{22}$$

Note that equations 7 and 3 integrate to

$$X = \frac{X_0 A}{AY e^{-AKT}} \tag{23}$$

in comparison to

$$X = \frac{2YBX_0}{KTRX_0 + 2Y\beta e^{-\beta T}}$$

If R is set equal to zero, the term $KTRX_0$ vanishes and β reduces to AK with substantial identity between equations 22 and 23. In equations 22 or 23

$$\lim_{t \to 0} X(t) = X_0$$

and

$$\lim_{t \to \infty} X(t) = 0$$

as required.

Now,

$$\frac{\partial X(t)}{\partial t} = \frac{2Y\beta X_0 \left(\frac{\partial}{\partial T} KTRX_0 + 2Y\beta e^{-\beta T}\right)}{(KTRX_0 + 2Y\beta e^{-\beta T})^2}$$

$$= \frac{2Y\beta X_0(KRX_0 + 2Y\beta^2 e^{-\beta T})}{(KTRX_0 + 2Y\beta e^{-\beta T})^2} \tag{24}$$

and when $dx/dt = 0$ (at maximum growth)

$$-KRX_0 = 2Y\beta^2 e^{-\beta T} \tag{25}$$

and β = slope of the log growth curve (Fig. 2) $= AK - R$.

From the definition of R:

$$R = -1/X \; dx/dt \text{ when } \frac{ds}{dt} = 0$$

or

$$\frac{dx}{X} = -R\,dt$$

$$\log X = -RT + C$$

$$X = e^{-RT} + C \tag{26}$$

where equation 26 is $ds/dt = 0$ (max. growth).

which is resolved to

$$\frac{dZ}{dt} + n\Phi(t)Z = n\Psi(t)$$

or

$$\frac{dZ}{dt} + Z(AK - R) = \frac{K}{Y}\left(1 + \frac{RT}{2}\right) \tag{14}$$

This is a linear first order differential equation in Z of the form

$$\frac{dZ}{dt} + P(t) = Q(t) \tag{15}$$

where

$$P(t) = AK - R = \beta,$$

and

$$Q(t) = \frac{K}{Y}\left(1 + \frac{RT}{2}\right)$$

The solution requires the integrating factor

$$e^{\int P(t)\,dt} = e^{\int \beta dt} = e^{\beta T + c} \text{ or } e^{\beta T}$$

where $\beta = AK - R$

Thus,

$$Ze^{\beta T} = \int e^{\beta T}\left(\frac{K}{Y} + \frac{KRT}{2Y}\right)dt \tag{16}$$

$$= K/Y\left(\int e^{\beta T}\,dt + \frac{R}{2}\int e^{\beta T}\,T\,dt\right)$$

$$= \frac{K}{\beta Y}e^{\beta T} + \frac{KR}{2Y}\left(\frac{\beta T - 1}{\beta^2}e^{\beta T}\right) + C \tag{17}$$

$$Z = 1/X = \frac{K}{\beta Y} + \frac{KR(\beta T - 1)}{2Y\beta^2} + Ce^{-\beta T} \tag{18}$$

$$\frac{2Y\beta^2}{X} = 2\beta K + KR(\beta T - 1)T + 2Y\beta^2 Ce^{-\beta T}$$

or

$$X = \frac{2Y\beta^2}{2\beta K + KR(\beta T - 1) + 2Y\beta^2 Ce^{-\beta T}} \tag{19}$$

when $X = X_0$, $T = 0$, and

$$2X_0\,\beta K - X_0\,KR + X_0\,2Y\beta^2\,C = 2Y\beta^2$$

or

$$C = \frac{2Y\beta^2 + X_0\,KR - 2X_0\,\beta K}{X_0\,2Y\beta^2} \tag{20}$$

such that (19) becomes

$$X = \frac{2Y\beta^2 X_0}{2X_0\beta R + KRX_0\beta T - KRX_0 + e^{-\beta T}(2Y\beta^2 + X_0 RK - 2X_0\beta K)} \tag{21}$$

and

$$X = X_0 e^{\beta T} = X_0 e^{(Ks_0 - R)T}$$

$$\frac{\ln (X/X_0)}{T} = Ks_0 - R \tag{30}$$

because

$$\beta = AK - R = s_0 K + \frac{X_0}{Y} K - R \cong Ks_0 - R$$

Thus, the slope of the log phase in equation (30) is $Ks_0 - R$ from Fig. 4a, and a plot of β vs. s_0 gives an intercept on β of R and a slope of K, where K is the rate constant ($l \cdot$mg substrate^{-1} days^{-1}).

Units

X: mg cell mass$\cdot l^{-1}$

s: mg substrate$\cdot l^{-1}$

T: days

Y: mg cell mass$\cdot$mg substrate^{-1}

A: $s_0 + \frac{Y_0}{Y} \approx s_0$ in mg substrate$\cdot l^{-1}$

R: days^{-1}

K: $l \cdot$mg substrate$^{-1} \cdot$days^{-1}

β: days^{-1}

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Equations 22 and 26 are inconsistent; (26) predicts exponential die-off whereas (22) shows an almost linear behavior. The discrepancy lies in the approximation of

$$\int_0^T x\,\mathrm{d}t,$$

equation 10A, but 22 is highly questionable in any event in the die-off region. A solution for $s(t)$ is as follows:

From equation (8)

$$\mathrm{d}x/\mathrm{d}t + RX = -Y\frac{\mathrm{d}s}{\mathrm{d}t}$$

and

$$\frac{-\mathrm{d}x}{Y} - \frac{RX\,\mathrm{d}t}{Y} = \mathrm{d}s \tag{27}$$

or

$$\int_{s_0}^{s} \mathrm{d}s = -\frac{R}{Y}\int_{T_0}^{T} x\,\mathrm{d}t - \frac{1}{Y}\int_{X_0}^{X} \mathrm{d}x \tag{28}$$

but in equation 10A

$$\int_{T_0}^{T} x\,\mathrm{d}x \overset{0}{=} \frac{X}{2}(T - T_0)$$

and

$$s - s_0 = -\left(\frac{x - x_0}{Y}\right) - \frac{RXT}{2Y}$$

$$s = s_0 + \frac{X_0}{Y} - X\left(\frac{2 + RT}{2Y}\right) = A - X\left(\frac{2 + RT}{2Y}\right)$$

$$= A - \frac{\beta^2 X_0(2 + RT)}{KX_0[R(\beta T - 1) + 2\beta]} \tag{29}$$

In the early stages of growth, equation 22 can be transformed as follows:

$$X = \frac{2Y\beta X_0}{KRTX_0 + 2Y\beta\,\mathrm{e}^{-\beta T}}$$

$$= \frac{2Y\beta X_0\,\mathrm{e}^{\beta T}}{KRTX_0\,\mathrm{e}^{\beta T} + 2Y\beta}$$

$$= X_0\,\mathrm{e}^{\beta T}\left(\frac{2Y\beta}{KRTX_0\,\mathrm{e}^{\beta T} + 2Y\beta}\right) \qquad \text{(Modified 22)}$$

But at small X_0 and small T,

$$\frac{2Y\beta}{KRTX_0\,\mathrm{e}^{\beta T} + 2Y\beta} \overset{0}{=} 1$$

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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 Section 10(d) (1) (A), (B) or (C)

COMPANY: AVA Chemical Ventures LLC.

COMPANY AGENT: [signature] Date: 7/20/2000

SUBMITTER: [signature] Date: 7/20/2000

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FINAL REPORT

AVACHEM SUCROSE OCTANOATE

HONEY BEES ACUTE CONTACT TOXICITY

DATA REQUIREMENT:	EPA Ecological Effects Test Guidelines. OPPTS 850.3020, Honey Bee Acute Contact Toxicity. Public Draft. April, 1996.
PROTOCOL NUMBER:	WSU Protocol No. 00-004
SPONSOR:	AVA CHEMICAL VENTURES LLC. 80 Rochester Avenue Portsmouth, NH 03801
PRINCIPLE CONTACT:	Tony Barrington (603) 431-4242 FAX: (603) 430-8029
STUDY CONDUCTED BY:	The Bee Group Washington State University 24106 North Bunn Road Prosser, WA 99350 (509) 781-0552 FAX: (509) 786-9370 email: mayerd@wsu.edu
PRINCIPAL INVESTIGATOR:	Dr. Daniel F. Mayer
STUDY COMPLETION DATE:	6 July 2000

Ron Britt & Associates, Inc.
P.O. Box 8336
Yakima, WA 98908
Phone /fax (509)966-9681

QUALITY ASSURANCE STATEMENT

COMPANY: The Bee Group - WSU/AVA Chemical Ventures

RESIDUE TRIAL NUMBER: 00-004

CHEMICAL: Avachem: Sucrose Octanoate

TEST SYSTEM: Honey Bees

INSPECTION DATE	INSPECTION TYPE	DATE REPORTED TO RESEARCH DIRECTOR	DATE REPORTED TO STUDY DIRECTOR	DATE REPORTED TO STUDY MANAGEMENT	INITIALS
June 16, 2000	Dosing of Honey Bees	6/28/00	6/28/00	6/28/00	KB

Kathleen Britt
Officer, Quality Assurance Unit

7/6/00
Date

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Final Report Title:

AVACHEM Sucrose Octanoate: Honey Bees Acute Contact Toxicity

Washington State University Bee Protocol Number: WSU 00-004

Date study initiated: 13 June 2000

Date biological test completed: 18 June 2000

In accordance with Washington State University's Bee Group intent that this study be conducted by our facilities following good laboratory practice, the principal investigator herein confirms that the study was conducted in compliance with U.S. EPA FIFRA (40 CFR Part 160) and/EPA TSCA (40 CFR 792) Good Laboratory Practice Standards, which are consistent with the OEDC Principals of Good Laboratory Practice (OECD ENV/MC/CHEM(98)17) and MAFF Japan Good Laboratory Practice Standards (59 NohSan No.3850).

Daniel F. Mayer,
Principal Investigator — 7/6/00 Date

Sponsor — 7/10/00 Date

Submitter — 7/11/00 Date

SUMMARY

SPONSOR: AVA Chemical Ventures LLC.

TEST SUBSTANCE: AVACHEM Sucrose Octanoate 40%

WASHINGTON STATE UNIVERSITY'S
BEE GROUP PROTOCOL NUMBER: WSU 00-004

STUDY: **AVACHEM SUCROSE OCTANOATE HONEY BEES ACUTE CONTACT TOXICITY**

SUMMARY:

Five geometrically spaced dosages of AVACHEM Sucrose Octanoate 40% were administered to groups of 30 adult worker honey bees. At initiation of the study each bee received a single dose of the chemical. The dose was applied to the mesoscutum with an Eppendorf micropipette. The bees were observed after 4, 24 and 48 hours for toxicological response and the number of bees that were alive and the number that were dead recorded at 24 and 48 hours. A LD_{50} with 95% confidence limits could not be calculated because there was little or no mortality with any of the rates tested.

RESULTS:

Acute toxicity of negative control

Acute Toxicity Bioassay on *Apis mellifera* L, order Hymenoptera

The percent mortality at 24 hours and 48 hours, respectively was 10% and 10%.

Acute toxicity of solvent control

Acute Toxicity Bioassay on *Apis mellifera* L, order Hymenoptera

STUDY CONTRIBUTORS

The following contributed to this report in the capacities indicated:

Name	Title
Tony Barrington	Sponsor Representative
Daniel F. Mayer	Study Director
Daniel F. Mayer	Principal Investigator
Jeff Lunden	Research Technologist Supervisor
Kathy Britt	Quality Assurance

The percent mortality at 24 hours and 48 hours, respectively was 13.3% and 16.7%.

CONCLUSION:

AVACHEM Sucrose Octanoate is not hazardous to honey bees when applied directly to adult bees. The LD_{50} of greater than 80 ug/bee was used to classify the toxicity of the pesticide according to the classification scheme of Johansen & Mayer (1990).

Highly Toxic:	Less than 2 micrograms per bee
Moderately Toxic:	Greater than or equal to 2 micrograms per bee but less than 11 micrograms per bee
Relatively Non-Toxic:	Greater than or equal to 11 micrograms per bee virtually non-toxic

AVACHEM Sucrose Octanoate 40% is in the virtually non-toxic class.

TEST DATES: Experimental Start - 16 June 2000
Experimental Termination - 18 June 2000

BIOLOGICAL TEST COMPLETION: 18 June 2000

The percent mortality at 24 hours and 48 hours, respectively was 3.3% and 6.7%.

Acute toxicity of the 5 ug/bee solution

Acute Toxicity Bioassay on *Apis mellifera* L, order Hymenoptera

The percent mortality at 24 hours and 48 hours, respectively was 3.3% and 3.3%.

Acute toxicity of the 10 ug/bee solution

Acute Toxicity Bioassay on *Apis mellifera* L, order Hymenoptera

The percent mortality at 24 hours and 48 hours, respectively was zero and zero.

Acute toxicity of the 20 ug/bee solution

Acute Toxicity Bioassay on *Apis mellifera* L, order Hymenoptera

The percent mortality at 24 hours and 48 hours, respectively was zero and zero.

Acute toxicity of the 40 ug/bee solution

Acute Toxicity Bioassay on *Apis mellifera* L, order Hymenoptera

The percent mortality at 24 hours and 48 hours, respectively was zero and zero.

Acute toxicity of the 80 ug/bee solution

Acute Toxicity Bioassay on *Apis mellifera* L, order Hymenoptera

INTRODUCTION

The biological portion of the study was conducted by Washington State University's Bee Group for AVA Chemical Ventures LLC to determine the acute contact toxicity of AVACHEM Sucrose octanoate 40% to adult worker honey bees (*Apis mellifera* L.) using the topical treatment method. The study was conducted by The Bee Group at Washington State University, Irrigated Agricultural Research and Extension Center, 24106 North Bunn Road, Prosser, WA 99350 using honey bees belonging to the Bee Group. The test was conducted from 16 June to 18 June, 2000. A certified copy of the protocol, a certified copy of the raw data and a certified copy of the final report are filed under Project Number 00-004 in Dan Mayer's (Principal Investigator) archives located at the station.

GENERAL STUDY INFORMATION

Study Objectives

* to determine the acute contact toxicity of AVACHEM Sucrose Octanoate 40% to adult worker honey bees using the topical treatment method, and

* to calculate a LD_{50} with 95% confidence limits for honey bees.

An end-use formulation of AVACHEM Sucrose Octanoate 40% was applied to the honey bees.

Test Site

The field and in-life portions of this study were performed by the Bee Group at the Irrigated Agricultural Research & Extension Center, Washington State University, 24106 North Bunn Road, Prosser, WA 99350.

Study Personnel

Sponsor Representative:	Tony Barrington Managing Member
Study Director:	Dr. Daniel F. Mayer Entomologist

Washington State University's Bee Group

Principal Investigator:	Daniel F. Mayer, Entomologist
Research Technologist Supervisor:	Jeff Lunden

FIGURES

TABLES

APPENDICES

according to good beekeeping practices (Johansen and Burgett 1983).

Worker honey bees 3 to 4 weeks old and apparently healthy were collected from top lids for the test. Honey bees were collected from each of 3 different colonies into a holding box. The holding box with the bees inside was transported to the laboratory. The bees were anaesthetized with carbon dioxide in the holding box until they dropped to the bottom of the box. Ten bees were counted out from the holding box and put in each of 24 different Dixie cups. The number of test chambers per dose was 3 for a total of 21. Two extra Dixie cup of bees were prepared so that the bees were available if there was a need for more bees.

The honey bees in the Dixie cups (one cup per test chamber) were allowed to begin moving. Just prior to treatment the bees in each cup were reanaesthetized with carbon dioxide for approximately 15 to 30 seconds. Immediately after reanaesthtizing the 10 bees from each cup were placed on their abdomen on a paper towel on the work bench. The test solution was then applied using a calibrated Eppendorf microsyringe. For each bee, 2 ul of solution were drawn into the tip and then gently dispensed onto the top surface of each bee's mesoscutum. Three groups of 10 bees (total of 30 bees) was treated with each solution concentration. Three groups of solvent control were treated as described above but received only 2 ul acetone. Three groups of negative control were treated as described above but were not treated with acetone.

The negative control was done first followed by the solvent control followed by treatments with the low dose (5 ug/bee) done first.

The disposable tip on the Eppendorf was changed after each treatment.

Prior to putting the honey bees in each test chamber a cotton wad (5 x 5 cm) soaked with 50% sugar syrup was placed on the bottom of each test chamber for the bees to feed on. In order to control bias, bees were impartially distributed to Dixie cups and test chambers.

Physical System

Test Units

Test chambers were constructed with the tops and bottoms of 150 x 15 mm plastic petri plates. Wire screen was cut into a strip 46 x 5 cm and the ends stapled to form a cylinder (Figure 1). Petri plates served as top and bottom of the cage.

The test chambers were labeled by dose and treatment number.

Test Conditions

The temperature was recorded in the laboratory at irregular intervals during the

MATERIALS AND METHODS

Test Guidelines

The methods, species used and route of exposure described was based on procedures specified in the Environmental Protection Agency Registration Guidelines Pesticide Assessment Guidelines, FIFRA Subdivision L, 850.3020, Hazard evaluation: Nontarget Insects and in the June, 1996, draft proposal for a new guideline, OECD Guidelines for the Testing of Chemicals, Honeybees, Acute Contact and Oral toxicity Tests.

The droplet application to the mesoscutum method allows for a precise exposure of the test substance that simulates a possible route of field exposure of the bees and other non-target insects. Exposure is most likely to occur from overspraying.

At initiation of the test, three replicate test groups, each of ten bees were dosed with each concentration of chemical and rates.

TEST SYSTEM

Chemical System

The test substance received from AVA Chemical Ventures LLC on 16 June 2000 was a liquid formulation of AVACHEM Sucrose Octanoate EUP [40%] which contained approximately 40% active substance per gallon of formulation. It was identified on the label as: AVACHEM, AVA Sucrose octanoate, End Use Product, Batch #503299. It was assigned The Bee Groups identification number 00-004 upon receipt and logged on the GLP Test Substance Form and stored dark and dry in Dan Mayer's locked pesticide storage room at 60^0 to 80^0 F.

Solution Calculation and Preparation

The appropriate amount of AVACHEM Sucrose Octanoate 40% was calculated for each dosing solution (Appendix I). AVACHEM Sucrose Octanoate 40% was then mixed in distilled water with 0.02% Tween-20 so that a given quantity, 2 microliters/bee of solution, containing either 5, 10, 20, 40 or 80 ug active ingredient (ai) of chemical was applied (Appendix II).

Biological Systems

The studies were conducted using European honey bees (*Apis mellifera* L.) belonging to The Bee Group, Washington State University. The colonies are located on North Bunn Road, approximately 0.5 miles south of the intersection of King Tull Road and North Bunn Road. All honey bee colonies used in this study were adequately fed, healthy, queen-right, disease-free colonies and were maintained

REFERENCES

Johansen, C. and M. Burgett. 1983. Beekeeping. Wash. St. Univ. Coop. Ext. PNW 79. 27 pp.

Johansen, C.A. and D.F. Mayer. 1990. Pollinator protection: A bee and pesticide handbook. Wicwas Press, Cheshire, CT. 212 pp.

Russell, R.M. and J.L. Robertson. 1979. Programming probit analysis. Bull. Entomol. Soc. Amer. 23;209-213.

study and the records are included in the raw data.

After all the honey bees were treated and put into the test chambers the chambers were placed in a dark rearing chamber. The high temperature in the rearing chamber during the biological study was 26 degrees C. and the low temperature was 21 degrees C. Records are in the raw data. The high humidity in the rearing chamber during the study was 66% and the low humidity was 61%. Records are in the raw data.

Observations

Observations for mortality and toxicological responses were made at approximately 4 hours after exposure. At approximately 24 hours after exposure of bees, observations for mortality and toxicological responses were made and the number of dead and live bees in each cage were recorded and the percent mortality determined. At approximately 48 hours after exposure of bees, observations for mortality and toxicological responses were made and the number of dead and live bees in each cage were recorded and the percent mortality determined.

RESULTS

Mortality and Toxic Signs

No abnormal behavior was observed. The total number of honey bees exposed, live bees, dead bees and percent mortality for each replication at 24 and 48 hours is given in Appendix III. The mean percent mortality is summarized in Table 1. The mortality of the untreated checks was within the normal range limits.

Statistical Analysis

LD_{50} values (ug/bee) were to be calculated using a computerized probit analysis program (Polo-PC Probit and Logit Analysis (Russell & Robertson, 1979)). However, there was little or no mortality with any of the rates of AVACHEM Sucrose Octanoate 40% tested and the LD_{50} was greater than 80 ug/bee. The LD_{50} was used to classify the toxicity of the pesticide according to the scheme of Johansen and Mayer (1990).

Highly Toxic:	Less than 2 micrograms per bee
Moderately Toxic:	Greater than or equal to 2 micrograms per bee but less than 11 micrograms per bee
Relatively Non-Toxic:	Greater than or equal to 11 micrograms per bee

CONCLUSION

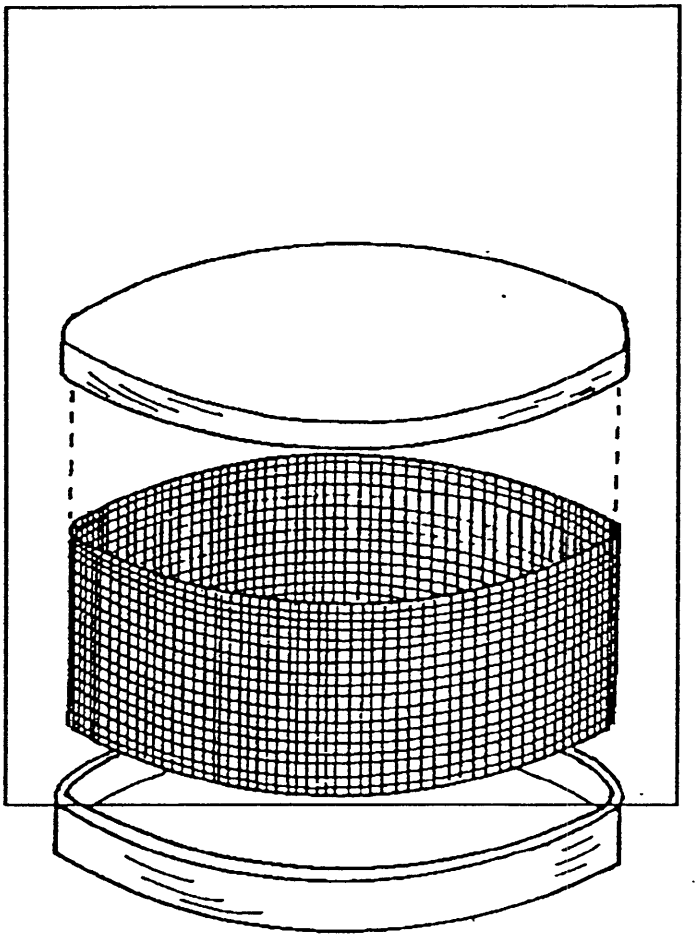
AVACHEM Sucrose Octanoate 40% is not toxic to honey bees.

Table 1. The mean percent mortality of honey bees treated with different rates of AVACHEM Sucrose Octanoate 40%, negative control and solvent control.

	Post Treatment		
Treatment	4 hours	24 hours	48 hours
5 g/bee	0	3.3	3.3
10 g/bee	0	0	0
20 g/bee	0	0	0
40 g/bee	0	0	0
80 g/bee	13.3	13.3	16.7
Negative control	6.7	10.0	10.0
Solvent control	0	3.3	6.7

FIGURE 1

DIAGRAM OF TEST CAGES



Disposable cage formed from two top or bottom halves of 150 x 15 mm petri plates and 18 x 2 inch (45.7 x 5.1 cm) wire screen strip stapled to form cylinder.

plus 20 ml of acetone

Dose: 5 ug/2 ml

Use 20 ml of 10 ug/2 ul
plus 20 ml of acetone

APPENDIX II

SOLUTION CALCULATIONS

Stock Solution

Concentration: 80 micrograms active ingredient/2 microliters

Volume: 40 milliliters acetone (40,000 microliters)

$\frac{X \text{ ug a.i.}}{40{,}000 \text{ ul}} = \frac{80 \text{ ug a.i.}}{2 \text{ ul}}$ X = 1,600,000 ug a.i.

1,600,000 ug a.i. = 1.6 gm a.i.

$\frac{1.6 \text{ gm a.i.}}{0.0327}$ = 48.929 gm material

Test substance active ingredient concentration = 40%

Test substance material needed for stock solution = $\frac{1.6}{0.40}$ gm = 4.0 gm

To make stock solution use:

4.0 gm material plus 40 ml acetone

Dose Dilution Calculations

Stock Solution: 80 ug a.i./2 ul

Dose: 40 ug/2 ul

Use 20 ml stock solution plus
20 ml of acetone

Dose: 20 ug/2 ul

Use 20 ml of 40 ug/2 ml solution
plus 20 ml of acetone

Dose: 10 ug/2 ml

Use 20 ml of 20 ug/2 ul

APPENDIX IV

HONEY BEE MORTALITY BY REPLICATE

Mortalities of honey bees 24 hours after being treated with different doses of AVACHEM Sucrose Octanoate 40%. Applications done 16 June. Prosser, WA.

Treatment	No. exposed	No. alive	No. dead	% Mortality
5 ug/bee				
Rep. 1	10	10	0	0
Rep. 2	10	9	1	10
Rep. 3	10	10	0	0
Total	30	29	1	3.3
10 ug/bee				
Rep. 1	10	10	0	0
Rep. 2	10	10	0	0
Rep. 3	10	10	0	0
Total	30	30	0	0
20 ug/bee				
Rep. 1	10	10	0	0
Rep. 2	10	10	0	0
Rep. 3	10	10	0	0
Total	30	30	0	0
40 ug/bee				
Rep. 1	10	10	0	0
Rep. 2	10	10	0	0
Rep. 3	10	10	0	0
Total	30	30	0	0
80 ug/bee				
Rep. 1	10	9	1	10
Rep. 2	10	8	2	20
Rep. 3	10	9	1	10
Total	30	26	4	13.3
Solvent Control				
Rep. 1	10	10	0	0
Rep. 2	10	9	1	10
Rep. 3	10	10	0	0
Total	30	29	1	3.3
Negative Control				
Rep. 1	10	9	1	10
Rep. 2	10	9	1	10
Rep. 3	10	9	1	10
Total	30	27	3	10

APPENDIX III

SOLUTION MIX PREPARATION

The solutions were prepared as follows:

Stock Solution

The stock solution was made first using 4.0 gm AVACHEM Sucrose Octanoate plus 40 ml distilled water plus 0.2% Tween 20 followed by the dilutions.

The 40 ug/2 ul solution was made using 20 ml stock solution plus 20 ml of distilled water plus 0.2% Tween 20.

The 20 ug/2 ul solution was made using 20 ml of 40 ug/2 ml solution plus 20 ml of distilled water plus 0.2% Tween 20.

The 10 ug/2 ml solution was made using 20 ml of 20 ug/2 ul plus 20 ml of distilled water and plus 0.2% Tween 20.

The 5 ug/2 ml solution was made using 20 ml of 10 ug/2 ul plus 20 ml of distilled water plus 0.2% Tween 20.

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AVACHEM SUCROSE OCTANOATE

HONEY BEES ACUTE CONTACT TOXICITY

DATA REQUIREMENT: EPA Ecological Effects Test Guidelines. OPPTS 850.3020, Honey Bee Acute Contact Toxicity. Public Draft. April, 1996.

PROTOCOL NUMBER: WSU Protocol No. 00-004

SPONSOR:
AVA CHEMICAL VENTURES LLC.
80 Rochester Avenue
Portsmouth, NH 03801

PRINCIPLE CONTACT:
Tony Barrington
(603) 431-4242
FAX: (603) 430-8029
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STUDY CONDUCTED BY:
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(509) 781-0552
FAX: (509) 786-9370
email: mayerd@wsu.edu

PRINCIPAL INVESTIGATOR: Dr. Daniel F. Mayer

PROPOSED STUDY INITIATION	June, 2000
PROPOSED EXPERIMENTAL START DATE	June, 2000
PROPOSED EXPERIMENTAL TERMINATION DATE	June, 2000
PROPOSED STUDY DATE COMPLETION	July, 2000

PROTOCOL APPROVAL:

STUDY DIRECTOR Daniel F. Mayer DATE 6/13/00
Daniel F. Mayer

SPONSOR REPRESENTATIVE _____ DATE _____
Tony Barrington

23 DFM 7/6/00

Mortalities of honey bees 48 hours after being treated with different doses of AVACHEM Sucrose Octanoate 40%. Applications done 16 June. Prosser, WA.

Treatment	No. exposed	No. alive	No. dead	% Mortality
5 ug/bee				
Rep. 1	10	10	0	0
Rep. 2	10	9	1	10
Rep. 3	10	10	0	0
Total	30	29	1	3.3
10 ug/bee				
Rep. 1	10	10	0	0
Rep. 2	10	10	0	0
Rep. 3	10	10	0	0
Total	30	30	0	0
20 ug/bee				
Rep. 1	10	10	0	0
Rep. 2	10	10	0	0
Rep. 3	10	10	0	0
Total	30	30	0	0
40 ug/bee				
Rep. 1	10	10	0	0
Rep. 2	10	10	0	0
Rep. 3	10	10	0	0
Total	30	30	0	0
80 ug/bee				
Rep. 1	10	9	1	10
Rep. 2	10	8	2	20
Rep. 3	10	8	2	20
Total	30	25	5	16.7
Solvent Control				
Rep. 1	10	10	0	0
Rep. 2	10	8	2	20
Rep. 3	10	10	0	0
Total	30	28	2	6.7
Negative Control				
Rep. 1	10	9	1	10
Rep. 2	10	9	1	10
Rep. 3	10	9	1	10
Total	30	27	3	10

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At initiation of the test three replicate test groups, each of ten bees will be dosed with each concentration of chemical and rates. Chemical will be mixed in acetone so that a given quantity, 2 microliters/bee of solution, will contain rates ug active ingredient (ai) of chemical.

The droplet application to the mesoscutum method allows for a precise exposure of the test substance that simulates a possible route of field exposure of the bees and other non-target insects. Exposure is most likely to occur from overspraying.

IDENTIFICATION OF TEST SUBSTANCE

The test substance will be AVACHEM Sucrose Octanoate EUP [40%] which contains 40% per gallon of active ingredient. The sponsor will supply all necessary information specifying the purity of the test substance. When the insecticide is received it will be logged on the GLP Test Substance Form and stored dark and dry in Dan Mayer's locked pesticide storage room at 60^0 to 80^0 F.

TEST INSECTS

The study will be conducted using European honey bees (*Apis mellifera* L.) belonging to Washington State University, IAREC, Prosser, WA. All hives will be maintained according to good beekeeping practices (Johansen and Burgett 1983).

Adult honey bees (3 to 5 weeks old) will be collected from the top lid and/or broodless top frames of three different colonies. One frame or lid with healthy adult worker honey bees will be removed from the top super of each adequately fed, healthy, queen-right, disease-free colony with known history and physiological status and placed in a holding box. The bees in the holding box will be taken to the laboratory.

The bees in the holding box will be anaesthetized with carbon dioxide to facilitate handling and transferred to separate Dixie cups (10 bees per cup). The bees will be held inside the cups until all bees in each one begin to move.

In order to control bias, bees will be impartially distributed to the Dixie cups and test cages. Tests will be conducted as soon after collection of bees as possible.

After the bees inside each cup begin to move and just prior to treatment they will be reanaesthetized with carbon dioxide for approximately 15 to 45 seconds. The 10 bees from each cup will be placed on their abdomen on a paper towel on the work bench. The test solution will then be applied using a calibrated Eppendorf or Gibson microsyringe and disposable tips. For each bee, 2 ul of solution will be drawn into the tip and then gently dispensed onto the top surface of the bee's mesoscutum. Three groups of 10 bees (total of 30 bees) will be treated with each solution concentration. Three groups of control bees will be treated as described above with 2 ul acetone. Three groups of negative control

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OBJECTIVE

The purpose of this test is to determine the acute contact toxicity of AVACHEM Sucrose Octanoate 40% end use product (40%) to adult worker honey bees (*Apis mellifera* L.) using the topical treatment method. A LD_{50} with 95% confidence limits will be calculated if possible.

These data are in partial fulfillment of EPA Ecological Effects Test Guidelines. OPPTS 850.3020, Honey Bee Acute Contact Toxicity. Public Draft. April, 1996.

SUMMARY

Five geometrically spaced dosages of AVACHEM Sucrose Octanoate 40% will be administered to groups of 30 adult worker honey bees. At initiation of the study each bee will receive a single dose of the chemical. The dose will be applied to the mesoscutum with an Eppendorf or Gibson micropipette.The bees will be observed after 4, 24 and 48 hours for toxicological response. A LD_{50} with 95% confidence limits will be calculated using Polo-PC Probit and Logit Analysis (Russell & Robertson, 1979).

JUSTIFICATION OF THE TEST SYSTEM

The honey bee is useful in evaluating the potential nontarget hazards of agricultural chemicals to nontarget insects since it is an important pollinator of various agricultural crops. There is also a substantial agrichemical data base on honey bees for which to compare the potential toxicities.

TREATMENTS

Treatment		Form	Dose Rates (ug/bee)
1.	AVACHEM Sucrose Octanoate	EUP [40%]	5, 10, 20, 40, 80
2.	Solvent Control	--	acetone
3.	Negative Control	--	none

METHODS AND MATERIALS

The methods, species used and route of exposure described in this protocol are based on procedures specified in the Environmental Protection Agency Ecological Effects Test Guidelines. OPPTS 850.3020, Honey Bee Acute Contact Toxicity. Public Draft. April, 1996.

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- the average mortality for the total number of control must not exceed 10 per cent at the end of the test.

RECORDS TO BE MAINTAINED

Records will be maintained according to guidelines set out in 158 CFR Subdivision L hazard evaluation: non-target insects and shall include but not be limited to the following:

1. A copy of the signed protocol and any and all amendments.
2. Dates of initiation, termination and duration of study.
3. Bee source, strain, conditions of colonies and age of bees.
4. Identification and characterization of the test substance as provided by company.
5. Environmental conditions during the study: in field and laboratory as related to pesticide applications and maintenance of the bees.
6. Dosage calculations, preparation and application methods.
7. Observation records.
8. Statistical calculations.
9. A copy of the final report.
10. Dates and study phases audited by the Quality Assurance Unit and the audit reports.
11. Sample chain of custody.

FINAL REPORT

Upon completion and analysis, a report will be issued to document the laboratory results and present appropriate statistical analyses necessary for an EPA review of the data. This report will be written according to EPA Data Reporting Guidelines (DRGs) and Standard Evaluation Procedure (SEPs). Original raw data will be sent to the sponsor with the final report.

A draft report of the results of the study will be prepared and submitted to the sponsor within 30 working days after the study is completed. A report will include, but not be limited to, the following:

1. Name and address of the facilities performing the study.
2. Objectives and procedures stated in the approved protocol. If changes are made in the original protocol those changes will be included.
3. Dates on which the study was initiated and completed.
4. If provided by company, the name, chemical abstracts number or code number, strength, purity and composition of the material.
5. A description of the methods used.
6. A description of the test system used. The final report shall include the number of bees used, source of supply, species, approximate age, dosage regimen, and method of application.

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~~00-002~~ 00-004 DFM 6/16/00

bees will be handled as described above but will not be treated. After treatment each group of bees will be transferred to cages.

TEST SOLUTIONS

The test substance will be mixed in acetone so that a given quantity, 2 microliters/bee of solution, will contain a known amount of the active ingredient (ai) of the chemical. The most concentrated solution will be prepared first, and appropriate dilutions made from it. All insecticide solutions will be adjusted to 100% active ingredient.

OBSERVATION DESIGN

The cages will be made from a circular insert formed from strips of metal screens (6.7 meshes/cm) 45 cm long and 5 cm wide. For the top and bottom 15 cm diameter petri plates will be used.

Bees will be fed during testing by providing cotton squares (5 x 5 cm) soaked with 50% sucrose solution (1:1 ratio) and placed in the cages.

Bees in the test cages will be held in the dark at 70 to 85 degrees F. and relative humidity between 50 and 80 percent during the test period.

Bees will be observed for mortality and toxicological responses at approximately 4, 24 and 48 hours after dosing and responses recorded by dosage level. The number of live and dead bees will be determined at 4, 24 and 48 hours after the bees have been treated. Mortality will be expressed in percent.

STATISTICAL ANALYSIS

LD_{50} values (ug/bee) will be calculated using a computerized probit analysis program (Polo-PC Probit and Logit Analysis (Russell & Robertson, 1979)). The LD_{50} will be used to classify the toxicity of the pesticide according to the scheme of Johansen and Mayer (1990).

Highly Toxic:	Less than 2 micrograms per bee
Moderately Toxic:	Greater than or equal to 2 micrograms per bee but less than 11 micrograms per bee
Relatively Non-Toxic:	Greater than or equal to 11 micrograms per bee

VALIDITY OF THE TEST

For the test to be valid the following conditions apply:

~~00-002~~
00-004 DFM 6/16/00

REFERENCES

Johansen, C.A. and D.M. Burgett. 1883. Beekeeping. Wash. St. Univ. Coop. Ext. Publ. 79. 27 pp.

Johansen, C.A. and D.F. Mayer. 1990. Pollinator protection: A bee and pesticide handbook. Wicwas Press, Cheshire, CT. 212 pp.

Russell, R.M. and J.L. Robertson. 1979. Programming probit analysis. Bull. Entomol. Soc. Amer. 23;209- 213.

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~~00-002~~ 00-004 DFM 6/16/00

7. A description of all circumstances that may have affected the quality of integrity of the data.
8. Statistical methods employed for analyzing the data.
9. The name of the Principal Investigator and the names of other professionals and supervisory personnel involved in the study.
10. A summary and analysis of the data, and a statement of the conclusion drawn from the analysis.
11. The location where all specimens, raw data, and the final reports are to be stored.
12. A signed statement prepared by the QA Officer listing the dates that study inspections were made and the phases of the study inspected.
13. If it is necessary to make corrections or additions to a final report after it has been accepted, such changes shall be in the form of amendment by the Study Director. The amendment should clearly identify the part of the final report that is being added to or corrected and the reasons for the correction or addition. The amendment shall be signed and dated by the person(s) responsible.
14. If appropriate a copy of the Certificate of analysis as an appendix.

QUALITY ASSURANCE

The study will be conducted according to Good Laboratory Practices in 40 CFR Part 160 and examined by the Quality Assurance Officer for compliance of the GLP's and the specified protocol. Kathy Britt of Ron Britt & Associates will be the Quality Assurance Officer for this project.

AMENDMENTS

No changes will be made without the specific written consent of the Study Director in the form of a protocol amendment. An assessment of the affect of the deviations on the study must also be given. All protocol amendments will be signed by the Study Director and Sponsor and included as the raw data in the achieved report.

SOPS

1.6 Protocol Contents, Deviations and Amendments
1.7 Documentation of Phone Calls and Correspondence
3.0 Pesticide Receipt and Storage
3.1 General Procedures-Archives
4.0 Calibration of Sartorius B310s Electronic Scale
4.5 Calibration of Thermometers
4.8 Calibration of Eppendorf Digital Pipette
6.0 Maintenance of Honey Bee Colonies
6.1 Procedures for LD_{50} Studies on Honey Bees (Apis mellifera)
8.0 Collection and Handling of Raw Data

28 DFM 7/16/00

GLP TEST SUBSTANCE USE LOG octanote

Name AVACHEM Sucrose Octanote DFM 6/13/00

Manufacturer AVA Chemical Ventures LLC

Formulation End use Product

AI Information 40 %

#\Description of Containers 1 glass bottol

Amount Recieved 100 gms

Lot # Batch # 503299

MSDS yes ✓ no ____

Date Recieved 6/13/00

Reciever D. Mayes

Date Returned ____

Amount Returned ____

Condition upon Reciept good

EPA Registration # ____

Physical Description of Material brown liquid

EPA Established # ____

Date	Amount Used	Amount Left	Initial
6/16/00	4 ~~ml~~ gm 6/16 DFM WB	96 gms	DFM
6/16/00	4 gms	92 gms	DFM

COMMENTS ____

~~00-002~~
00-004 (WR)
DFM
6/16/00

DEVIATION NUMBER ONE TO PROTOCOL

Study Title.

AVACHEM SUCROSE OCTANOATE HONEY BEES ACUTE CONTACT TOXICITY

Project Identification Code.

Bee Group WSU No. 00-004

Description of Deviation

On page 3 of the protocol in the first paragraph, the second sentence is as follows, "Chemical will be mixed in acetone so that a given quantity, 2 microliters/bee of solution, will contain rates ug active ingredient (ai) of chemical". SOP 6.1, revision 5 also states the chemical should be mixed in acetone.

The chemical was mixed in distilled water plus 0.2% Tween 20.

Reason for the Deviation

The chemical would not dissolve in acetone. Distilled water plus Tween is an acceptable and often used in bee toxicity studies. This was also a deviation from

Impact of the Change on the Study

The change had no impact on the study.

Study Director Daniel F. Mayer Date 6/16/00

Daniel F. Mayer

Principal Investigator Daniel F. Mayer Date 6/16/00

Daniel F. Mayer

Sponsor Anthony Bartlett Date 6/27/00

ANTHONY BARRETT

6/16/00

ANACHEM Sucrose Octanoate 40% JDL
Acute Contact

Stock Solution

Concentration: 80 ug ai / 2 ul
Volume : 40 ml acetone (40,000 ul)

$\frac{x \text{ ug ai}}{40{,}000 \text{ ul}} = \frac{80 \text{ ug ai}}{2 \text{ ul}}$ $x = 1{,}600{,}000 \text{ ug ai}$

1,600,000 ug ai = 1.6 gm ai

$\frac{1.6 \text{ gm ai}}{0.40} =$ 4.0 gm material

To make stock solution use:
4.0 gm material plus
40 ml acetone

Octanoate

WSU # 00-004

D-Mays 6/13/00

6/13/00 Recived AVA chem Sucrose octanoate & entered information on GLP substance log & put chemical in Pesticide Storage DFM

6/14/00 Checked honey bee colonies DFM

6/15/00 Calibrated sartorius scale
Calibrated max-min themometers - lab., rearing Chamber, chemical storage
Calibrated psycometer
Calibrated Eppendorf Maxipetter DFM

6/16/00 Jeff calibrated micropetpetter
Dan mixed 900 ml of Western Family pure granuated sugar with 900 ml of distilled water
made cages

9:05 Jeff calculated amounts & started making solutions

9:20: Weighed out test substance and diluted with 40 ml acetone. Made serial dilutions with acetone. TDL

9:49 Two high rates settled out - so remixed & weighed out test substance and diluted with 40 ml acetone, Made serial dilutions with acetone. DFM

10:05 Weighed out 4.002 gm
The ai again settled out in acetone - decided to use distilled water + 0.2% Tween-20

D Mayer 6/16/00

6/16/00

Tween 20 = Polyoxyethylene sorbitan monolaurate - Sigma Ultra P-7949 Lot 87H0992 6005-6R-5

calibrated Blue push button Eppendorf microsyringe to measure out the tween

Put 400 ul of Tween 20 into 200 mls of water & put on stirer to mix for about 1 minute

11:00 started to mix weighted out test substance & diluted with 40 ml of tween water mix, Made serial dilutions from stock.

The solutions have some sudgying. Done making solutions at 11:08.

started treating bees at 11:10

Dose Dilution Calculations 6/16/00
JDL

Stock Solution: 80 ug ai / 2 ul

Dose: 40 ug / 2 ul
Use 20 ml stock solution plus
20 ml acetone.

Dose: 20 ug / 2 ul
Use 20 ml of 40 ug / 2 ul solution
plus 20 ml acetone.

Dose: 10 ug / 2 ul
Use 20 ml of 20 ug / 2 ul solution
plus 20 ml acetone.

Dose: 5 ug / 2 ul
Use 20 ml of 10 ug / 2 ul solution
plus 20 ml of acetone

DMcCoy
6/16/00

6/16/00

GLP FORM FOR LD50 TEST SOLUTION PREPARATION

WSU STUDY NUMBER 00-004 DATE: 6/16 No. of Treatments 5

Eppendorf Maxi Microsyringe Calibration: Date 6/15 Init DFM JDL

Eppendorf Microsyringe Calibration Date 6/16 Init DFM + JDL

Sartorius Scale Calibration Date 6/15 Init DFM JDL

Solute ✓ Acetone _____ Water

Test Substance Avachem Sucrose octanoate Formulation liquid

Percent Active Ingredient 40%

Treatment #	Rate (ug ai/bee)	ug ai needed for 20 ml solution	Amount stock solution needed (ml)	Amount of solute needed (ml)
1	80	800,000	40 ml	0
2	40	400,000	20 ml	20
3	20	200,000	20 ml of Trt # 2	20
4	10	100,000	20 ml of Trt # 3	20
5	5	50,000	20 ml of Trt # 4	20
6				
3				

Stock Solution Preparation Volume 40 ml

Concentration Required 40 ug/ul

Calculations

Stock Solution concentration 80 ug ai / 2 ul

" " Volume 40 ml (40,000 ul)

$\frac{x \text{ ug ai}}{40{,}000 \text{ ul}} = \frac{80 \text{ ug ai}}{2 \text{ ul}}$ $x = 1{,}600{,}000 \text{ ug ai} = 1.6 \text{ gm ai}$

Test substance active ingredient concentration = 40%

Test substance material needed for stock solution = $\frac{1.6 \text{ gm}}{0.40}$

$= 4.0 \text{ gm}$

To make stock solution use:

4.0 gm material plus

40 ml acetone

D. Mayer
6/16/00

Tween Concentration

Stock solution 100 ml
Tween 1 ml

200 ml Tween 20 in 100 ml
400 ml " " " 200 ml

in Laboratory DFM 6/22/00 (WR)

WR 6/22/00 DFM

GLP FORM FOR TEMPERATURES AND HUMIDITY IN REARING CHAMBER

WSU STUDY NUMBER 00-004 Signature D. Mayer Date 6/15/00

Laboratory
~~Rearing chamber~~ thermometer calibrated by D. Mayer Date 6/15

~~Rearing chamber~~ psycometer calibrated by D. Mayer Date 6/15

Temperature in Rearing Chamber:

DATE 6/16 TIME 1030 MAX TEMP. 23 MIN TEMP. 22 INT. DFM
DATE 6/16 TIME 1145 MAX TEMP. 24 MIN TEMP. 22 INT. DFM
DATE ____ TIME ____ MAX TEMP. ____ MIN TEMP. ____ INT. ____
DATE ____ TIME ____ MAX TEMP. ____ MIN TEMP. ____ INT. ____
DATE ____ TIME ____ MAX TEMP. ____ MIN TEMP. ____ INT. ____
DATE ____ TIME ____ MAX TEMP. ____ MIN TEMP. ____ INT. ____
DATE ____ TIME ____ MAX TEMP. ____ MIN TEMP. ____ INT. ____
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DATE ____ TIME ____ MAX TEMP. ____ MIN TEMP. ____ INT. ____
DATE ____ TIME ____ MAX TEMP. ____ MIN TEMP. ____ INT. ____
DATE ____ TIME ____ MAX TEMP. ____ MIN TEMP. ____ INT. ____
DATE ____ TIME ____ MAX TEMP. ____ MIN TEMP. ____ INT. ____

Relatinve Humidity in Rearing Chamber:

DATE 6/16 TIME 12:15 RH 52 INT. DFM
DATE 6/16 TIME 6 pm RH 60 INT. DFM
DATE 6/18 TIME 2 pm RH 63 INT. DFM
DATE ____ TIME ____ RH ____ INT. ____
DATE ____ TIME ____ RH ____ INT. ____
DATE ____ TIME ____ RH ____ INT. ____
DATE ____ TIME ____ RH ____ INT. ____
DATE ____ TIME ____ RH ____ INT. ____
DATE ____ TIME ____ RH ____ INT. ____
DATE ____ TIME ____ RH ____ INT. ____
DATE ____ TIME ____ RH ____ INT. ____
DATE ____ TIME ____ RH ____ INT. ____
DATE ____ TIME ____ RH ____ INT. ____

8

GLP FORM FOR COLLECTING HONEY BEES

WSU STUDY NUMBER 00-004 TYPE OF HONEY BEE: European

DATE: 6/16/00 SIGNATURE D. Maye

HOW LONG AFTER APPLICATION:

2 HR RESIDUES _____ 8 HR RESIDUES _____

24 HR RESIDUES _____ HR RESIDUES _____

LD50 STUDY ✓

TIME COLLECTED HONEY BEES: 9:25 PERSON DF3

NUMBER OF COLONIES HONEY BEES COLLECTED FROM: 3

BEES COLLECTED FROM FRAMES: _____
OR LID TOPS: ✓

TIME ANAESTHETIZED BEES: 9:35 50cc

NUMBER OF BEES PER CUP: 10 PERSON Dan + Jeff

HONEY BEES REANETHETIZED _____ Yes or No ✓

TIME STARTED COUNTING BEES & PUT IN CUPS: 9:38

TIME FINISHED COUNTING BEES & PUT IN CUPS: 9:45

IMPARTIALLY PUT BEES IN CHAMBERS: ✓ Yes or No _____

TIME STARTED PUTTING BEES IN CHAMBERS: 11:10 PERSON DFM & JD

TIME FINISHED PUTTING BEES IN CHAMBERS: 11:50

TIME PUT BEES IN REARING CHAMBER: 11:55

AS PER PROTOCOL YES ✓ NO _____

IF NO, WHY? -

D. Mayr
6/20/00

WSU STUDY NO. WSU Study #00-004 6/20/00

LD50 ACUTE TOXICITY

12:00 noon HOURS				6/18 DATE				_____ HOURS				_____ DATE			
Trts	Dead	Live	%	Trts	Dead	Live	%	Trts	Dead	Live	%	Trts	Dead	Live	%
Neg. Control		0 6/20/00	10	20	gms										
1	1	9	10	1	0	10	0								
2	1	9	↓	2	0	10	0								
3	1	9	10	3	0	10	0								
	3	27	10		0	30	0								
Solvent Control				10	gms										
1	0	10	0	1	0	10	0								
2	2	8	20	2	0	10	0								
3	0	10	0	3	0	10	0								
	2	28	6.7		0	30	0								
80 gms				5	gms										
1	1	9	10	1	0	10	0								
2	2	8	20	2	1	9	10								
3	2	8	20	3	0	10	0								
	5	25	16.7		1	29	3.3								
40 gms															
1	0	10	0												
2	0	10	0												
3	0	10	0												
	0	30	0												

DFM 6/22 (WR) Rearing chamber

GLP FORM FOR TEMPERATURES IN ~~LABORATORY~~ Rearing chamber & CHEMICAL STORAGE

WSU STUDY NUMBER 00-004 SIGNATURE D. Mayer Date 6/15/00

DFM 6/22/00 (WR) ~~Laboratory~~ Rearing Chamber thermometer calibrated by D. Mayer Date 6/15/00

Chemical storage thermometer calibrated by D. Mayer Date 6/15/00

DFM 6/22 (WR) ~~Laboratory~~ Rearing chamber: ____ F° or C° ✓

D.FM 6/22/00 (WR) should be 24

DATE	TIME	TEMP.	INT.
6/15	1pm	24	DFM
6/16	0830	26 ~~20~~	DFM
6/16	4:00 pm	~~24~~ 25	JDL
6/17	12:00 noon	21-25	JDL
6/18	12:00 noon	23-26	JDL

CHEMICAL STORAGE: ✓ F° or C° ✓

DATE	TIME	MAX TEMP.	MIN TEMP.	INT.
6/15	1:30pm	25 C	22 C	DFM
6/16	0900	75° F	68 F	DFM F

a

D. Moyer 6/20/00

Phone Correspondence Log

Study name and number 00-004 Avachem Sucrose octanoate

Date	Time	Person Spoke To	Matter Concerning
6/20	11:00	Tom's answering machine	Biol. part of test done - result
6/22		"	How much material did he send
6/23	11:30	my voice mail	100 gms
6/27		Anthony	Managing Member - sign divulgation

D Mayr

WSU STUDY NO. 00-004 6/16/00

6/20/00

LD50 ACUTE TOXICITY

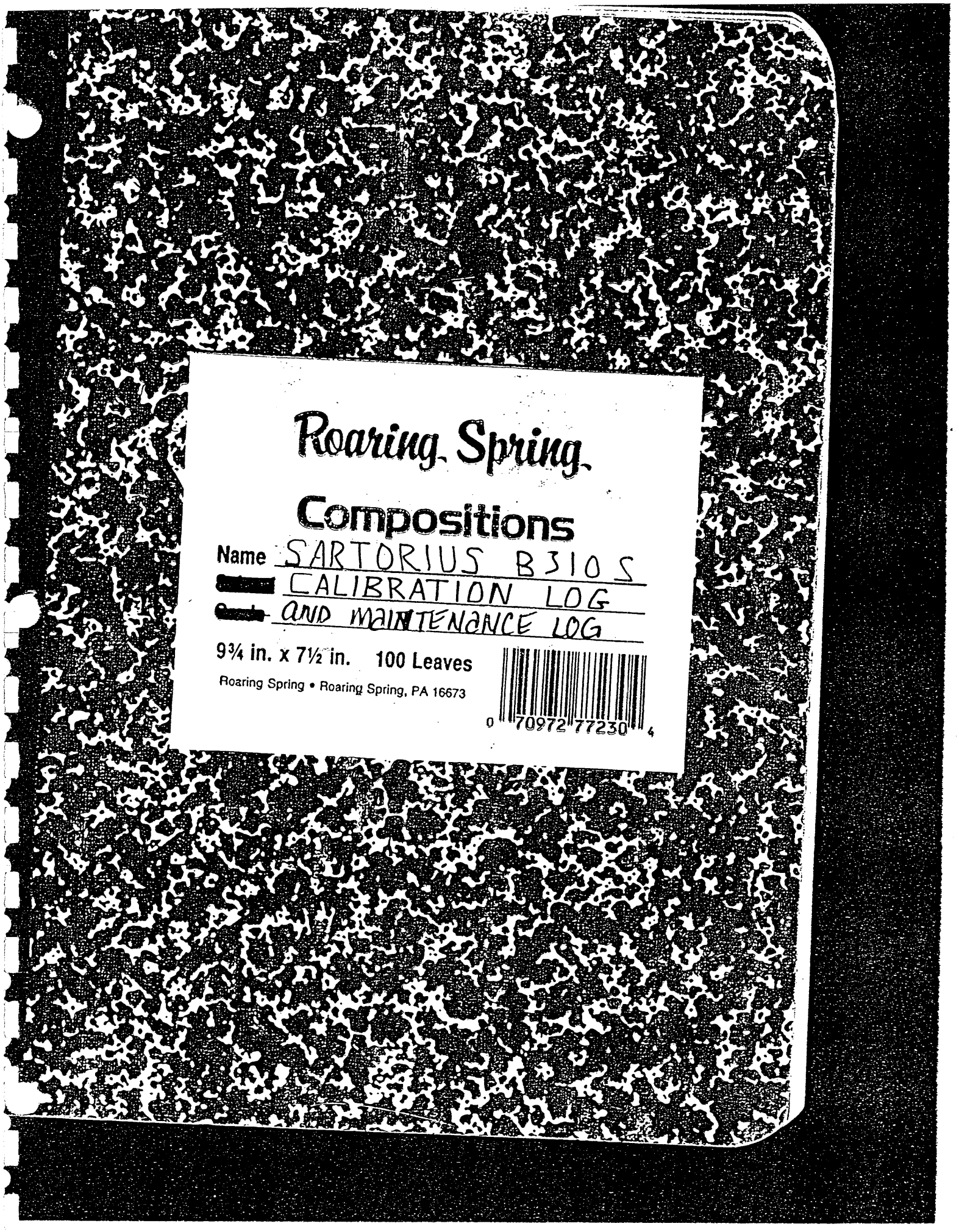
4:00 pm HOURS 6/16/00 DATE

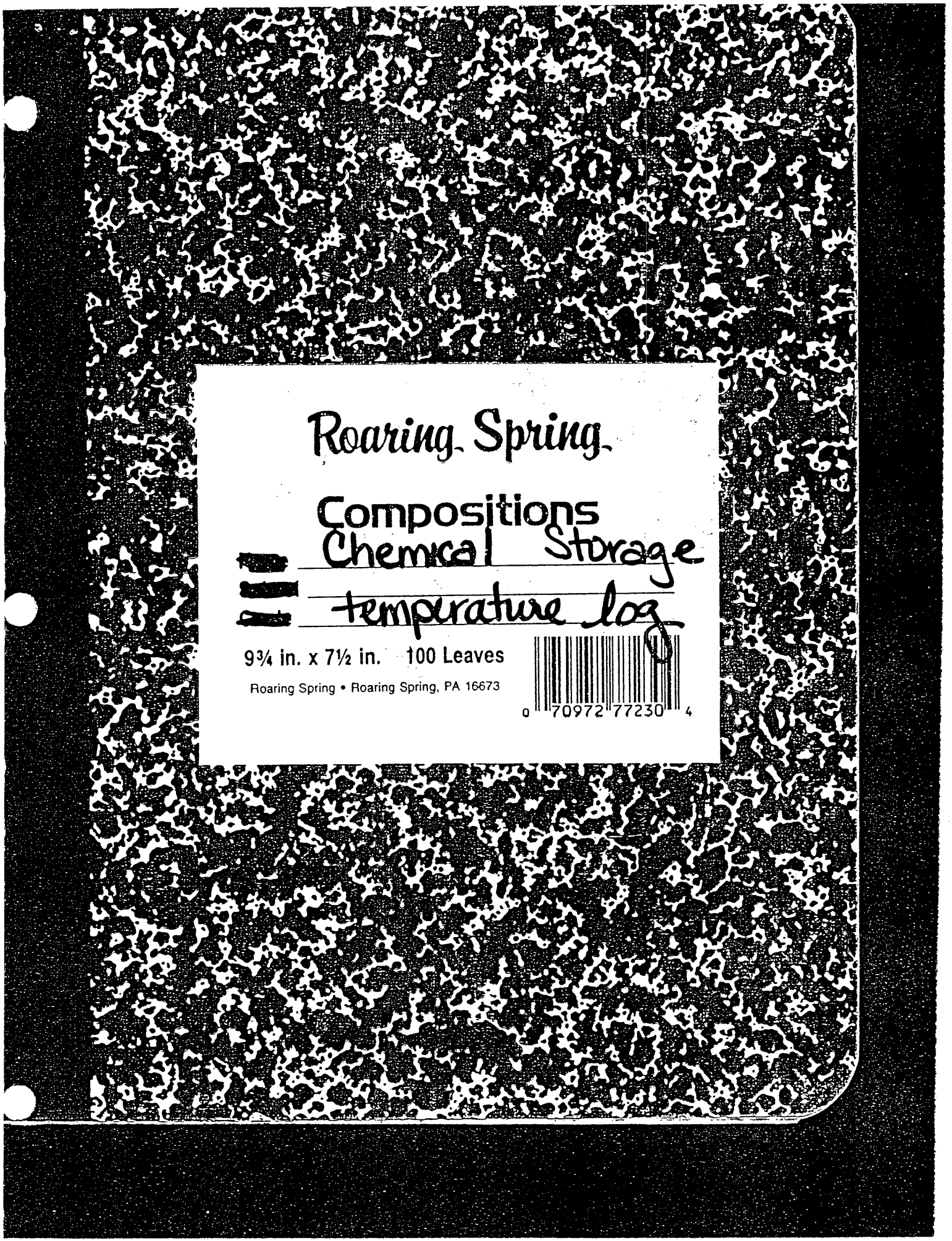
Trts	Dead	Live	%	Trts	Dead	Live	%
Neg Control				20 gms			
1	0	10	0	1	0	10	0
2	1	9	10	2	0	10	0
3	1	9	10	3	0	10	0
	2	28	6.7		0	30	0
Solvent Control				10 gms			
1	0	10	0	1	0	10	0
2	0	10	0	2	0	10	0
3	0	10	0	3	0	10	0
	0	30	0		0	30	0
80 gms				5 gms			
1	1	9	10	1	0	10	0
2	1	9	10	2	0	10	0
3	2	8	20	3	0	10	0
	4	26	13.3		0	30	0
40 gms							
1	0	10	0				
2	0	10	0				
3	0	10	0				
	0	30	0				

12:00 noon HOURS 6/17 DATE

Trts	Dead	Live	%	Trts	Dead	Live	%
Neg control				20 gms			
1	1	9	10	1	0	10	0
2	1	9	10	2	0	10	0
3	1	9	10	3	0	10	0
	3	27	10		0	30	0
Solvent Control				10 gms			
1	0	10	0	1	0	10	0
2	1	9	10	2	0	10	0
3	0	10	0	3	0	10	0
	1	29	3.3		0	30	0
80 gms				5 gms			
1	1	9	10	1	0	10	0
2	2	8	20	2	1	9	10
3	1	9	10	3	0	10	0
	4	26	13.3		1	29	3.3
40 gms							
1	0	10	0				
2	0	10	0				
3	0	10	0				
	0	30	0				

MM/dd/yr	1gr	5g	10g	50g (Calibrated Mass (cc))	Initials	Recal. br. ?
08/09/99	1.000	5.000	10.001	100.013	JDL	No
08/27/99	1.000	5.000	10.001	100.003	DFM	No
6/15/00	1.001	5.000	10.001	50.003	DFM	No


Roaring Spring
Compositions
Name SARTORIUS B310S
CALIBRATION LOG
AND MAINTENANCE LOG
9¾ in. x 7½ in. 100 Leaves
Roaring Spring • Roaring Spring, PA 16673
0 70972 77230 4


Roaring Spring
Compositions
Chemical Storage
temperature log
9¾ in. x 7½ in. 100 Leaves
Roaring Spring • Roaring Spring, PA 16673
0 70972 77230 4

Roaring Spring

BLUE RUTTON

Compositions

Name [illegible]URF DIGITAL

School PIPETTE 4710

Grade CALIBRATION LOG

9¾ in. x 7½ in. 100 Leaves

Roaring Spring • Roaring Spring, PA 16673

0 70972 77230 4

Roaring Spring

Compositions

Name Psychrometer

School

Grade RH

9¾ In. x 7½ in. 100 Leaves

Roaring Spring • Roaring Spring, PA 16673

D. Max 6/15/00

		Max	min
DFM	6/7/99	76° ~~76°~~ F	72°F
DFM	6/11/99	75°F	71°F
DFM	6/14/99	75°F	72°F
DFM	6/16/99	76°F	71°F
DFM	6/23/99	78F	73°F
DFM	8/2/99	76°F	72°F
DFM	8/10/99	77°F	72°F
DFM	8/16/99	78°F	73°F
DFM	8/23/99	78°F	72°F
DFM	8/30/99	79°F	73°F
DFM	9/10/99	79°F	72°F
DFM	9/23/99	78°F	71°F
DFM	7/9/99 late entry DFM 12/14/99	74F	73F

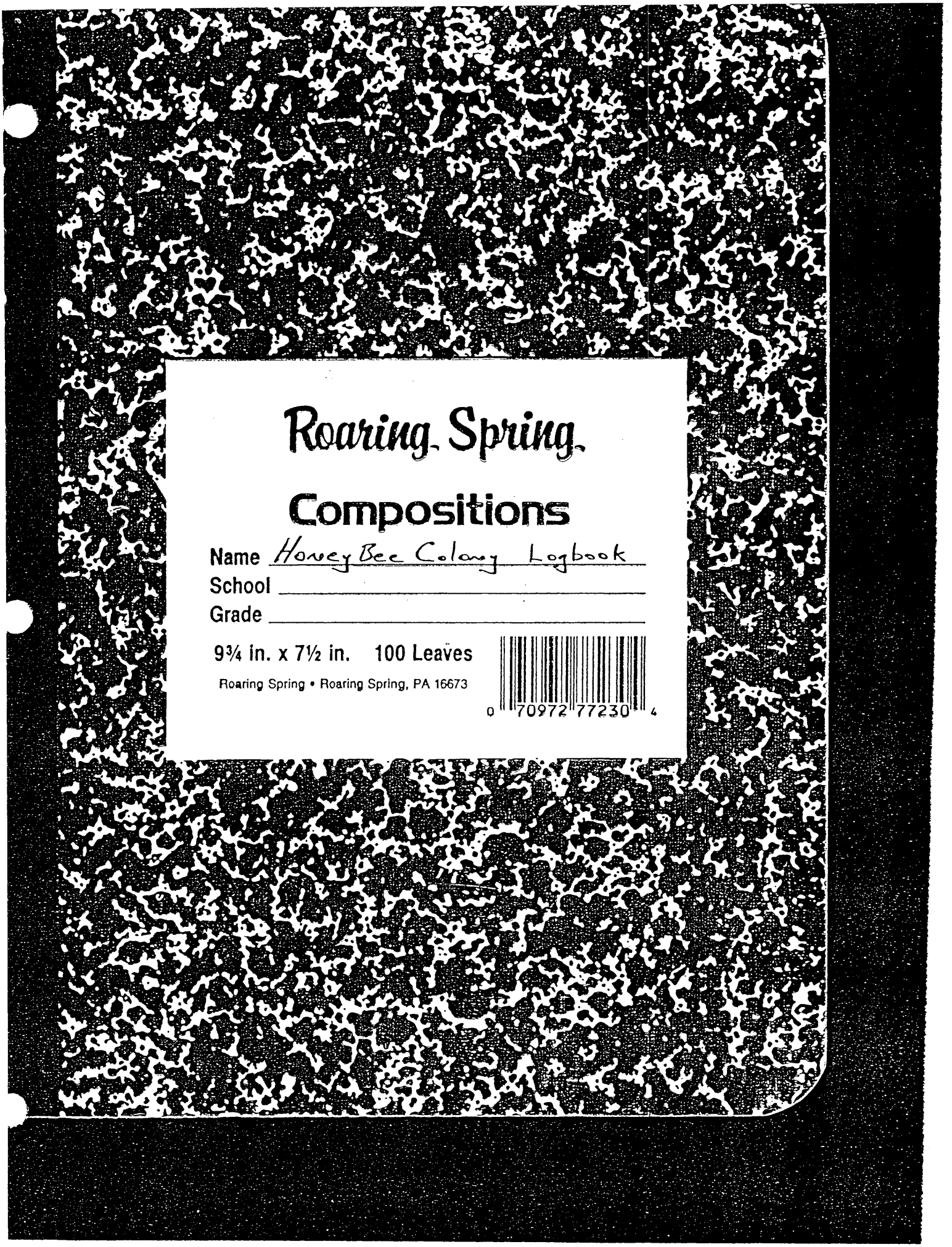
6/15/00 Calibration of max-min. thermometer in chemical storage

GLP Thermometer #1 : -23°C

GLP Thermometer #2 -23°C

max-min Thermometer -23°C

DFM 6/15


Roaring Spring
Compositions
Name Honey Bee Colony Logbook
School
Grade
9¾ in. x 7½ in. 100 Leaves
Roaring Spring • Roaring Spring, PA 16673
0 70972 77230 4

8/27/99 Calibration of Psychrometer

DMerryl GLP thermometer #1 - 23°C

" " #2 - 23°C

Psychrometer - 23°C

6/15/00 Calibration of Psychrometer

DMerryl GLP Thermometer #1 - 24°C

GLP Thermometer #2 - 24°C

Psychrometer - 23.8°C

Roaring Spring

Compositions

Name Eppendorf Maxipettor

[redacted] CALIBRATION LOG

[redacted] #1

9¾ in. x 7½ in. 100 Leaves

Roaring Spring • Roaring Spring, PA 16673

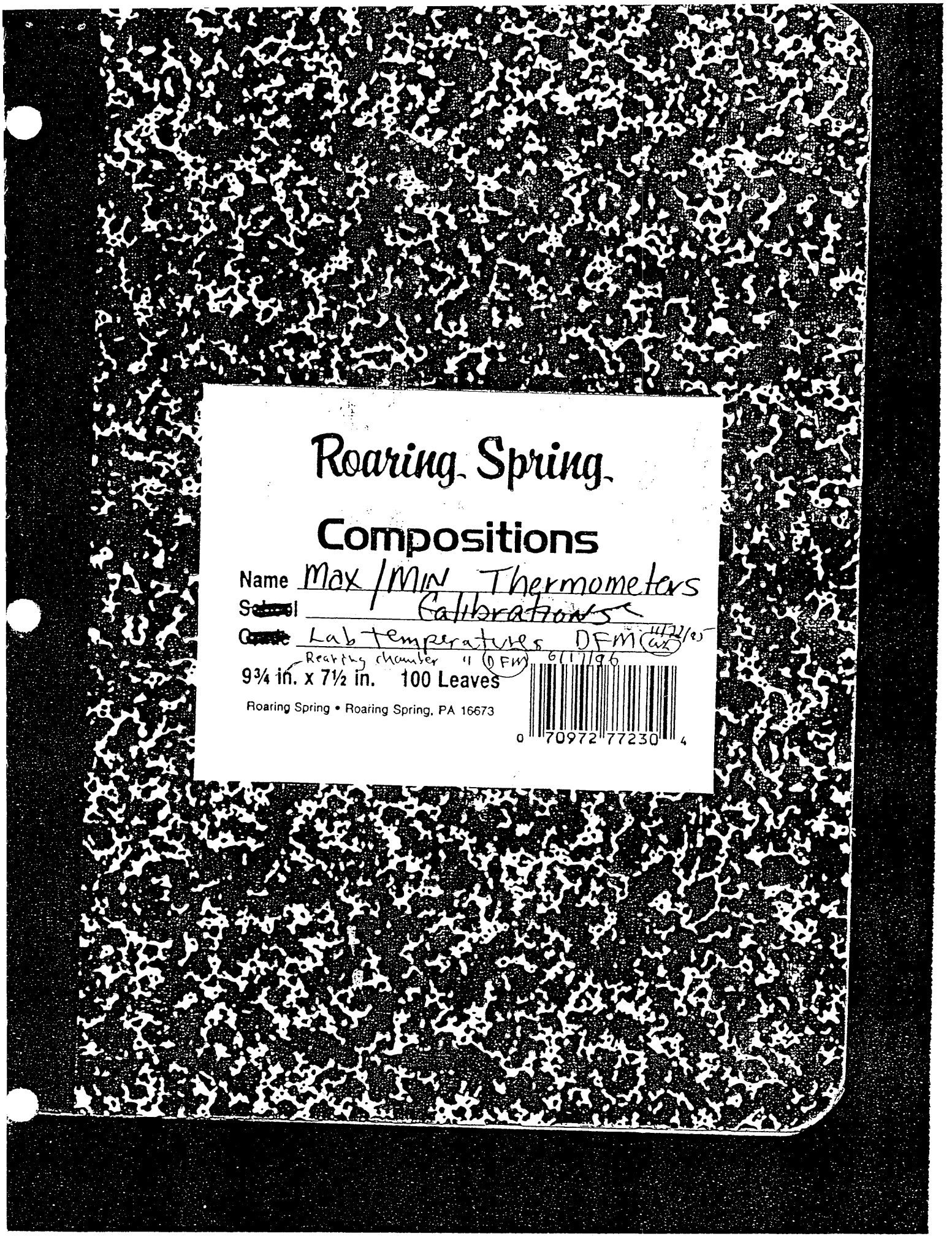
6/14/99 Inspected all colonies for health.
All appeared healthy except the colony
in the southwest corner. 6/14/99
D Mayer

7/1/99 Inspected all honeybee colonies for
health. All appeared healthy. Colonge
in southwest corner is growing +
appers to be healthy now. Do
not use this colony for GLP
tests yet however. Jeff Lucas

8/7/99 Inspected all HB colonies for health.
All appeared healthy. D. Mayer

9/23/99 Inspected all HB colonies for health
& all appeared healthy. D Mayer

6/14/00 Inspected the 3 HB colonies we will
use bees from on test to be done on
Fri. All three were healthy, queenright
& had eggs. they are about 10 frames
of bees/colony. D Mayer


Roaring Spring
Compositions
Name Max / Min Thermometers
School Calibrations
Course Lab temperatures DFM 11/22/95
Roaring chamber 11 DFM 6/17/96
9¾ in. x 7½ in. 100 Leaves
Roaring Spring • Roaring Spring, PA 16673
0 70972 77230 4

Omaxe 6/15/98

Calibration for AVA Study #00-004

Dispensing #	ug/gm
1	4.998
2	4.997
3	5.002
4	4.997
5	4.998
6	5.003
7	5.002
8	4.997
9	4.999
10	5.002

49.995 ÷ 10 = 4.9995

% error $\frac{1 - (4.995)}{5} \times 100$

= 0.190

DANIEL F. MAYER, PH.D.
THE BEE GROUP
24106 NORTH BUNN ROAD
PROSSER, WA 99350
(509) 781-0552
EMAIL: mayerd@wsu.edu

6 July 2000

Kathleen Britt
RON BRITT & ASSOCIATES
POB 8336
Yakima, WA 98908
(509) 966-9681

Dear Kathy:

I am responding to your Audit of 6 July 2000 of the first draft of the final report for WSU Bee Group Study #00-004, AVACHEM SUCROSE OCTANOATE HONEY BEES ACUTE CONTACT TOXICITY.

Page Number

#4 QA statement (statement & CV to be submitted) which you will submit.

#8 No Date should be No Data

Date has been changed to Data. Spelling error.

#11 Solution Calc./Prep..... (ai) of chemical <was applied>

was applied has been added to the sentence for greater clarity.

#12 1rst paragraph was > were
2nd paragraph was > were

Was has been changed to were in both sentences as per verb agreement.

#19 5 ul # alive = 29

Under the 5 ul column the 30 has been changed to a 29 as per the raw data.

6/15/00 Calibration for max-min thermometers in lab & rearing chamber

DFM GLP Thermometer #1 - 24°C

GLP Thermometer #2 - 24°C

Lab max-min - 24°C

Rearing chamber max-min - 24°C

Calibration for

CURRICULUM VITAE FOR RON BRITT & ASSOCIATES PERSONNEL

NAME & TITLE

Kathleen Britt
Comptroller, QA

EDUCATION

SCHOOL	CERTIFICATE OR DEGREE	DATES OF ATTENDANCE
Central Washington University	BA Education	1973-1974
Washington State University	BA Foreign Languages	1970-1972
Yakima VAlley College	AA	1968-1970
Lakes High School		1965-1968

PROFESSIONAL EXPERIENCE

POSITION/TITLE	INSTITUTION/COMPANY	DATES
Office Manager	Ron Britt & Associates, Inc.	1976 – Present
OA for outside businesses	Ron Britt & Associates, Inc	1998 - Present

TRAINING/CERTIFICATION

Small Business Workshop	1979
QA Basic Training	1989
QA Advanced Training	1990

PROFESSIONAL MEMBERSHIP/ACTIVITIES

Past:

PTSA President	1988-1989, 1989-1990 School Years.
PTSA Secretary	1987-1988
PTSA Legislative Rep	1986-1987
Counselor for Summitview counselling program	1985-1986
PTSA Volunteer Chairman	1985-1986
Enrichment Program at Summitview	1984-1987
WAFLT	1974-1976

The original doc...
KB ... 7/6/00

#22 also deviation from SOP 6.1 rev 5

Also a deviation from SOP 6.1, revision 5 has been added to complete the sentence.

#9 raw data chem. storage temp.
6/16 4 pm 24-25 (24 is a write over)

The 24 is a write over and the correction has been dated, initialed and error code added.

Sus. Use Amount left and initials

The amount left has been entered and duteously initialed.

Are you returning the material left? if retaining - where?

I plan to return the material.

Chem. Storage Log - 2000 date 6/7/99: max temp. is a write over.

The max temp. is a write over and the correction has been dated as a late entry, duteously initialed and error code added.

Thank you for your help on this project and the rapid response on the audit of the first draft of the final report.

Sincerely,

Daniel F. Mayer, Ph.D.
Entomologist

Kathleen Britt

CONTINUING TRAINING/EXPERIENCE HISTORY

10-10-89	QA Basic Training Course - San Diego	8 Hrs.
10-11,12,13-89	SQA Conference - San Diego	3 Days
11-6,7,8-89	NAICC Conference - Las Vegas	2 1/2 Days
1-90	Reviewed & Audited all Company SOPs	1 Week
5-3-90	MAAG Audit	3 Hrs.
7-6-90	Safety Meeting	1/2 Hr.
9-26-90	Uniroyal Audit	1 Hr.
10-2-90	QA Advanced Training - Orlando	3 3/4 Hrs.
10-2,3,4-90	SQA Conference - Orlando	2 1/2 Days
2-27,28-91	Ciba Geigy GLP/QA Training Course	2 days
3-5-91	ICI Audit	5 hours
3-25-91	First Aid Class	8 hours
6-4-91	ETI Audit	4 hours
8-7-91	AmCy Audit	4 hours
10-15-91	SQA Professional Enhancement Seminar	3 hours
10-16,17,18-91	SQA Meetings	3 days
10-24-91	Information Meeting - Merck	3 hours
11-10,11,12-91	NAICC Meetings	3 days
1-6-92	Pheromone Meeting with Dr.Howell	1 1/2 hours
1-17-92	Simplot Grower Meeting	5 hours
2-2,3,4,5-92	AAIE Meetings	4 days
9/15-17/92	EPA Audit	2.5 days
12/09/92	QA Presentation, Rohm & Haas	1 hr
2/1-4/93	AAIE Meetings	4 days

CONTINUING TRAINING/EXPERIENCE HISTORY

Kathleen Britt

1981 & 1982	Vole Conference	3 Days each
1983	Bee Conference	1 Day
1976-1989	Recording field recommendations	
1977-1989	Assist in report writing, typing, proof reading, re-write and charts.	
1977-1989	Assist with studies in technical capacities.	
January 1989	Read EPA proposed regulations & sample SOP's.	
March 1988	Read Ron Britt & Associates, Inc. SOP's.	
April 1988	Started reading protocols for 1988 studies in order to chart activity dates.	
4-13-88	Attended Pan Ag Audit	3 1/2 Hrs.
6-3-88	Ron Collins Audit	2 Hrs.
6-14-88	Hoechst/Roussel Audit	1 Hr.
7-5-88	Monsanto Audit	2 Hrs.
11-4-88	Pan Ag QA Audit	2 1/2 Hrs.
5-16-89	Safety Meeting	1/2 Hr.
6-21-89	Valent QA	2 3/4 Hrs.
8-3-89	Rhone-Poulenc Audit	2 Hrs.
8-5-89	Attended Corn Pest Management Course by Del Monte, WSU and Ron Britt & Associates, Inc.	3 Hrs.
8-14-89	Uniroyal Audit	2 1/2 Hrs.
8-17-89	Merck Visit	2 Hrs.
8-23-89	Stewart Ag Audit	2 1/2 Hrs.
8-29-89	DuPont visit & review of requirements	4 Hrs.
8-30-89	Valent office visit	2 Hrs.

Kathleen Britt

Continuing Training/Experience History

9/19/96	Uniroyal Audit by Study Director	3 hrs.
10/14-16/96	Baltimore SQA Meetings	3 days
2/2/97	AAIE Meetings	4 hrs.
3/24/97	PCRCSQA Meeting in Seattle	6 hrs.
4/17-18/97	Uniroyal Audit	9 hrs.
5/9/97	Merck Audit	6 hrs.
8/12/97	ABG Audit	2 hr.
8/15/97	FMC Audit	6 hr.
10/21-24/97	SQA in Seattle	4 days
3/27/98	Novartis QAU – Selected Field Topics	8 hr.
6/22/98	PRCSQA in Seattle	6 hr.
9/15/98	SISTEM ~~SCRIP~~ Audit – Judy Speas – Yakima, WA	6 hrs.
10/21-23/98	SQA in Denver, CO	3 days
3/10-12/99	Novartis Training Sessions- Basic, QA, Advanced Bbrown, Tcox, Kprice, Bnorth at Colorado Springs, CO	3 days

STATEMENT

For purposes of GLP compliance, I acknowledge this to be a correct curriculum vitae.

Name: Kathleen Britt Initials: KB Date: 6/8/99

Kathleen Britt

CONTINUING TRAINING/EXPERIENCE HISTORY

4/26-28/93	First Aid Class	10 hrs
7/8/93	Abbott Audit	3 hrs
10/4/93	SQA Professional Enhancement (Training the Trainer)	3 hrs
10/4-7/93	SQA Meetings	3 days
5/3/94	Rhone Poulenc audit	7 hrs.
8/19/94	Merck audit	4 hrs.
9/20/94	Ciba audit	4 hrs.
10/3/94	SQA retook basic training	7 hrs.
10/4-6/94	SQA annual meeting	3 days
2/6/95	AAIE meetings	1 day
4/10/95	Atochem Audit	5 hrs.
5/2/95	Agribusiness Audit	5 hrs.
8/3/95	Agribusiness Audit	1 hr.
9/19,20/95	EPA Audit	1.5 days
1/12/96	Microsoft Access, level 1	8 hrs.
1/26/96	National Alliance ICC - moderator	2.5 hrs.
2/16/96	Quicken for Windows, level 2	8 hrs.
3/11/96	Microsoft Works (Win), level 1	8 hrs.
3/22-23/96	GLPs for the Field & QA for Field Residue Trials	2 days
5/10/96	Microsoft Access, level 2	8 hrs.
6/19/96	SISTEM Facility Audit	6 hrs.
8/30/96	Sandoz Audit	6 hrs.
9/5/96	SCRIP Audit	5 hrs.

surface active agents, however, one should anticipate the potential for eye irritation and skin irritation with large scale exposure or in sensitive individuals.

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Section 3 - Physical Data

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Melting Point:	Not Available
Boiling Point:	Decomposes above 200°C
Density:	Approximately 1.21 grams/cm^3
Solubility in Water:	Miscible with water (surfactant)
Appearance:	Brown solid
Odor:	Minimal odor - slightly sweet
Vapor Pressure:	NA
Other Factors:	Material will darken with exposure to high heat as sugars are oxidized. Rate of darkening increases with heat.

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Section 4 - Fire and Explosion Hazard Data

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Flash Point:	Decomposes
Flammable Limits:	Liquid will support combustion
Thermal Decomposition:	Begins to decompose above 100°C.
Hazardous Decomposition Products:	Carbon dioxide and Carbon monoxide
Extinguishing Media:	Water, Dry Chemical, Carbon Dioxide, and Foam

Water may be used to keep exposed containers cool. Handling similar to sugar and edible oil mixture.

For large quantities involved in a fire, one should wear full protective clothing and a NIOSH approved self contained breathing apparatus with full face piece operated in the pressure demand or positive pressure mode as for a situation where lack of oxygen and excess heat are present.

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Section 5 - Health Hazard Data

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Threshold Limit Value: Not determined.

Effects of Overexposure:	Not Determined. Based on analogous compounds can produce irritation to mucous membranes and upper respiratory tract.
Possible Symptoms:	Irritation of upper respiratory system and eyes.

Material is approved for food use but complete data on all aspects of long term exposure are not available. Material can be expected to break into octanoic and similar fatty acids and sucrose upon ingestion and no harmful effects are expected.

Eye or respiratory contact is expected to cause some irritation.

Tony Barrington

From: "Tracy Palmer" <Tracy@appliedpowerconcepts.com>
To: <avachem@gsinet.net>
Sent: Monday, December 08, 2003 2:23 PM
Subject: MSDSsucrose octanoate

MATERIAL SAFETY DATA SHEET

Last Revised: June 24, 1999

****

Section 1 - Material Identification

****

Supplier: Applied Power Concepts, Inc.
411 E. Julianna Street
Anaheim, CA 92801

Telephone: (714) 502-1150
Fax: (714) 502-2450

Chemical Name: α-D-Glucopyranoside, β-D-fructofuranosyl, monoctanoate
α-D-Glucopyranoside, β-D-fructofuranosyl, dioctanoate
α-D-Glucopyranoside, β-D-fructofuranosyl, trioctanoate

CAS Registry Number: 42922-74-7
58064-47-4

Chemical Family: Organic Chemical - sucrose esters of fatty acids.

Trade Name: Sucrose Octanoate. A mixture containing C_8 and C_{10} fatty acid mono-, di- and triesters of sucrose octanoate.

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Section 2 - Hazardous Ingredients

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Composition (% by weight):
Contains variable percentages of the various fatty acid esters with 2 to 16% n-butanol.

All handling requirements would be similar to those for sugar and edible grease or oil.

Sucrose esters have been approved by the Food and Drug Administration for food use. Since they are

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Section 8 - Special Protection or Handling

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Should be stored in plastic lined steel, plastic, glass, aluminum, stainless steel, ore reinforced fiberglass containers.

Protective Gloves:	Vinyl or Rubber
Eyes:	Splash Goggles or Full Face Shield
	Area should have approved means of washing eyes.
Ventilation:	General exhaust.
Storage:	Store in cool, dry, ventilated area.

Protect from incompatible materials.

****

Section 9 - Other Information

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Materials containing reactive chemicals should be used only by personnel with appropriate chemical training.

The information contained in this document is the best available to the supplier as of the time of writing. Some possible hazards have been determined by analogy to similar classes of material. No separate tests have been performed on the toxicity of this material. The items in this document are subject to change and clarification, as more information becomes available.

Handling: Avoid continued contact with skin. Avoid contact with eyes.

In any case of any exposure which elicits a response, a physician should be consulted immediately.

First Aid Procedures:

Inhalation: Remove to fresh air. If not breathing give artificial respiration. In case of labored breathing give oxygen. Call a physician.

Ingestion: No effects expected. Do not give anything to an unconscious person. Call a physician immediately.

Skin Contact: Flush with plenty of water. Contaminated clothing may be washed or dry-cleaned normally.

Eye contact: Wash eyes with plenty of water for at least 15 minutes lifting both upper and lower lids. Call a physician.

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Section 6 - Reactivity Data

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Stability: Material is stable unless heated above 200°C. Under normal conditions of storage and use material is stable.

Conditions to Avoid: Strong oxidizing agents

Hazardous Polymerization: None known

Further Information: May blacken and dehydrate upon exposure to concentrated acid

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Section 7 - Spill, Leak or Accident Procedures

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After Spillage or Leakage: Neutralization is not required. Sweep up or soak up with absorbent material such as paper, rags or sawdust. May be disposed of as with a mixture of sugar and grease or edible oil.

Disposal: Laws and regulations for disposal vary widely by locality. Observe all applicable regulations and laws. This material may be disposed of in solid waste in a manner similar to sugar and edible oil or grease.

No requirement for a reportable quantity (CERCLA) of a spill is known.