PETITION TO ALLOW USE OF SORBITOL OCTANOATE AS A SYNTHETIC SUBSTANCE IN ORGANIC CROP PRODUCTION

SUBMITTED

TO

NATIONAL ORGANIC STANDARDS BOARD

BY

AVA CHEMICAL VENTURES, L.L.C.

JUNE, 2006

June 13, 2006

CHEMICAL VENTURES, L.L.C.

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Mr. Arthur Neal
USDA/AMA/TM/NOP
U.S Department of Agriculture
1400 Independence Avenue
Room # 4008 South
Washington, D.C. 20250

SUBJECT: PETITION TO ALLOW USE OF SORBITOL OCTANOATE AS A SYNTHETIC SUBSTANCE IN ORGANIC CROP PRODUCTION

Dear Mr. Neal:

I am pleased to submit two copies of the subject petition.

I would like to draw your attention to the fact that the Environmental Protection Agency (EPA) has recently classified Sorbitol Octanoate, along with Sucrose Octanoate Esters, as "Octanoate Esters". This classification is recorded in the EPA document titled, Biopesticides Registration Action Document, a copy of which is included in Attachment 1 of the petition.

I would be pleased to answer any questions you or your colleagues may have.

Very truly yours

AVA CHEMICAL VENTURES, L.L.C.

Anthony Barrington
Managing Member

TABLE OF CONTENTS

FOLLOWS TAB

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JUNE, 2006

1. COMMON NAME

The subject of this petition is the biochemical insecticide/miticide active ingredient, Sorbitol Octanoate. The substance belongs to the organic chemical family of "sugar esters."

2. MANUFACTURER'S NAME

Sorbitol Octanoate 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.
714-502-1150, Extn. 110
farone@appliedpowerconcepts.com

3. INTENDED USE

Sorbitol Octanoate is a contact-type biochemical insecticide/miticide that is registered for foliar spray application to greenhouse, nursery and field crops.

4. CROP LIST & APPLICATION METHOD

Please see **Attachment 1** which includes the EPA-approved labels for Sorbitol Octanoate. Other EPA documents relating to the Sorbitol Octanoate pesticide registration are also included in **Attachment 1.**

5. SOURCE AND MANUFACTURING PROCESS

Sorbitol Octanoate is a synthesis of sorbitol 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 sugar ester active ingredients for pest control purposes. 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 Sorbitol Octanoate active ingredient (AI) that is the subject of this petition Is manufactured from sorbitol and from food-grade C-8 fatty acids derived from tropical vegetable oils.

Sorbitol is a polyol (sugar alcohol) that occurs naturally in a wide variety of fruits and berries. It is commercially produced by the hydrogenation of glucose. 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 Sorbitol Octanoate is described in U.S Patent # 6,419,941, titled, " Polyol Ester Insecticides and Method of Synthesis", dated July 16, 2002. Please see **Attachment 3** for a copy of the patent.

The manufacturing process is designed to produce an ester with high monoester content, as this has been demonstrated to have the highest efficacy against various types of insect pests.

Steps in Manufacturing Process

The manufacturing process for Sorbitol Octanoate involves a single chemical reaction that is solvent-free and has no post-reaction purification steps, other than the optional removal of the esterification catalyst. One of the objectives is to have an environmentally acceptable process that produces no toxic by-products.

1. Octanoic acid is charged to the reactor at a temperature sufficiently high to keep it in liquid form.

2. Sorbitol is added in an amount that allows the production of monoester stoichiometrically, plus an additional 10 per cent to drive the reaction essentially to completion.

3. An esterification catalyst (sulfuric acid or phosphoric acid) is added. Phosphoric acid is preferred since neutralization at the completion of the reaction provides a phosphate salt that can either be left in the product (since phosphorus is an essential plant nutrient), or removed by filtration.

4. The reactor is held at a temperature sufficiently high along with a pressure sufficiently low to allow water to be removed as the esterification reaction proceeds.

5. The reaction is allowed to proceed until the remaining octanoic acid reaches a low equilibrium value.

6. At the completion of the reaction the solution is neutralized with an amount of base that is sufficient to neutralize all of the mineral acid used as a catalyst plus bring the solution to a desired pH for subsequent use. If calcium hydroxide is used as the base, calcium phosphate can be filtered out of the product.

Discussion of Raw Materials and Manufactured Product:

Raw Materials

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

Octanoic Acid (CAS # 124-07-02)

Sorbitol (CAS # 50-70-4)

Sulfuric Acid (CAS # 7664-93-9)
or Phosphoric Acid (CAS # 7664-38-2)

Calcium Hydroxide (CAS # 1305-62-0)

All of the above appear on the EPA **List 4- Inerts of Minimal Concern.**

Manufacturing Use Product (MUP)

Sorbitol Octanoate is manufactured to the specification contained in the Confidential Statement of Formula (CSF), a copy of which his contained in **Attachment 4 – Confidential Business Information (CBI).** The CSF lists the following components of the MUP:

Sorbitol Octanoate (CAS # 108175-15-1). This is the active ingredient (AI) and is the main component of the MUP.

Other components of the MUP are un-reacted sorbitol and octanoic acid and calcium phosphate unless the latter is filtered out.

End Use Product (EUP)

Sorbitol Octanoate EUP is formulated by diluting MUP to achieve a specific AI concentration with Sucrose Octanoate Esters, another sugar ester that is a registered insecticide/miticide and/or with one or more items from EPA's **LIST 4- Inerts of Minimal Concern.**

6. PREVIOUS REVIEWS

Sorbitol Octanoate has not previously been reviewed for use in organic crop production.

7. EPA, FDA AND STATE REGULATORY AUTHORITY REGISTRATIONS

EPA: Sorbitol Octanoate is registered by EPA as a biochemical insecticide/miticide for use as a foliar spray on greenhouse, ornamental and field crops. It has also been granted a waiver from the requirement for a tolerance for all food crops. Copies of the EPA-approved labels and other documents relating to the registration/waiver of tolerance are contained in **Attachment 1.**

All of the starting materials in Sorbitol Octanoate as well as the manufacturing impurities appear on EPA's **LIST 4- Inerts of Minimal Concern.**

The MUP registration number is 70950-4 and the EUP registration numbers are 70950-3 and 70950-5.

FDA: The FDA has not reviewed Sorbitol Octanoate or other sorbitol esters but has reviewed sorbitan esters, sorbitol and octanoic acid.

Sorbitol esters, including Sorbitol Octanoate, are similar in chemical structure to the sorbitan esters approved by the FDA for use as food additives and as personal care product ingredients. The only difference between the sorbitol ester that is the subject of this petition and a sorbitan ester is in the degree to which water is removed from the main sorbitol structure during the manufacturing process. Sorbitan is derived from sorbitol by "dehydration", ie, the removal of one molecule of water from the sorbitol molecule.

As noted in the study contained in **Attachment 5,** Sorbitol Octanoate in water hydrolyzes quickly to sorbitol and octanoic acid, making these substances of interest for purposes of assessing the health effects of sorbitol esters.

The FDA has approved several sorbitan esters made with long-chain fatty acids for direct food additive use. Please see **Attachment 7** for details. Sorbitan esters made with long-chain fatty acids do not to have pest control properties; hence the use of octanoic acid in the Sorbitol Octanoate ester that is the subject of this petition.

Both sorbitol and octanoic acid - the breakdown products of Sorbitol Octanoate – are classified by the FDA as, "Direct Food Substances Affirmed as Generally Recognized as Safe (GRAS)" (21CFR§184.1).

California Department of Pesticide Regulation (CDPR): A petition to register Sorbitol Octanoate has been field with the CDPR. As of this date, the review is in process.

Other State Registrations: As of this date, none have been filed.

8. CAS NUMBERS & LABELS

The CAS Registry Number for Sorbitol Octanoate is: **108175-15-1.**

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

9. PHYSICAL PROPERTIES AND CHEMICAL MODE OF ACTION

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

Chemical Mode of Action: Sorbitol Octanoate is a contact-type insecticide/miticide that is rapidly toxic to soft-bodied arthropods including mites, aphids, whiteflies 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: Sorbitol Octanoate is a non-ionic surfactant. Field trials have demonstrated that it has synergistic pest control properties when used in conjunction with other active ingredients and adjuvents. No negative chemical reactions have been observed.

(b). Toxicity and Environmental Persistence: As noted in the study contained in **Attachment 5,** Sorbitol Octanoate in water quickly hydrolyzes to sorbitol and octanoic acid. This finding is consistent with an earlier study of sucrose fatty acid

esters which found they do not persist in the environment and biodegrade within approximately five days at approximately 20-27 °C, in both aerobic and anaerobic conditions.[1] Sucrose fatty acid esters, like Sorbitol Octanoate, are not soluble in water.

Based on the information submitted in connection with the pesticide registration petition, including the documents referred to in the previous paragraph, EPA concluded that Sorbitol Octanoate 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 label directions. Waivers were granted for studies of Avian Acute Oral Toxicity, Avian Dietary, Freshwater Fish and Freshwater Invertebrate.

(c). Environmental Impacts from Use and Manufacture: As noted under (b) above, Sorbitol Octanoate quickly hydrolyzes to sorbitol and octanoic acid. The Sorbitol Octanoate manufacturing use product (MUP) is manufactured by a 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 that describes the manufacturing process).

(d). Effects on Human Health: As noted in Section 7 above, the only differences between the sorbitan esters that are FDA –approved for food additive use and Sorbitol Octanoate are in a), the degree to which water is removed during the manufacturing process and b), the specific fatty acid that is used to make the esters. Octanoic acid is used to make Sorbitol Octanoate; the FDA-approved sorbitan esters are manufactured with mixtures of several longer-chain fatty acids.

Attachment 7 contains a report by the Joint FAO/WHO Expert Committee on Food Additives (FOOD ADDITIVES SERIES NO. 17) that summarizes acute animal feeding studies conducted with various sorbitan esters. Based on the acute feeding studies summarized in the report, as well as others that examined longer-term effects on animals and humans, the Expert Committee on Food Additives determined that the daily intake level of sorbitan esters that causes no toxicological effect in the rat to be 50,000 ppm (5%), which is equivalent to 2500 mg/kg bw. The Committee set an estimate of acceptable daily intake in man of 0-25mg/kg bw.

Attachment 5 contains a report of a study on the degradation of Sorbitol Octanoate. It concludes that Sorbitol Octanoate hydrolyzes rapidly to sorbitol and octanoic acid.

Attachment 7 contains a copy of WHO FOOD ADDITIVES Series No. 5, a toxicological evaluation of sorbitol. It reports LD_{-50} values ranging from 7,300

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

mg/kw bw to 25,700 mg/kw bw. for sorbitol administered orally or intravenously to mice and rats. **Attachment 7** contains a copy of a WHO/FAO report (WHO Food Add. /67.29) on sorbitol that concludes there is no need for limitation of sorbitol in the human diet on toxicological grounds.

Attachment 7 contains a FAO/WHO report (WHO FOOD SERIES 40) that documents the acute toxicity of octanoic acid. It reports the LD_{50} of octanoic acid administered by gavage to the male rat was found in one study to be 1283 mg/kg bw and in a second study involving both male and female rats the LD_{50} was found to be 10,080 mg/kg. bw.

Attachment 7 contains a copy of a report reviewed by the Cosmetic Ingredient Review Expert Panel (CIREP) that contains information on the animal and human toxicology of a range of sorbitan esters, including Sorbitan Caprylate (Octanoate). The report concludes:

> " The results of oral toxicity studies of the Sorbitan Fatty Acid Esters indicated that these Sorbitans at low concentrations were relatively non toxic via ingestion. The lowest LD_{50} for the rat in the 20 Sorbitan Ester studies (reviewed) was 31 g/kw bw for Sorbitan Stearate." (p. 102)

The report also reviewed animal toxicity studies based on fatty acids and concludes:

> " Little acute toxicity was observed when Oleic, Lauric, Palmitic, Myristic or Stearic Acid, or cosmetic formulations containing these fatty acids at concentrations of 2.2% to 13% were given to rats orally at doses of 15 to 19 g/kg body weight." (p. 102).

Sorbitol is classified by the FDA as a "substance added directly to human food affirmed as generally recognized as safe (GRAS)". (21CFR§184.1835).

Octanoic acid is classified by the FDA as a "substance added directly to human food affirmed as generally recognized as safe (GRAS)". (21CFR§184.1025).

Both sorbitol and octanoic acid are included in **EPA's List 4- Inerts of Minimal Concern**.

When tested for acute eye irritation, Sorbitol Octanoate manufacturing use product (MUP) caused corneal opacity in six of six rabbits tested at 24 hours after dosing with the MUP at 0.1 ml/eye. Corneal opacity resolved by day 14 after dosing. Eyes of all six rabbits also showed conjunctival irriation, and five of six rabbits showed iritis at one hour post-dosing. These signs also resolved by day 14. Sorbitol octanoate MUP is severely irritating to the eye and is classified as Toxicity Category II.

(e). Effects on Soil Organisms, Crops and Livestock: Sorbitol Octanoate is EPA-registered for use as a foliar spray to control insects on a wide range of crops. It has been tested on a number of crops, including pear, tobacco, peach, apple and strawberry with no reported phytotoxicity.

More extensive testing has been carried out with a closely related compound, Sucrose Octanoate Esters (SOE). That compound has been tested on almond, apple, pear, citrus, cotton, grape, peach, lettuce, tomato, potato, soybean, tomato, mint, cabbage, melon, strawberry and several ornamental crops, including rose and poinsettia. Even at dosages several time the label dose, no phytotoxicity has been observed.

SOE has also been tested against beneficial insects, including honey bees and lady beetles, without causing mortality. Given the similarity of the chemistry, it is reasonable to conclude that Sorbitol Octanoate will not harm beneficial insects.

10. SAFETY INFORMATION

A Material Safety Data Sheet for Sorbitol Octanoate is included as **Attachment 8.**

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 to add a synthetic substance, Sorbitol Octanoate, to the National List for use as an insecticide/miticide on crops. As a synthesis of sorbitol and food-grade fatty acids derived from tropical vegetable oils manufactured by a "zero discharge" process, Sorbitol Octanoate is a commercially-viable version of naturally-occurring sugar esters found in several plant species. It is manufactured by a process that has considerably less environmental impact than a process based on the cultivation of plants containing sugar esters and their extraction for use as natural pesticides.

Sorbitol Octanoate is effective primarily against soft-bodied insects (eg., whiteflies, aphids, mealybugs) and mites. Applicant notes that there are a very limited number of materials approved for organic production that are effective against these categories of pest. One approved substance is Sucrose Octanoate

Esters (SOE), which has chemical and usage profiles that are very similar to those of Sorbitol Octanoate.

Lower treatment Cost: Like SOE, Sorbitol Octanoate is applied on a volume-to-volume basis (active ingredient of 0.03-.0.4%) rather than on a per-acre basis. This is because both substances are contact insecticides/miticides, where good coverage is essential to achieving a high level of efficacy. As the crop type and/or crop growth stage set the volume of water needed per-acre to achieve good coverage, the amount of active ingredient used per acre varies widely. This affect the economics of using the products; crops that require a low spray volume will have a low cost per-acre to the grower, whereas crops that have a high spray volume will have a high cost per-acre.

The advantage of Sorbitol Octanoate relative to SOE and to other insecticides approved for organic use is that it is relatively inexpensive to manufacture; approximately half the cost of SOE. This will allow growers of crops that require high spray volumes to achieve lower per-acre treatment costs than would be possible with SOE.

Other Pesticides Registered for Similar Uses: The petitioned substance is an EPA-approved insecticide/miticide that is registered for use against soft-bodied insects and mites on a wide range of greenhouse, nursery and field crops. The attached Table 1 lists seven OMRI-approved substances that are registered for generally similar uses.

Label limitations: With respect to efficacy against particular insect types/life stages, the following limitations of the materials listed in Table 1, based on a review of their labels, are noted:

- #1 and #6 are not labeled for mites.
- #1 is an insect growth regulator, killing only the larval stages of insects and #3 is most effective when applied before insects or eggs are present in large numbers.
- The label for #3 recommends that no more than two consecutive applications be made, followed by a t least two applications of an alternative chemistry.
- The label for #4 contains instructions with respect to the timing of application that, for many crop/pest combinations, limit use of the product to when crops are dormant.

With respect to impacts on non-target organisms the following limitations, based on language contained in the respective labels, are noted:

- #5 is toxic to fish and #6 is highly toxic to fish

- #2 is a bee hazard and should not be applied when bees are actively visiting the treatment area.

- The label for #7 contains extensive warnings about phytotoxicity.

Integrated Pest Management: Increasing attention is being given in both organic and non-organic agriculture to the use of integrated pest management (IPM) involving a range of cultural methods , including promotion of beneficial insects to act as natural predators and rotation of pesticides to avoid resistance build-up in target insect populations. This is an additional reason for expanding the range of pest control products available to organic agriculture; IPM relies heavily on rotating pesticides.

Summary: The petitioned substance is an effective insecticide/miticide; it can be used at all plant growth stages; and it is not harmful to fish or beneficial insects. It is significantly less costly to apply than its organically-approved sister compound, SOE. It therefore compliments the existing substances approved for pest control in organic agriculture from an efficacy standpoint, particularly within the framework of IPM systems, while avoiding the negative environmental effects of some of these substances.

SECTION 6517 (C) (1) (B) (i)

The petitioned substance is a soap derived from coconut oil fatty acids or palm kernel oil fatty acids.

13. COMMERCIAL COFIDENTIAL INFORMATION STATEMENT:

Confidential information has been assembled in **Attachment 4- CBI.** The documents included are the EPA-approved Physical and Chemical Properties of Sorbitol Octanoate; the Confidential Statement of Formula (CSF) for the Manufacturing Use Product; raw material specifications; a discussion of product identity and composition; analysis of samples; and analytical methods for certified limits. This information is commercially valuable; is used in the applicant's business to manufacture Sorbitol Octanoate; and is maintained in secrecy by the applicant and its manufacturing contractor.

TABLE 1: OMRI-APPROVED INSECTICIDES/MITICIDES WITH AGRICULTURAL USE LABELING SIMILAR TO SUCROSE OCTANOATE

OMRI GENERIC CATEGORY	REFERENCE NUMBER	ACTIVE INGREDIENT	BRAND NAME	COMMENTS
Neem Extract and Derivatives	#1	Azadirachtin	Neemix 4.5	1. Not labeled for mites 2. Kills larval stages of insects only.
	#2	Extract of Neem Oil	Trilogy	1. Most effective when applied before insects or eggs are present in large numbers. 2. Bee hazard; do not apply when bees are actively visiting the treatment area.
Oils- Nonsynthetic Source	#3	Cotton oil/clove oil/ garlic oil	GC-Mite	
	#4	Soybean oil	Golden Pest Spray Oil	
Oils-Petroleum-Based - Narrow Range	#5	Parafinic oil	Organic JMS Stylet-Oil	Toxic to fish.
Pyrethrum	#6	Pyrethrins	PyGanic Crop Protection EC 1.4	1. not labeled for mites 2. Highly toxic to fish
Soap	#7	Potassium salts of fatty acids	M-Pede	1. Label contains extensive warning about phytotoxicity.

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:
70950-3	1-17-06

Term of Issuance: Unconditional

Name of Pesticide Product:
AVACHEM SORBITOL OCTANOATE [90.0%]

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 modifications to your label before releasing your product for shipment:

   a. Revise the "EPA Reg. No." entry to become 70950-3.

Signature of Approving Official: (See second page for signature)

Date: 1-17-06

EPA Form 8570-6

2. Submit three (3) 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)

Enclosures (2)

AVACHEM SORBITOL OCTANOATE [90.0%]

Biochemical Insecticide/Miticide for Greenhouse, Nursery & Field Crop Use

ACTIVE INGREDIENT:
Sorbitol Octanoate..... 90.0%

OTHER INGREDIENTS:..... 10.0%

Total 100.0%

(For 1 Gallon size: This container will treat up to 0.6 acre at the highest volume application rate)

EPA Reg. No. 70950-G EPA Est. No. 075197-CA-001

KEEP OUT OF REACH OF CHILDREN

WARNING

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.

Have the product container or label with you when calling a poison control center or doctor, or going for treatment.

For information about this product call 1-888-229-7414

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

Net Contents: 1 Gallon, 2.5 Gallons

~8290626.doc
REVISED 1/12/'06, 1/13/'06 @2:15pm & at 2:30pm. 1/17/'06 @ 10:30am

PRECAUTIONARY STATEMENTS
Hazards 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 and wash contaminated clothing before reuse.

Personal Protective Equipment (PPE):
Applicators and other handlers must wear: Long sleeved shirt and long pants, shoes plus socks and protective eyewear.

Discard clothing and other absorbant materials that have been drenched or heavily contaminated with this product's concentrate. Do not reuse them.

Follow the manufacturer's instructions for cleaning/ maintaining PPE. If no such instructions for washables, use detergent and hot water. Keep and wash PPE separately from other laundry.

User Safety Recommendations:
Users should:

- Wash hands before eating, drinking, chewing gum, using tobacco, or using the toilet.
- Remove clothing/PPE immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.
- Remove PPE immediately after handling This product. As soon as possible, wash thoroughly and change into clean clothing.

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 disposing of equipment wash water or rinsate.

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 State/Tribal agency responsible for pesticide regulation.

AGRICULTURAL USE REQUIREMENTS

Use this product only in accordance with its labeling and with the Worker Protection Standard, 40 CFR Part 170. This standard contains requirements for the protection of agricultural workers on farms, forests, nurseries, and greenhouses, and handlers of agricultural pesticides. It contains requirements for training, decontamination, notification, and emergency assistance. It also contains specific instructions and exceptions pertaining to the statements on this label about personal protective equipment (PPE), and restricted-entry interval. The requirements in this box only apply to uses of this product that are covered by the Worker Protection Standard.

Do not enter or allow worker entry into treated areas during the restricted-entry interval (REI) of 24 hours.

PPE required for early entry to treated areas that is permitted under the Worker Protection Standard and that involves contact with anything that has been treated, such as plants, soil, or water, is:

- coveralls,
- shoes plus socks,
- protective eyewear, and
- waterproof gloves.

GENERAL INFORMATION

AVACHEM SORBITOL OCTANOATE [90.0%] is a biochemical insecticide/miticide for use against the soft-bodied insects listed on this label, similar insect types, mites and thrips. It is primarily a contact insecticide with limited residual activity.

- Do not apply this product through any type of irrigation system.
- Apply as soon as infestation begins.
- Use in sufficient water to achieve adequate coverage.
- Repeat applications, as necessary, at intervals of 7 - 10 days, to maintain control.
- Thorough spray coverage of plant foliage, including the undersides of leaves, is essential for good control of the pest.
- May be applied up to and including day of harvest.

PESTS CONTROLLED

This product is used as a foliar spray to control or suppress soft-bodied pests which include: adelgid, aphids, caterpillars, glassy-winged sharpshooter, lace bug, leafhopper, mealy bug, plant bug, psyllid, soft scale, mites, tent caterpillar, thrips and whitefly.

MIXING AND APPLICATION

Shake or stir before use.

AVACHEM SORBITOL OCTANOATE [90.0%] is miscible 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 0.5% v/v solution of this product

Use between 25 and 325 gallons of total mix volume per acre, or 6-18 gallons per 10,000 square feet. The amount of total mix required to achieve adequate coverage will vary with the type, growth stage and/or spacing of the treated crop.

Use the following Rate Tables to calculate total mix volumes.

Rate Table (Per Acre)

Total Mix Volume	% v/v Solution	Amount Of Product English (Metric)
25 gal. (94.75 liters)	0.5%	16 Fl. Oz. (2 Cups) (474 ml)
50 gal. (189.50 liters)	0.5%	2 pints (4 Cups) (948 ml)
100 gal. (379.00 liters)	0.5%	2 quarts (8 Cups) (1.90 liters)

Rate Table (Per 10,000 Square Feet)

Total Mix Volume	% v/v Solution	Amount of Product English (Metric)
6 gal. (22.74 liters)	0.5%	4 Fl. Oz. (1/2 Cup) (119 ml)
18 gal. (68.22 liters)	0.5%	12 Fl. Oz. (1.5 Cups) (356 ml)

Timing of Application

Initiate applications as soon as pest is observed. Repeat applications, as necessary, at intervals of 7 - 10 days to maintain control.

Test Application

This product has been tested on many crops and ornamentals. However, it is not possible to evaluate all plant species or varieties for tolerance to this product. Test AVACHEM SORBITOL OCTANOATE [90%] for possible phytotoxic

responses by treating a few plants at the label use rate prior to large scale use.

Mixing

The user at his discretion can tank mix pesticides currently labeled for the same crop unless the product labels specifically prohibit such use. AVACHEM SORBITOL OCTANOATE [90.0%] has not been extensively tested for use in tank mixes with many commonly used insecticides, fungicides or spray tank adjuvents. Test all tank mix combinations for physical compatibility by first performing a jar compatibility test using proper proportions of chemicals and water.

- Always determine the compatibility of water, pesticides and other tank additives, such as penetrators, spreader stickers or activators, gibberellic acid, calcium nitrate or diatomaceous earth, foliar nutrients and alkaline based chelating agents such as EDTA, with this product prior to addition to the spray tank.
- For best results, use freshly mixed spray made with soft water. If water has high mineral content (hardness >300 ppm or 17.5 grains/gallon), check for compatibility as described above. If needed, add a compatibility agent to the tank prior to the addition of this product. Do not lower the pH of the final spray mixture below 7.0.
- If foaming occurs in sprayers equipped with an agitator, use a defoaming agent.
- Use freshly prepared spray solutions.

  When using this product in a tank mix, read and follow all product labels. Mix in this order:
  - Compatibility agent (if needed)
  - Wettable and soluble powders
  - Flowable liquids
  - Emulsifiable oils and concentrates
  - AVACHEM SORBITOL OCTANOATE [90.0%]

- Application of spray mixtures must conform to use precautions and directions for all products included in the tank mix; follow the most restrictive label text. Spray promptly after mixing and keep combinations agitated to achieve and maintain the suspension.

Application

- Apply this product with ground spray equipment. Do not apply this product through any type of irrigation system.
- This product must come into contact with the targeted pests to be effective. Complete coverage of plant foliage is essential for maximum control.
- If mixing with other pesticides, apply promptly to avoid or reduce alkaline hydrolysis of certain pesticides.

INSECTICIDAL/MITICIDAL USES

VEGETABLES, HERBS AND SPICES
(Greenhouse and Outdoor)

Vegetables: Artichoke, Asparagus, Brassica (cole), (such as: bok choy, broccoli, broccoli raab, Brussels sprouts, cabbage, cauliflower, Chinese broccoli, Chinese cabbage, collard greens, kale, kohlrabi, mustard greens and napa cabbage), **Bulb** (such as: green onion, leeks, garlic, onion and shallot), **Cucurbit** (such as: cucumber, melon, pumpkin and summer squash), **Fruiting** (such as: eggplant, pepper and tomato), **Leafy** (such as: celery, endive, escarole, lettuce, spinach and Swiss chard), **Legume** (such as: bean, pea and soybean), **Root and tuber** (such as: beet, carrot, horseradish, potato, radish, rutabaga, sugarbeet and yam), **Herbs and spices** (such as: basil, chive, dill, marjoram, mint, parsley and sage), **Watercress.**

Pests Controlled: Aphids, caterpillars, leafhoppers, mites, thrips, whiteflies.

FRUIT AND NUT CROPS

Citrus fruits (such as: citron, grapefruit, kumquat, lemon, lime, tangelo, and tangerine), **Pome fruits** (such as: apple, crabapple, pear and quince), **Stone fruits** (such as: apricot, cherry, nectarine, peach, plum and prune), **Small Fruits and Berries** (such as: blackberry, blueberry, cane berries, cranberry, currants, grape, raspberry, strawberry, boysenberry, and olallieberry) **Tree nuts** (such as: almond, chestnut, filbert, pecan, pistachio, black and English walnut).

Pests Controlled: Aphids, glassy-winged sharp-shooter, leafhoppers, mites, mealy bug, psyllid, soft scale, thrips, whiteflies.

FIELD CROPS

Alfalfa, Canola, Cotton, Peanut, Soybean, Tobacco and Wheat

Pests Controlled: Aphids, caterpillars, mites, thrips, whiteflies.

HOPS, COFFEE, BANANA AND PINEAPPLE

Pests Controlled: Aphids, leafhoppers, mealy bug, mites, plant bug, soft scale, thrips, whiteflies.

ORNAMENTAL LANDSCAPE TREES AND SHRUBS, CHRISTMAS TREES, ROSES, FLOWERS AND BEDDING PLANTS

Pests Controlled: Adelgid, aphids, lace bug, leafhopper, mealy bug, mites, plant bug, psyllid, soft scale, tent caterpillar, thrips, whiteflies.

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 must 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

AVA Chemical Ventures, L.L.C. (AVA Chemical) seeks to present reliable information concerning the composition, properties and use of the product; however, to the extent permitted by law: (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 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) 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.

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:
70950-4	1-17-06

Term of Issuance: Unconditional

Name of Pesticide Product:
AVACHEM SORBITOL OCTANOATE MANUFACTURING USE PRODUCT

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 modifications to your label before releasing your product for shipment:

   a. Revise the "EPA Reg. No." entry to become 70950-4.

Signature of Approving Official: JLH
(See second page for signature)

Date: 1-17-06

EPA Form 8570-6

2. Submit three (3) 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)

Enclosures (2)

AVACHEM SORBITOL OCTANOATE MANUFACTURING USE PRODUCT

BIOCHEMICAL INSECTICIDE/MITICIDE FOR FORMULATING USE ONLY

ACTIVE INGREDIENT:

Sorbitol Octanoate.....90.00%

OTHER INGREDIENTS:..... 10.00%

Total: 100.00%

EPA Reg. No. 70950-U

EPA Est. No. 075197-CA-001

KEEP OUT OF REACH OF CHILDREN

WARNING

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.
Have the product container or label with you when calling a poison control center or doctor, or going for treatment.	

For information about this product call: 1-888-229-7414

ACCEPTED

JAN 17 2006

Under the Federal Insecticides, Fungicide, and Rodenticide Act, as amended, for the pesticide registered under EPA Reg. No. 70950-4

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

Net Contents: 900 Gallons

~1460740.doc
Revised 1/12/'06, 1/13/'06

PRECAUTIONARY STATEMENTS

HAZARDS 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 and wash 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, bedding and greenhouse plants.

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: Wastes resulting from the use of this product must 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

AVA Chemical Ventures, L.L.C. (AVA Chemical) seeks to present reliable information concerning the composition, properties and use of the product; however, to the extent permitted by law: (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.

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:
70950-5	1-17-06

Term of Issuance: Unconditional

Name of Pesticide Product:
AVACHEM OCTA-SP [50.0%]

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 modifications to your label before releasing your product for shipment:

   a. Revise the "EPA Reg. No." entry to become 70950-5.

Signature of Approving Official:
(See second page for signature)

Date: 1-17-06

EPA Form 8570-6

2. Submit three (3) 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)

Enclosures (2)

AVACHEM OCTA-SP [50.0%]

Biochemical Insecticide/Miticide for Greenhouse, Nursery & Field Crop Use

ACTIVE INGREDIENTS:
Sorbitol Octanoate..... 41.7%

Sucrose Octanoate Esters (α-D-Glucopyranosyl - ß-D-fructofuranosyl - octanoate), mono-, di-, and triesters of sucrose octanoate 8.3%

OTHER INGREDIENTS: 50.0%

TOTAL 100.0%

(for 1 Gallon size: This container will treat up to 0.6 acre at the highest volume application rate)

EPA Reg. No. 70950-xxxx EPA Est. No. 075197-CA-001

KEEP OUT OF REACH OF CHILDREN

WARNING

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.
Have the product container or label with you when calling a poison control center or doctor, or going for treatment.		

For information on this product call: 1-888-229-7414

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

ACCEPTED
JAN 17 2006
Under the Federal Insecticide, Fungicide, and Rodenticide Act, as amended, for the pesticide registered under EPA Reg. No. 70950-5

Net Contents: 1.0 Gallon, 2.5 Gallons

PRECAUTIONARY STATEMENTS
Hazards 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 and wash contaminated clothing before reuse.

Personal Protective Equipment (PPE):
Applicators and other handlers must wear: Long sleeved shirt and long pants, shoes plus socks and protective eyewear.

Discard clothing and other absorbant materials that have been drenched or heavily contaminated with this product's concentrate. Do not reuse them.

Follow the manufacturer's instructions for cleaning/ maintaining PPE. If no such instructions for washables, use detergent and hot water. Keep and wash PPE separately from other laundry.

User Safety Recommendations:
Users should:

- Wash hands before eating, drinking, chewing gum, using tobacco, or using the toilet.
- Remove clothing/PPE immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.
- Remove PPE immediately after handling this product. As soon as possible, wash thoroughly and change into clean clothing.

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 disposing of equipment wash water or rinsate.

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 State/Tribal agency responsible for pesticide regulation.

AGRICULTURAL USE REQUIREMENTS

Use this product only in accordance with its labeling and with the Worker Protection Standard, 40 CFR Part 170. This standard contains requirements for the protection of agricultural workers on farms, forests, nurseries, and greenhouses, and handlers of agricultural pesticides. It contains requirements for training, decontamination, notification, and emergency assistance. It also contains specific instructions and exceptions pertaining to the statements on this label about personal protective equipment (PPE), and restricted-entry interval. The requirements in this box only apply to uses of this product that are covered by the Worker Protection Standard.

Do not enter or allow worker entry into treated areas during the restricted-entry interval (REI) of 48 hours.

PPE required for early entry to treated areas that is permitted under the Worker Protection Standard and that involves contact with anything that has been treated, such as plants, soil, or water, is:

- coveralls,
- shoes plus socks,
- protective eyewear, and
- waterproof gloves.

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.

For ground boom applications, apply with nozzle height no more than 4 feet above the ground or crop canopy and when wind speed is 10 mph or less at the application site as measured by an anemometer. Use fine or coarser spray according to ASAE 572 definition for standard nozzles or VMD for spinning atomizer nozzles.

For orchard/vineyard airblast applications, do not direct spray above trees/vines and turn off outward pointing nozzles at row ends and outer rows. Apply only when wind speed is 3-10 mph at the application site as measured by an anemometer outside of the orchard/vineyard on the upwind side.

The applicator also must use all other measures

necessary to control drift.

GENERAL INFORMATION

AVACHEM OCTA-SP [50.0%] is a biochemical insecticide/miticide for use against the soft-bodied insects listed on this label, similar insect types, mites and thrips. It is primarily a contact insecticide with limited residual activity.

- Do not apply this product through any type of irrigation system.
- Apply as soon as infestation begins.
- Use in sufficient water to achieve adequate coverage.
- Repeat applications, as necessary, at intervals of 7 - 10 days, to maintain control.
- Thorough spray coverage of plant foliage, including the undersides of leaves, is essential for good control of the pest.
- May be applied up to and including day of harvest.

PESTS CONTROLLED

This product is used as a foliar spray to control or suppress soft-bodied pests which include: adelgid, aphids, caterpillars, glassy-winged sharpshooter, lace bug, leafhopper, mealy bug, plant bug, psyllid, soft scale, mites, tent caterpillar, thrips and whitefly.

MIXING AND APPLICATION

Shake or stir before use.

AVACHEM OCTA-SP [50.0%] is miscible 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 0.5% v/v solution of this product.

Use between 25 and 325 gallons of total mix volume per acre, or 6-22 gallons per 10,000 square feet. The amount of total mix required to achieve adequate coverage will vary with the type, growth stage and/or spacing of the treated crop.

Use the following Rate Tables to calculate total mix volumes.

Rate Table (Per Acre)

Total Mix Volume	% v/v Solution	Amount of Product English (Metric)
25 gal. (94.75 liters)	0.5%	16 Fl. Oz. (2 Cups) (474ml)
50 gal. (189.50 liters)	0.5%	2 pints (4 Cups) (948 ml)
100 gal. (379.00 liters)	0.5%	2 quarts (8 Cups) (1.90 liters)

Rate Table (Per 10,000 Square Feet)

Total Mix Volume	% v/v Solution	Amount of Product English (Metric)
6 gal. (22.74 liters)	0.5%	4 Fl.Oz. (1/2 Cup) (119 ml)
18 gal. (68.22 liters)	0.5%	12 Fl. Oz. (1.5 Cups) (356 ml)

Timing of Application

Initiate applications as soon as pest is observed. Repeat applications, as necessary, at intervals of 7 - 10 days to maintain control.

Test Application

This product has been tested on many crops and ornamentals. However, it is not possible to evaluate all plant species or varieties for tolerance to this product. Test AVACHEM OCTA-SP [50.0%] for possible phytotoxic responses by treating a few plants at the label use rate prior to large scale use.

Mixing

The user at his discretion can tank mix pesticides currently labeled for the same crop unless the product

labels specifically prohibit such use. AVACHEM OCTA-SP [50.0%] has not been extensively tested for use in tank mixes with many commonly used insecticides, fungicides or spray tank adjuvents. Test all tank mix combinations for physical compatibility by first performing a jar compatibility test using proper proportions of chemicals and water.

- Always determine the compatibility of water, pesticides and other tank additives, such as penetrators, spreader stickers or activators, gibberellic acid, calcium nitrate or diatomaceous earth, foliar nutrients and alkaline based chelating agents such as EDTA, with this product prior to addition to the spray tank.
- For best results, use freshly mixed spray made with soft water. If water has high mineral content (hardness >300 ppm or 17.5 grains/gallon), check for compatibility as described above. If needed, add a compatibility agent to the tank prior to the addition of this product. Do not lower the pH of the final spray mixture below 7.0.
- If foaming occurs in sprayers equipped with an agitator, use a defoaming agent.
- Use freshly prepared spray solutions.

When using this product in a tank mix, read and follow all product labels. Mix in this order:

- Compatibility agent (if needed)
- Wettable and soluble powders
- Flowable liquids
- Emulsifiable oils and concentrates
- AVACHEM OCTA-SP [50.0%]

- Application of spray mixtures must conform to use precautions and directions for all products included in the tank mix; follow the most restrictive label text. Spray promptly after mixing and keep combinations agitated to achieve and maintain the suspension.

Application

- Apply this product with ground spray equipment. Do not apply this product through any type of irrigation system.
- This product must come into contact with the targeted pests to be effective. Complete coverage of plant foliage is essential for maximum control.
- If mixing with other pesticides, apply promptly to avoid or reduce alkaline hydrolysis of certain pesticides.

INSECTICIDAL/MITICIDAL USES

VEGETABLES, HERBS AND SPICES
(Greenhouse and Outdoor)
Vegetables: Artichoke, Asparagus, Brassica (cole), (such as: bok choy, broccoli, broccoli raab, Brussels sprouts, cabbage, cauliflower, Chinese broccoli, Chinese cabbage, collard greens, kale, kohlrabi, mustard greens and napa cabbage.), **Bulb** (such as: green onion, leeks, garlic, onion and shallot), **Cucurbit** (such as: cucumber, melon, pumpkin and summer squash), **Fruiting** (such as: eggplant, pepper and tomato), **Leafy** (such as: celery, endive, escarole, lettuce, spinach and Swiss chard), **Legume** (such as: bean, pea and soybean), **Root and tuber** (such as: beet, carrot, horseradish, potato, radish, rutabaga, sugarbeet and yam), **Herbs and spices** (such as: basil, chive, dill, marjoram, mint, parsley and sage), **Watercress.**

Pests Controlled: Aphids, caterpillars, leafhoppers, mites, thrips, whiteflies.

FRUIT AND NUT CROPS
Avocado
Citrus fruits (such as: citron, grapefruit, kumquat, lemon, lime, tangelo, and tangerine), **Pome fruits** (such as: apple, crabapple, pear and quince), **Stone fruits** (such as: apricot, cherry, nectarine, peach, plum and prune), **Small Fruits and Berries** (such as: blackberry, blueberry, cane berries, cranberry, currants, grape, raspberry, strawberry, boysenberry, and olallieberry) **Tree nuts** (such as: almond, chestnut, filbert, pecan, pistachio, black and English walnut).

Pests Controlled: Aphids, glassy-winged sharp-shooter, leafhoppers, mites, mealy bug, psyllid, soft scale, thrips, whiteflies.

FIELD CROPS
Alfalfa, Canola, Cotton, Peanut, Soybean, Tobacco and Wheat

Pests Controlled: Aphids, caterpillars, mites, thrips, whiteflies.

HOPS, COFFEE, BANANA AND PINEAPPLE

Pests Controlled: Aphids, leafhoppers, mealy bug, mites, plant bug, soft scale, thrips, whiteflies.

ORNAMENTAL LANDSCAPE TREES AND SHRUBS, CHRISTMAS TREES, ROSES, FLOWERS AND BEDDING PLANTS

Pests Controlled: Adelgid, aphids, lace bug, leafhopper, mealy bug, mites, plant bug, psyllid, soft scale, tent caterpillar, thrips, whiteflies.

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 must 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

AVA Chemical Ventures, L.L.C. (AVA Chemical) seeks to present reliable information concerning the composition, properties and use of the product; however, to the extent permitted by law: (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 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) 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.

(b) * * *

(2) * * *

(ii) The progress which has been made toward registration of the proposed use, if a repeated specific or public health exemption is sought. It shall be presumed that if a complete application for registration of a use, which has been under a specific or public health exemption for any 3 previous years, or any 5 previous years if the use is supported for registration by the IR-4 program, has not been submitted, reasonable progress towards registration has not been made.

■ 7. Section 166.30 is amended by revising paragraph (a)(1), removing paragraph (b), and redesignating paragraph (c) as paragraph (b).

§ 166.30 Notice of Agency decision.

(a) * * *

(1) *Incomplete applications.* The Agency may discontinue the processing of any application that does not address all of the requirements of § 166.20 until such time the additional information is submitted by the applicant.

* * * * *

■ 8. Section 166.32 is amended by revising the introductory text of paragraph (b) to read as follows:

§ 166.32 Reporting and recordkeeping requirements for specific, quarantine, and public health exemptions.

* * * * *

(b) *Interim and final reports.* A final report summarizing the results of pesticide use under any specific, quarantine, or public health exemption must be submitted to the Agency within 6 months from the expiration of the exemption unless otherwise specified by the Agency. For quarantine exemptions granted for longer than 1 year, interim reports must be submitted annually. When an application for renewal of the exemption is submitted before the expiration of the exemption or before submission of the final report, an interim report must be submitted with the application. The information in interim and final reports shall include all of the following:

* * * * *

■ 9. Section 166.40 is amended by revising paragraph (a), removing the period at the end of paragraph (b) and adding a semi-colon and the word "and" at the end of paragraph (b), and adding paragraph (c) to read as follows:

§ 166.40 Authorization.

* * * * *

(a) An unpredictable emergency condition exists;

* * * * *

(c) EPA has provided verbal confirmation that, for food uses, a tolerance or exemption from the requirement of a tolerance can be established in a timely manner, responsive to the projected timeframe of use of the chemical and harvest of the commodity, and that, for any use, the Agency has no other objection.

■ 10. Section 166.43 is amended by revising paragraphs (a)(1) and (b) to read as follows:

§ 166.43 Notice to EPA and registrants or basic manufacturers.

(a) * * *

(1) The State or Federal Agency issuing the crisis exemption must notify the Administrator in advance of utilization of the crisis provisions.

* * * * *

(b) *Contents of notice.* Information required to be provided in notices shall include all of the following:

(1) The name of the product and active ingredient authorized for use, along with the common name and CAS number if available, including a copy of the EPA registered label and use directions appropriate to the authorized use;

(2) The site on which the pesticide is to be used or is being used;

(3) The use pattern;

(4) The date on which the pesticide use is to begin and the date when the use will end;

(5) An estimate of the level of residues of the pesticide expected to result from use under the crisis exemption;

(6) Earliest anticipated harvest date of the treated commodity;

(7) Description of the emergency situation; and

(8) Any other pertinent information available at the time.

§ 166.47 [Removed]

■ 11. Section 166.47 is removed.

■ 12. Section 166.49 is amended by revising paragraph (a) to read as follows:

§ 166.49 Public notice of crisis exemptions.

(a) *Periodic notices.* At least quarterly, the Administrator shall issue a notice in the **Federal Register** announcing issuance of crisis exemptions. The notice shall contain all of the following:

(1) The name of the applicant;

(2) The pesticide authorized for use;

(3) The crop or site to be treated; and

(4) The name, address, and telephone number of a person in the Agency who can provide further information.

* * * * *

[FR Doc. 06–743 Filed 1–26–06; 8:45 am]

BILLING CODE 6560–50–S

ENVIRONMENTAL PROTECTION AGENCY

40 CFR Part 180

[EPA–HQ–OPP–2005–0515; FRL–7757–2]

Sorbitol Octanoate; 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 the biochemical sorbitol octanoate on all food commodities when applied/used in accordance with label directions. 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 of 1996 (FQPA), requesting an exemption from the requirement of a tolerance. This regulation eliminates the need to establish a maximum permissible level for residues of sorbitol octanoate.

DATES: This regulation is effective January 27, 2006. Objections and requests for hearings must be received on or before March 28, 2006.

ADDRESSES: To submit a written objection or hearing request follow the detailed instructions as provided in Unit VIII. of the **SUPPLEMENTARY INFORMATION.** EPA has established a docket for this action under Docket identification (ID) number EPA–HQ–OPP–2005–0515. All documents in the docket are listed on the www.regulations.gov website. (EDOCKET, EPA's electronic public docket and comment system was replaced on November 25, 2005, by an enhanced federal-wide electronic docket management and comment system located at *http://www.regulations.gov/.* Follow the online instructions.) Although listed in the index, some information is not publicly available, i.e., CBI or other information whose disclosure is restricted by statute. Certain other material, such as copyrighted material, is not placed on the Internet and will be publicly available only in hard copy form. Publicly available docket materials are available either electronically in EDOCKET or in hard copy at the Public Information and Records Integrity Branch (PIRIB), Rm. 119, Crystal Mall #2, 1801 S. Bell St., Arlington, VA. This docket facility is open from 8:30 a.m. to 4 p.m., Monday through Friday, excluding legal holidays. The docket telephone number is (703) 305–5805.

FOR FURTHER INFORMATION CONTACT: Denise Greenway, Biopesticides and Pollution Prevention Division (7511C), Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington, DC 20460–0001; telephone number: (703) 308–8263; e-mail address: *greenway.denise@epa.gov.*

SUPPLEMENTARY INFORMATION:

I. General Information

A. Does this Action Apply to Me?

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

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

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

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

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

II. Background and Statutory Findings

In the **Federal Register** of September 29, 2004 (69 FR 58166) (FRL–7679–1), EPA issued a notice pursuant to section 408(d)(3) of the FFDCA, 21 U.S.C. 346a(d)(3), announcing the filing of a pesticide tolerance petition (PP 2E6389) by AVA Chemical Ventures, L. L. C., 80 Rochester Avenue, Suite 214, Portsmouth, NH, 03801. The petition requested that 40 CFR part 180 be amended by establishing an exemption from the requirement of a tolerance for residues of sorbitol octanoate. 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.

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 exemption is "safe." Section 408(c)(2)(A)(ii) of the FFDCA defines "safe" to mean that "there is a reasonable certainty that no harm will result from aggregate exposure to the pesticide chemical residue, including all anticipated dietary exposures and all other exposures for which there is reliable information." This includes exposure through drinking water and in residential settings, but does not include occupational exposure. Pursuant to section 408(c)(2)(B), in establishing or maintaining in effect an exemption from the requirement of a tolerance, EPA must take into account the factors set forth in section 408(b)(2)(C), which require 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...." Additionally, section 408(b)(2)(D) of the FFDCA requires that the Agency consider "available information concerning the cumulative effects of a particular pesticide's residues" and "other substances that have a common mechanism of toxicity."

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

III. Toxicological Profile

Consistent with section 408(b)(2)(D) of the FFDCA, EPA has reviewed the available scientific data and other relevant information in support of this action and considered its validity, completeness, and reliability, and the relationship of this information to human risk. EPA has also considered available information concerning the variability of the sensitivities of major identifiable subgroups of consumers, including infants and children.

Sorbitol octanoate is a fatty acid ester made from sorbitol and caprylic acid. Caprylic acid, also known as octanoic acid, is a common fatty acid in plants that is derived from edible oils or fats. It also is produced in small quantities in the human body and is marketed as a human dietary supplement (Ref. 1). Sorbitol, a food grade sweetener with about half the sweetness of sucrose, is a hexahydric alcohol and occurs naturally in fruits such as apples, plums, pears, cherries, dates, peaches, and apricots (Ref. 2). Both sorbitol and octanoic acid are on the Agency's List 4 Inerts of Minimal Concern. Sorbitol is cleared for food use in unlimited quantities as an antidusting agent (40 CFR 180.910). While sorbitol octanoate is the subject of this final rule, the raw materials from which it is made are common in crops eaten regularly by humans and animals.

Furthermore, sorbitol octanoate is chemically and toxicologically similar to certain groups of compounds, namely certain sorbitan esters and certain sucrose octanoate esters that have been FDA-approved since 1983 when used as direct additives in food, as emulsifiers in certain processed foods, and as post-harvest protective coatings for certain fruits (21 CFR 172.836, 172.838, 172.840, 172.842 and 172.859). In 1995, FDA expanded the range of foods in which sucrose octanoate esters (SOEs) are permitted (August 29, 1995, 60 FR 44756). Sorbitol octanoate and SOEs both are fatty acid esters, and both are made by reacting sugars with octanoic acid (i.e., both are non-ionic surfactants manufactured by esterifying C_8 fatty acid with a sugar: sorbitol in the case of sorbitol octanoate and sucrose in the case of SOEs). Sorbitol octanoate and SOEs have similar solubility in water, similar degrees of stability, and require a similar concentration to achieve droplet spread. FDA-approved sorbitan esters are different from sorbitol octanoate only in that sorbitol has one more water molecule than sorbitan. Therefore, the toxicological data associated with SOEs and sorbitan esters can be used to support an exemption from the requirement of a tolerance for sorbitol octanoate.

The applicant collected and summarized the toxicological data associated with the cited FDA food-use approvals for SOEs which included sorbitan esters (as they are chemically similar), and submitted this information in support of an earlier tolerance exemption request (64 FR 49010, September 9, 1999) for SOEs (Ref. 3). In turn, the Agency reviewed and accepted both the summaries and the underlying data in granting the tolerance exemption for SOEs (67 FR 60146, September 25, 2002). Because of the substantial similarity between the two active

ingredients (i.e., sorbitol octanoate and SOEs), the Agency allowed the applicant to "bridge" to that previously-submitted data/information to support the tolerance exemption requested for sorbitol octanoate.

Toxicity information/data submitted in support of this tolerance exemption are referenced below. Toxicity data requirements that relate to or aggregate with human dietary risk were addressed by requests for data waivers, which were based on publically available information/data that were previously submitted by the applicant, and reviewed and accepted by the Agency, in support of the tolerance exemption that the Agency granted for the chemically-similar SOEs (Refs. 3, 4, and 6). In addition, the Agency found relevant data from additional public sources, including EPA's National Toxicology Program, which contributed to the Agency's review (Ref. 1). All of this information/data, which, in combination, was 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), was deemed adequate to support the waiver requests. Sorbitan esters and sucrose fatty acid esters, which are used as food emulsifiers and as post-harvest fruit protectants, have been found to be of no particular toxic concern in studies used to support their safety to the FDA. Sorbitol octanoate is different from the FDA-approved sorbitan esters in that octanoate is the sole fatty acid component and sorbitan anhydrides are derived from sorbitol by removal of one molecule of water. Therefore, results from studies on sorbitan esters can be used to support lack of toxicity concern with sorbitol octanoate. Sorbitol octanoate also rapidly hydrolyzes to sorbitol and octanoic acid, both of which are common human dietary components of no toxicological concern. Both sorbitol and octanoic acid are included in EPA's List 4 inert ingredients, and thus are of minimal concern. Sucrose octanoate has previously been registered by EPA (EPA Reg. No. 70950–2). The rationales for waiver requests for all required mammalian toxicological studies are acceptable. More detailed analyses of these data and information can be found in specific Agency reviews of the studies and technical literature (Refs. 1, 6, 7, 8 and 9).

1. *Acute oral toxicity waiver (OPPTS 870.1100) 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 (Refs. 3 and 4). The data contained in these reports demonstrated that sorbitan esters and sucrose octanoate esters had extremely low oral toxicity (in laboratory studies), even at concentrations substantially higher than are found in human food.

In studies with rats and humans, it was demonstrated that sorbitan esters and sucrose octanoate esters were rapidly hydrolyzed and absorbed by the body. Sorbitol octanoate is different from the sorbitan esters approved by FDA for direct addition to food for human consumption in the degree to which water is removed during the manufacturing process and the specific fatty acid that is used to make the esters. Sorbitan is a generic name for anhydrides (cyclic ether tetrahydric alcohols) derived from sorbitol by removal of one molecule of water. Octanoic acid is used to make sorbitol octanoate, but the sorbitan esters are made with mixtures of several longer-chain fatty acids. Sorbitan monopalmitate in the diet of rats; sorbitan monostearate in the diet of rats; sorbitan tristearate administered to rats by gavage; and sorbitan monopalmitate, sorbitan monostearate, and sorbitan tristearate in rats (maximum oral dose) caused no toxic symptoms/mortality. The acute oral LD_{50}s for monoleate and sorbitan monolaurate in rats were 39.8 and 37.5 grams/kilogram (g/kg), respectively. An estimate of acceptable daily intake in man of 0-25 milligrams/kilogram (mg/kg) was set by the Expert Committee on Food Additives. Sorbitol octanoate hydrolyzed rapidly to sorbitol and octanoic acid. The LD_{50}s for sorbitol in mice/rats dosed intravenously or orally ranged from 7,100 to 25,700 mg/kg, respectively. The oral LD_{50}s for octanoic acid were 1,283 mg/kg (one study, male rats) and 10,080 mg/kg (another study, male and female rats), amounts far greater than humans would encounter via the oral exposure route from pesticidal use of sorbitol octanoate. Sorbitol (21 CFR 184.1835) and ocatanoic acid (21 CFR 184.1025) are classified as GRAS by the FDA and are in EPA's List 4 - Inerts of Minimal Concern. Because sorbitol octanoate is chemically similar to SOEs, for which an exemption from tolerance already is established, and octanoic acid is a sorbitol octanoate constituent/degradate of no toxicological concern, the information/data described above support waiver from the data requirements for acute oral toxicity studies (classification: acceptable; Toxicity Category IV for the manufacturing-use product and end-use product).

2. *Acute dermal toxicity waiver (OPPTS 870.1200) 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 connection with the tolerance exemption granted for SOEs, which as noted above are chemically and toxicologically similar to sorbitol octanoate. Also, dermal toxicity data on the sorbitan esters is relevant to sorbitol octanoate. The only difference between the sorbitan esters used in cosmetics and sorbitol octanoate is in the degree to which water is removed during the manufacturing process. Sorbitan fatty acid esters were generally minimal to mild skin irritants in animals and humans. In addition, publically available sources list the rabbit dermal LD_{50} for octanoic acid (a sorbitol octanoate constituent/degradate of no toxicological concern) as > 5,000 mg/kg (Ref. 1), an amount far greater than humans would encounter via the dermal exposure route from pesticidal use of sorbitol octanoate and which places it in the Toxicity Category of no concern (IV) (classification: acceptable; Toxicity Category IV for the manufacturing-use product and end-use product).

3. *Acute inhalation toxicity waiver (OPPTS 870.1300) 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 connection with the tolerance exemption granted for SOEs, which as noted above are chemically and toxicologically similar to sorbitol octanoate (Refs. 1,3,4 and 6). No adverse effects have been reported by researchers working with sorbitol octanoate, and the compound is not volatile. The sorbitol octanoate constituents sorbitol and octanoic acid are classified as Generally Recognized as Safe (GRAS) by the FDA and are among EPA's List 4 Inerts of Minimal Concern. The chemically-similar sorbitan fatty acid esters are waxy solids or viscous liquids which cannot be inhaled (classification: acceptable; Toxicity Category IV for the manufacturing-use product and end-use product).

4. *Hypersensitivity study waiver (OPPTS 870.2600) MRID 455973–01.* No hypersensitivity incidents have been reported for laboratory workers regularly exposed to sorbitol octanoate for up to 7 years. Neither have there been reports of hypersensitivity from

those working with the chemically-similar sucrose octanoate. A waiver for conduct of a dermal sensitization study for sorbitol octanoate thus can be supported. 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).

5. *Genotoxicity and mutagenicity waiver (OPPTS 870.5300, 870.5195) 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 and sorbitol octanoate (because of their chemical and toxicological similarities) are not genotoxic and/or mutagenic, nor is the active ingredient structurally and/or chemically similar to known mutagens or known classes of mutagens (Refs. 3, 4 and 6). In addition, a study reported by EPA's National Toxicology Program shows octanoic acid, a sorbitol octanoate constituent/degradate of no toxicological concern, to be negative for genotoxicity/mutagenicity (Ref. 1) (classification: acceptable).

6. *Other data requirements waived.* Immune response and all remaining Tier I biochemical toxicology data requirements that relate to or aggregate with human dietary risk were waived (see OPPTS 880.3800 through 870.4200, MRID 444158–03 and amendment number 1) due to the low toxicity of the chemically similar SOEs, as reported in the open technical literature (Refs. 3, 4, 5, 6 and 7). In addition, octanoic acid, a sorbitol octanoate constituent/degradate of no toxicological concern, is considered a nonteratogenic compound even at the very high dose rate of 18.75 millimoles/kg (Ref. 1).

IV. Aggregate Exposures

In examining aggregate exposure, section 408 of the FFDCA directs EPA to consider available information concerning exposures from the pesticide residue in food and all other non-occupational exposures, including drinking water from ground water or surface water and exposure through pesticide use in gardens, lawns, or buildings (residential and other indoor uses).

A. Dietary Exposure

1. *Food.* An Acceptable Daily Intake (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 (Refs. 3, 4, and 6). There are no reasonably foreseeable circumstances in which the residue levels of SOEs or the chemically- and toxicologically-similar compound sorbitol octanoate would ever approach this amount. Sorbitol octanoate hydrolyzes into its constituents (sorbitol and octanoic acid) shortly after application and then biodegrades. In studies with rats and humans, it was demonstrated that SOEs were rapidly hydrolyzed and absorbed by the body (Ref. 6). Because sorbitol octanoate is made from sorbitol (present in certain fruits) and caprylic acid (derived from edible oils and fats), there is a great likelihood of exposure in the normal human diet to both SOEs (derived from sugar and edible tallow or edible vegetable oils) and sorbitol octanoate, and their components for most, if not all, individuals, including infants and children. Sorbitol and octanoic acid are common components of the human diet. Thus, sorbitol octanoate 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 either SOEs (which for years have been FDA-approved food emulsifiers) or the chemically-similar sorbitol octanoate. Even if there is a significant increase in dietary exposure to sorbitol octanoate due to its use as a pesticide, the acute toxicity information from the National Toxicology Program and the information submitted by the registrant demonstrating extremely low mammalian toxicity (Toxicity Category IV) for SOEs (which, again, are chemically similar to sorbitol octanoate) indicate that any possible risk associated with acute exposures by the oral, dermal and inhalation routes to sorbitol octanoate would be low to non-existent. Further, any increased exposure due to the proposed products would be negligible because the active ingredient sorbitol octanoate will rapidly hydrolyze into its constituent components (sorbitol and octanoic acid), which subsequently will be rapidly metabolized by soil bacteria, thus limiting the general public's contact with treated plants or food products.

2. *Drinking water exposure.* No drinking water exposure is expected. Sorbitol octanoate is not applied directly to water, does not persist in the environment and biodegrades following application/use. Even if sorbitol octanoate residues were to enter drinking water, we do not expect any significant risk since sorbitol octanoate will rapidly hydroloyze into its consituent components (sorbitol and octanoic acid), which then would biodegrade prior to consumption by microorganisms before the general public would contact drinking water containing residues of sorbitol octanoate.

B. Other Non-Occupational Exposure

The potential for non-dietary exposure to sorbitol octanoate residues for the general population, including infants and children, is unlikely because the uses are limited to applications to horticultural and agricultural crops. The sorbitol octanoate constituents sorbitol and octanoic acid are normal parts of the human diet. Sorbitol octanoate toxicity from a dietary exposure standpoint has been determined to be extremely low. Therefore, while there exists a great likelihood of prior exposure for most, if not all, individuals to both sorbitol octanoate and SOEs, any increased non-occupational exposure due to the proposed products would be negligible because the active ingredient sorbitol octanoate will rapidly hydrolyze into its constituent components (sorbitol and octanoic acid) which will be rapidly metabolized by soil bacteria, thus limiting the general public's contact with treated plants or food products via the dermal or inhalation routes.

V. Cumulative Effects

Section 408(b)(2)(D)(v) of FFDCA requires that, when considering whether to establish, modify, or revoke a tolerance, the Agency consider "available information" concerning the cumulative effects of a particular pesticide's residues and "other substances that have a common mechanism of toxicity." These considerations include the possible cumulative effects of such residues on infants and children.

Except through ocular exposure, which is only expected in the occupational setting and can be prevented by the use of protective eyewear, neither sorbitol octanoate nor SOEs are toxic, and it is not anticipated that there would be cumulative effects from common mechanisms of toxicity. EPA does not have, at this time, available data to suggest whether sorbitol octanoate has a common mechanism of toxicity with other substances. Unlike other pesticides for which EPA has followed a cumulative risk approach based on a common mechanism of toxicity, EPA has not made a common mechanism of toxicity

finding as to sorbitol octanoate and any other substances and sorbitol octanoate does not appear to produce a toxic metabolite produced by other substances. For the purpose of this tolerance action, therefore, EPA has not assumed that sorbitol octanoate has a common mechanism of toxicity with other substances. For information regarding EPA's efforts to determine which chemicals have a common mechanism of toxicity and to evaluate the cumulative effects of such chemicals, see the policy statements released by EPA's Office of Pesticide Programs concerning common mechanism determinations and procedures for cumulating effects from substances found to have a common mechanism on EPA's web site at *http://www.epa.gov/pesticides/cumulative.*

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

1. *U.S. population.* The Agency has determined that there is a reasonable certainty that no harm will result from aggregate exposure to residues of sorbitol octanoate to the U.S. population. This includes all anticipated dietary exposures and other non-occupational exposures for which there is reliable information. The Agency arrived at this conclusion based on the extremely low levels of mammalian dietary toxicity associated with SOEs and, by extension, sorbitol octanoate due to the fact it is nearly identical chemically. Accordingly, it is unlikely that any toxic effects will result from exposure to sorbitol octanoate via the oral, dermal or inhalation pathways when the registered sorbitol octanoate products are used according to proposed label directions (Ref. 6). Based upon the data submitted in connection with SOEs and, by extension, the chemically-similar compound sorbitol octanoate, the amount of sorbitol octanoate applied to food crops is many orders of magnitude lower than the concentrations of sorbitol octanoate needed to cause toxicological effects. Because the worst case scenario exposure is far below the level of any dietary toxicity known for SOEs or sorbitol octanoate, or their components and degradates, EPA has determined that residues will not pose a dietary risk under reasonably foreseeable circumstances and that granting a tolerance exemption is appropriate.

2. *Infants and children.* FFDCA section 408 provides that EPA shall apply an additional tenfold margin of exposure for infants and children in the case of threshold effects unless the Agency determines, based on reliable data, that a different margin is safe. Margins of exposure are referred to as uncertainty or safety factors, and are used to account for potential prenatal and postnatal toxicity and any lack of completeness in the data base. Based on all the reliable available information the Agency reviewed on SOEs and, by extension, sorbitol octanoate due to the fact that it is nearly identical chemically, the Agency concludes that sorbitol octanoate is practically non-toxic to mammals from a dietary standpoint, including infants and children. Thus, there are no threshold effects of concern and an additional margin of safety is not necessary to protect infants and children.

VII. 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, sorbitol octanoate 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 sorbitol octanoate. To date, there is no evidence to suggest that sorbitol octanoate affects the immune system, functions in a manner similar to any known hormone, or that it acts as an endocrine disruptor.

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 low toxicity and low exposure from the pesticidal use of sorbitol octanoate. For the same reasons, the Agency concludes that an analytical method is not required for enforcement purposes for sorbitol octanoate.

C. Codex Maximum Residue Level

There are no CODEX maximum residue levels for sorbitol octanoate.

VIII. Conclusions

Based on the toxicology information/data submitted and other information available to the Agency, there is a reasonable certainty that no harm will result from aggregate exposure to residues of sorbitol octanoate to the U.S. population, including infants and children, under reasonably foreseeable circumstances, when the biochemical pesticide is used in accordance with product label directions and good agricultural practices. This includes all anticipated dietary exposures and all other non-occupational exposures for which there is reliable information. The Agency has arrived at this conclusion based on the information/data submitted (and publically available) demonstrating negligible toxicity of the chemically-similar SOEs and sorbitan esters, and of sorbitol octanoate's constituents (sorbitol and octanoic acid). As a result, EPA is establishing an exemption from the tolerance requirements pursuant to FFDCA section 408(c) for residues of sorbitol octanoate in or on all food commodities.

IX. 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, EPA will continue to use those procedures, with appropriate adjustments, until the necessary modifications can be made. The new section 408(g) of the FFDCA provides essentially the same process for persons to "object" to a regulation for an exemption from the requirement of a tolerance issued by EPA under new section 408(d) of the FFDCA, as was provided in the old sections 408 and 409 of the FFDCA. However, the period

for filing objections is now 60 days, rather than 30 days.

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

You must file your objection or request a hearing on this regulation in accordance with the instructions provided in this unit and in 40 CFR part 178. To ensure proper receipt by EPA, you must identify docket ID number EPA–HQ–OPP–2005–0515 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 March 28, 2006.

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

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

2. *Copies for the Docket.* In addition to filing an objection or hearing request with the Hearing Clerk as described in Unit IX.A.1., you should also send a copy of your request to the PIRIB for its inclusion in the official record that is described in **ADDRESSES**. Mail your copies, identified by docket ID number EPA–HQ–OPP–2005–0515, to: Public Information and Records Integrity Branch, Information Technology and Resources Management Division (7502C), Office of Pesticide Programs, Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington, DC 20460–0001. In person or by courier, bring a copy to the location of the PIRIB described in **ADDRESSES**. You may also send an electronic copy of your request via e-mail to: *opp-docket@epa.gov*. Please use an ASCII file format and avoid the use of special characters and any form of encryption. Copies of electronic objections and hearing requests will also be accepted on disks in WordPerfect 6.1/8.0 or ASCII file format. Do not include any CBI in your electronic copy. You may also submit an electronic copy of your request at many Federal Depository Libraries.

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

A request for a hearing will be granted if the Administrator determines that the material submitted shows the following: There is a genuine and substantial issue of fact; there is a reasonable possibility that available evidence identified by the requestor would, if established resolve one or more of such issues in favor of the requestor, taking into account uncontested claims or facts to the contrary; and resolution of the factual issues(s) in the manner sought by the requestor would be adequate to justify the action requested (40 CFR 178.32).

X. 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. Lawson, M.E. 1997. Kirk-Othmer's Encyl Chem Tech. 4th Ed. J.I. Kroschwitz (ed). John Wiley & Sons, NY.

3. 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.

4. 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.

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

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

7. USEPA. Sucrose octanoate esters; A request for concurrence on a decision to waive the requirement for 90–day feeding study (870.3100) and Developmental Toxicity (Teratogenicity (870.3700) studies, based on the Registrant's correspondence of July 12, 2002. D. Greenway to R. S. Jones; August 7, 2002.

8. USEPA. Secondary Review of Data/ information submitted to support Registration of Sorbitol Octanoate R.D. Sjoblad to D. Greenway; December 29, 2004

9. USEPA. Endangered Species Risk Assessment for Sorbitol Octanoate. R. S. Jones to D. Greenway; September 13, 2005.

XI. Statutory and Executive Order Reviews

This final rule establishes an exemption from the tolerance requirement under section 408(d) of the FFDCA in response to a petition submitted to the Agency. The Office of Management and Budget (OMB) has exempted these types of actions from review under Executive Order 12866, entitled *Regulatory Planning and Review* (58 FR 51735, October 4, 1993). Because this rule has been exempted from review under Executive Order 12866 due to its lack of significance, this rule is not subject to Executive Order 13211, *Actions Concerning Regulations That Significantly Affect Energy Supply, Distribution, or Use* (66 FR 28355, May 22, 2001). This final rule does not contain any information collections subject to OMB approval under the Paperwork Reduction Act (PRA), 44 U.S.C. 3501 *et seq.*, or impose any enforceable duty or contain any unfunded mandate as described under Title II of the Unfunded Mandates Reform Act of 1995 (UMRA) (Public Law 104–4). Nor does it require any special considerations under Executive Order 12898, entitled *Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations* (59 FR 7629, February 16, 1994); or OMB review or any Agency action under Executive Order 13045, entitled *Protection of Children from Environmental Health Risks and Safety Risks* (62 FR 19885, April 23, 1997). This action does not involve any technical standards that would require Agency consideration of voluntary consensus standards pursuant to section 12(d) of the National Technology Transfer and Advancement Act of 1995 (NTTAA), Public Law 104–113, section 12(d) (15 U.S.C. 272 note). Since tolerances and exemptions that are established on the basis of a petition under section 408(d) of the FFDCA, such as the exemption in this final rule, do not require the issuance of a proposed rule, the requirements of the Regulatory Flexibility Act (RFA) (5 U.S.C. 601 *et seq.*) do not apply. In addition, the Agency has determined that this action will not have a

substantial direct effect on States, on the relationship between the national government and the States, or on the distribution of power and responsibilities among the various levels of government, as specified in Executive Order 13132, entitled *Federalism* (64 FR 43255, August 10, 1999). Executive Order 13132 requires EPA to develop an accountable process to ensure "meaningful and timely input by State and local officials in the development of regulatory policies that have federalism implications." "Policies that have federalism implications" is defined in the Executive order to include regulations that have "substantial direct effects on the States, on the relationship between the national government and the States, or on the distribution of power and responsibilities among the various levels of government." This final rule directly regulates growers, food processors, food handlers and food retailers, not States. This action does not alter the relationships or distribution of power and responsibilities established by Congress in the preemption provisions of section 408(n)(4) of the FFDCA. For these same reasons, the Agency has determined that this rule does not have any "tribal implications" as described in Executive Order 13175, entitled *Consultation and Coordination with Indian Tribal Governments* (65 FR 67249, November 6, 2000). Executive Order 13175, requires EPA to develop an accountable process to ensure "meaningful and timely input by tribal officials in the development of regulatory policies that have tribal implications." "Policies that have tribal implications" is defined in the Executive order to include regulations that have "substantial direct effects on one or more Indian tribes, on the relationship between the Federal Government and the Indian tribes, or on the distribution of power and responsibilities between the Federal Government and Indian tribes." This rule will not have substantial direct effects on tribal governments, on the relationship between the Federal Government and Indian tribes, or on the distribution of power and responsibilities between the Federal Government and Indian tribes, as specified in Executive Order 13175. Thus, Executive Order 13175 does not apply to this rule.

XII. Congressional Review Act

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

List of Subjects in 40 CFR Part 180

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

Dated: January 13, 2006.

James Jones,

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), 346a and 371.

■ 2. Section 180.1262 is added to subpart D to read as follows:

§ 180.1262 Sorbitol octanoate; exemption from the requirement of a tolerance.

An exemption from the requirement of a tolerance is established for residues of sorbitol octanoate in or on all food commodities when used in accordance with label directions.

[FR Doc. 06–756 Filed 1–26–06; 8:45 am]

BILLING CODE 6560–50–S

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Centers for Medicare & Medicaid Services

42 CFR Part 414

[CMS–1167–F]

RIN 0938–AN02

Medicare Program; Payment for Respiratory Assist Devices With Bi-Level Capability and a Backup Rate

AGENCY: Centers for Medicare & Medicaid Services (CMS), HHS.

ACTION: Final rule.

SUMMARY: This final rule clarifies that respiratory assist devices with bi-level capability and a backup rate must be paid as capped rental items of durable medical equipment (DME) under the Medicare program and not paid as items requiring frequent and substantial servicing (FSS), as defined in section 1834(a)(3) of the Social Security Act. Before 1999, respiratory assist devices with bi-level capability (with or without a backup rate feature) were referred to as "intermittent assist devices with continuous positive airway pressure devices" under the Medicare program and in the Healthcare Common Procedure Coding System (HCPCS). This final rule responds to public comments received on a proposed rule published in the **Federal Register** on August 22, 2003, and finalizes the policy in that proposed rule. The rule will ensure that respiratory assist devices are consistently and properly paid under Medicare as capped rental items.

DATES: The provisions of this final rule are effective on April 1, 2006.

FOR FURTHER INFORMATION CONTACT: Joel Kaiser, (410) 786–4499.

SUPPLEMENTARY INFORMATION:

I. Background

A. Legislative Authority for Payment for Durable Medical Equipment (DME)

Section 1834(a) of the Social Security Act (the Act) sets forth the payment methodology and requirements for payment for the purchase or rental of new and used durable medical equipment (DME) for Medicare beneficiaries under Medicare Part B (Supplementary Medical Insurance). In accordance with section 1834(a) of the Act, payment for DME is made on a fee schedule basis. Each item of DME that is paid under Medicare Part B is classified into one of the following payment categories:

- Inexpensive or other routinely purchased DME.
- Items requiring frequent and substantial servicing (FSS).
- Customized items.
- Oxygen and oxygen equipment.
- Other covered items (other than DME).
- Other items of DME (capped rental (CR) items).

Each category has its own unique payment rules. With the exception of customized items, for each item of DME that is identified by a code in the Healthcare Common Procedure Coding System (HCPCS), a fee schedule amount is calculated. The Medicare payment amount for a customized item of DME is based on the Medicare carrier's individual consideration of that item.

Section 1834(a) of the Act provides that Medicare payment for DME is equal

BIOPESTICIDES REGISTRATION ACTION DOCUMENT

Octanoate Esters

Sucrose Octanoate Esters
(PC Code 035300)

Sorbitol Octanoate
(PC Code 035400)

U.S. Environmental Protection Agency
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division
Sucrose Octanoate Esters
(PC Code 035300)
Sorbitol Octanoate
(PC Code 035400)

Table of Contents

3. Dietary Exposure and Risk Characterization
   a. Dietary
      i. Food
      ii. Drinking Water
   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
6. Aggregate Exposure from Multiple Routes Including Dermal, Oral, and Inhalation
7. Cumulative Effects
8. Risk Characterization

C. ENVIRONMENTAL ASSESSMENT

1. Ecological Effects Hazard Assessment
2. Environmental Fate and Ground Water Data
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
   c. Precautionary Labeling
2. Environmental Hazards Labeling
3. Application Rate

D. LABELING

V. Actions Required by Registrants

VI. Appendix A

VII. References

BIOPESTICIDES REGISTRATION ACTION DOCUMENT TEAM

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Biopesticides and Pollution Prevention Division

Biochemical Pesticides Branch

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

II. Executive Summary

This Biopesticides Registration Action Document (BRAD) was originally published in 2002 for a single active ingredient, Sucrose Octanoate Esters. With the addition of a new octanoate active ingredient (Sorbitol Octanoate), the BRAD is now revised and entitled Octanoate Esters. This edition of the BRAD now covers two active ingredients, Sucrose Octanoate Esters and Sorbitol Octanoate.

A. IDENTITY

The sucrose octanoate esters technical grade active ingredient (TGAI)/manufacturing-use product (MUP), AAvachem Sucrose Octanoate Manufacturing Use Product@ (EPA Registration Number 70950-1), registration was cancelled on July 21, 2005. It consisted 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), AAvachem Sucrose Octanoate [40.0%]@ (EPA Registration Number 70950-2), contains 40.0% sucrose octanoate esters.

The sorbitol octanoate TGAI/MUP, AAvachem Sorbitol Octanoate Manufacturing Use Product@ (EPA File Symbol 70950-U) and the EP, AAvachem Sorbitol Octanoate [90.0%]@ (EPA File Symbol 70950-G) both contain 90.0% sorbitol octanoate as the active ingredient. Sorbitol octanoate [D-Glucitol, octanoate] is not a naturally occurring compound, but is derived as a sugar ester synthesized via the condensation of a sorbitol (a naturally-occurring sugar alcohol) with octanoic acid (a naturally occurring fatty acid). The active ingredient is an oily liquid that is miscible in water, forming a stable emulsion when shaken.

B. USE/USAGE

AAvachem Sucrose Octanoate [40.0%]@ is applied as 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.

AAvachem Sorbitol Octanoate [90.0%]@ is to be used for spray treatments in greenhouses and on nursery and field crops to control or suppress soft-bodied pests (insects and mites).

C. RISK ASSESSMENT

No unreasonable adverse effects on humans or the environment are anticipated from aggregate exposure to: 1) AAvachem Sucrose Octanoate Manufacturing Use Product@ (now cancelled) or AAvachem Sucrose Octanoate [40.0%]@, or 2) AAvachem Sorbitol Octanoate Manufacturing Use Product@ or AAvachem Sorbitol Octanoate [90.0%]@. 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 registration.

Sucrose Octanoate Esters: Submitted information and data for the TGAI/MUP and the EP indicate Toxicity Category IV for acute oral, acute dermal, and acute inhalation toxicity; and for primary dermal irritation. Neither the TGAI/MUP (product registration now cancelled), nor the EP, 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 Toxicity Category II when the EP is tested and Toxicity Category I when the TGAI/MUP is used.

Sorbitol Octanoate: Submitted information and data for the TGAI/MUP and the EP indicate Toxicity Category IV for acute oral, acute dermal, and acute inhalation toxicity; and for primary dermal irritation. Neither the TGAI/MUP, nor the EP, is a dermal sensitizer. The data reported for primary eye irritation show that the test substance was severely irritating and is Toxicity Category II for both the TGAI/MUP and the EP.

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 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 octanoate esters products when used according to the label instructions. The Agency has considered 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 these products.

2. Ecological Risk Assessment

a. Ecological Toxicity Endpoints

Data waivers were requested and granted for ecological testing requirements because no toxic endpoints are expected for octanoate esters, 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 ingredients do not persist in the environment and biodegrade quickly. Sucrose octanoate degrades within approximately five days at approximately 20-27EC, in both aerobic and anaerobic conditions. Sorbitol octanoate biodegrades with an apparent post-application half-life of approximately 7 to 10 days.

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 octanoate esters products, used according to label directions, will not result in significant adverse effects to wildlife or other organisms.

D. DATA GAPS / LABELING

There are no data gaps. Certain precautionary labeling is required for octanoate esters products to mitigate risks associated with ocular exposure (results of primary eye irritation testing placed the active ingredients sucrose octanoate and sorbitol octanoate in Toxicity Categories I and II, respectively; see Labeling Rationale for details).

II. Overview

E. ACTIVE INGREDIENT OVERVIEW

Common Name:	Sucrose octanoate esters	Sorbitol octanoate
Chemical Name:	Sucrose octanoate esters [(α-D-glucopyranosyl, β-D-fructofuranosyl-octanoate), mono, di-, and triesters of sucrose octanoate]	Sorbitol octanoate [D-Glucitol, octanoate]
CAS Numbers:	42922-74-7 and 58064-47-4	108175-15-1
Trade and Other Names:	Avachem Sucrose Octanoate Manufacturing Use Product (cancelled), Avachem Sucrose Octanoate [40.0%]	Avachem Sorbitol Octanoate Manufacturing Use Product, Avachem Sorbitol Octanoate [90.0%]
OPP Chemical Code:	035300	035400
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 octanoate esters include the following:

	Sucrose octanoate esters	Sorbitol octanoate
Type of Pesticide:	Biochemical insecticide/miticide	Biochemical insecticide/miticide
Use Sites:	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.	Field, greenhouse, and nursery use on any type of agricultural commodity (including certain non-food ornamentals).
Formulation Types:	Liquid	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 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.	Apply by ground spray equipment (e.g. ground boom, air blast). Shake or stir before use, adding the appropriate quantity to water, with agitation. Maintain gentle agitation during application. The proposed label specifies application rates of 0.5% volume/volume (v/v) for all use sites.
Use Practice Limitations:	Do not allow workers into treated areas for 48 hours following application.	Do not allow workers into treated areas for 24 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.	Initiate applications as soon as pest pressure is observed. Repeat applications, as necessary, at intervals of 7 - 10 days.

C. ESTIMATED USAGE

Sucrose Octanoate Esters: The sucrose octanoate esters MUP (now cancelled) and EP were both granted section 3(c)(5) registration on September 16, 2002. 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.

Sorbitol Octanoate: None used yet since this is the first registration of this new active ingredient.

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 octanoate esters were fulfilled. Product analysis data requirements are adequately satisfied.

E. REGULATORY HISTORY

Sucrose Octanoate Esters:
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. A notice of 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 Asucrose 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 active ingredient) refer only to Asucrose octanoate esters [(α-D-glucopyranosyl-β-D-fructofuranosyl-octanoate), mono-, di-, and triesters of sucrose octanoate]. Hereinafter EPA uses the term Asucrose 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.

On September 25, 2002, EPA published a final rule (67 FR 60146) which established at 40 CFR ' 180.1222 an exemption from the requirement of a tolerance for residues of sucrose octanoate esters [(α-D-glucopyranosyl-β-D-fructofuranosyl-octanoate), mono-, di-, and triesters of sucrose octanoate] in or on all food commodities.

On December 4, 2002, EPA published a Notice of Registration Approval (67 FR 72172) announcing the September 16, 2002 section 3(c)(5) registration of the two applications for pesticide products [EPA Registration Numbers 70950-1(now canceled) and 70950-2] containing an active ingredient not included in any previously registered products.

Sorbitol Octanoate:
On December 5, 2001, EPA received an application from AVA Chemical Ventures, L.L.C. for two new products with the new active ingredient, sorbitol octanoate. A notice of receipt of the application for registration of sorbitol octanoate was published in the Federal Register on September 29, 2004 (69 FR 58164) with a 30-day comment period. No comments were received following this publication.

On September 29, 2004, EPA published a Notice of Filing a Pesticide Petition to Establish a Tolerance for a Certain Pesticide Chemical (sorbitol octanoate) in or on Food (2E6389, 69 FR 58166) with a 30-day comment period. No comments were received.

F. CLASSIFICATION

Sucrose Octanoate Esters: 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 Abiochemical pesticide@ designation to sucrose octanoate esters (Ref. 1).

Sorbitol Octanoate: On February 28, 2001, the Biochemical Classification Committee determined that the insecticide polyol esters (C_8/ C_{10} fatty acid esters of sorbitol) are very similar to sucrose octanoate esters (currently classified as a biochemical) and have a non-toxic mode of action. Sorbitol octanoate was designated as a biochemical.

G. FOOD CLEARANCES/TOLERANCES

Sucrose Octanoate Esters: On September 25, 2002, EPA published a final rule (67 FR 60146) which established at 40 CFR ' 180.1222 an exemption from the requirement of a tolerance for residues of sucrose octanoate esters [(α-D-glucopyranosyl-β-D-fructofuranosyl-octanoate), mono-, di-, and triesters of sucrose octanoate] in or on all food commodities. There are no Codex tolerances for sucrose octanoate esters.

Sorbitol Octanoate: On September 29, 2004, EPA published a Notice of Filing a Pesticide Petition to Establish a Tolerance for a Certain Pesticide Chemical (sorbitol octanoate) in or on Food (2E6389, 69 FR 58166) 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 sorbitol octanoate

III. Science Assessment

A. PHYSICAL/CHEMICAL PROPERTIES ASSESSMENT

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

1. Product Identity and Mode of Action

a. Product Identity:

Sucrose Octanoate: The technical grade active ingredient/manufacturing-use product, AAvachem Sucrose Octanoate Manufacturing Use Product@ (cancelled), consisted 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, AAvachem Sucrose Octanoate [40.0%],@ 40% sucrose octanoate esters.

Sorbitol Octanoate: The technical grade active ingredient/manufacturing-use product, AAvachem Sorbitol Octanoate Manufacturing Use Product@ and end use product, AAvachem Sorbitol Octanoate [90.0%]@ both consist of 90.0% sorbitol octanoate esters [D-Glucitol, octanoate].

b. Mode of Action:

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

Sorbitol Octanoate: The mode of action is a physical, surfactant effect that results in rapid suffocation and/or dewaxes the cuticle of the target pests, subsequently causing dessication via loss of body fluids. There are no neurological and/or physiological interactions with the target pest.

2. Physical And Chemical Properties Assessment

The physical and chemical characteristics of all products (TGAI/MUP and EP) were submitted to support registration. They are summarized in Table 1.

Table 1. Product chemistry data requirements.

Product Chemistry	Sucrose Octanoate Esters TGAI/MUP (MRID 444880-01, as amended by 451974-01, 451974-02, 454103-01 and, 454103-02)	Sucrose Octanoate Esters EP	Sorbitol Octanoate TGAI/MUP and EP (MRIDs 455974-01 and 455974-02 (and its amendment))
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 [40.0%], the end-use product, contains 40% sucrose octanoate esters	Avachem Sorbitol Octanoate Manufacturing Use Product and Avachem Sorbitol Octanoate [90.0%], the end-use product, both contain 90.0% sorbitol octanoate
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.	An acceptable description of the manufacturing processes was submitted.
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.	No impurities of toxicologic concern are formed during the formulation processes.

Product Chemistry	Sucrose Octanoate Esters TGAI/MUP (MRID 444880-01, as amended by 451974-01, 451974-02, 454103-01 and, 454103-02)	Sucrose Octanoate Esters EP	Sorbitol Octanoate TGAI/MUP and EP (MRIDs 455974-01 and 455974-02 (and its amendment))
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 was registered simultaneously with the EP.	Data obtained from the TGAI/MUP five-batch analysis demonstrate that the analytical method is adequate and without deficiency. No five-batch preliminary analysis data were submitted for the EP, but none are required since the EP is 100% MUP
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.	The certified limits for the active ingredient and other impurities are acceptable.
880.1800: Enforcement analytical method	The analytical method is high performance liquid chromatography (HPLC).	An acceptable liquid chromatography (HPLC) analytical method was submitted.	The analytical methods are high performance liquid chromatography (HPLC) and gas chromatography (GC)
Physical/Chemical Properties	**Sucrose Octanoate Esters TGAI/MUP (MRID 444158-02, as amended by MRIDs 446101-02, 447634-01, and 451974-02)**	**Sucrose Octanoate Esters EP**	**Sorbitol Octanoate TGAI/MUP and EP**
880.6302: Color	Amber	Amber	Amber
880.6303: Physical State	Liquid	Liquid	Liquid
880.6304: Odor	Faint sweet smell	Faint sweet smell	Faint sweet smell

Product Chemistry	Sucrose Octanoate Esters TGAI/MUP (MRID 444880-01, as amended by 451974-01, 451974-02, 454103-01 and, 454103-02)	Sucrose Octanoate Esters EP	Sorbitol Octanoate TGAI/MUP and EP (MRIDs 455974-01 and 455974-02 (and its amendment))
880.7200: Melting Point	NA, not a solid	Not required per 40 CFR ' 158.190	NA, not a solid
880.7220: Boiling Point	Decomposes above 105EC	Not required per 40 CFR ' 158.190	Decomposes above 105EC
880.7300: Density, Bulk Density, or Specific Gravity	8.75 to 9.50 lbs/gal	8.50 to 9.00 lbs/gal	9.30 to 9.90 lb/gal
880.7840: Solubility	Forms an emulsion with water	Not required per 40 CFR ' 158.190	Forms an emulsion with water
880.7050: Vapor Pressure	<5 mm Hg	Not required per 40 CFR ' 158.190	<5 mm Hg
880.7370: Dissociation constant	Not Applicable	Not Applicable	Not Applicable
880.7550: Octanol/water partition coefficient	Unknown	Not required per 40 CFR ' 158.190	Not Applicable
880.7000: pH	Not Applicable	7.0	7.0-7.3
880.6313: Stability	Stable below 40EC	Not required per 40 CFR '158.190	Stable below 40EC
880.6314: Oxidizing or Reduction Action	Not Applicable, does not contain an oxidizing or reducing agent	Not Applicable, does not contain an oxidizing or reducing agent	Not Applicable
880.6315: Flammability/Flame Extension	None; decomposes above 105EC	Not Applicable, does not contain a combustible liquid	Decomposes above 105EC
880.6316:	Not Applicable, is not	Not Applicable, is not	Non-explosive

Product Chemistry	Sucrose Octanoate Esters TGAI/MUP (MRID 444880-01, as amended by 451974-01, 451974-02, 454103-01 and, 454103-02)	Sucrose Octanoate Esters EP	Sorbitol Octanoate TGAI/MUP and EP (MRIDs 455974-01 and 455974-02 (and its amendment))
Explodability	potentially explosive	potentially explosive	
880.6317: Storage Stability	Not Applicable	At least 1year at 40EC based on shelf-life tests	At least 1year at 40EC based on shelf-life tests
880.7100: Viscosity	Not required per 40 CFR ' 158.190	500 to 2000 CP at 25EC	500 to 10,000 cP at 25EC
880.6319: Miscibility	Not Applicable, is not to be diluted with petroleum solvents	Totally miscible in water	Miscible in water
880.6320: Corrosion Characteristics	Not Applicable	Non-corrosive to metals, plastics and glass	Non-corrosive to metals, plastics and glass
880.6321: Dielectric Breakdown Voltage	Not required per 40 CFR ' 158.190	Not Applicable, is not to be used around electrical equipment	Not Applicable

B. HUMAN HEALTH ASSESSMENT

Information and data submitted to support registration of the octanoate esters technical grade/manufacturing-use and end-use products adequately satisfy the food and non-food use requirements set forth in 40 CFR ' 158.690 (c) for biochemical pesticides.

In general, for example with regard to sorbitol octanoate, the TGAI is not a naturally occurring substance, but is a sugar ester synthesized via the condensation of a sorbitol (a naturally-occurring sugar alcohol) with octanoic acid (a naturally-occurring fatty acid). Sorbitol octanoate is chemically similar to certain sorbitan esters and other sugar fatty acid esters that have been approved by FDA for direct food use as food emulsifiers and postharvest protective fruit coatings (see 21 CFR ' 172.836; 172,838; 172.840; and 172.842). Sorbitan esters are different from sorbitol octanoate only in that sorbitol has one more water molecule than sorbitan. Sorbitol octanoate is chemically similar to the active ingredient sucrose octanoate esters (found in EPA Registration Number 70950-2),and its effects on target pests are virtually identical (Ref. 9). Furthermore, sorbitol is a naturally-occurring carbohydrate found in apples, plums, pears, cherries, dates, peaches, apricots, and other fruits (Ref. 9). Octanoic acid (caprylic acid) is a naturally-occurring fatty acid found in plants, coconut oil, meat, and milk (Ref. 9). Sorbitol and octanoic acid are on EPA's List 4 Inerts of Minimal Concern. Sorbitol is cleared for food use in unlimited quantities as an anti-dusting agent under 40 CFR ' 180.910.

Excepting ocular exposure, the overall toxicological risk from human exposure to octanoate esters is negligible.

1. Toxicology Assessment

Adequate mammalian toxicology information and data are available and support registration of the products containing octanoate esters as the active ingredient. For sucrose octanoate esters, new studies were contracted by the registrant for primary eye irritation and primary dermal irritation. For sorbitol octanoate, new studies were contracted by the registrant for primary eye irritation only. Data waivers were requested and granted for all other toxicity data requirements (Ref. 10). 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 (Ref. 4). The submitted information/data, in combination, were found equivalent to what would normally be provided by guideline studies, and therefore are adequate to meet each toxicology requirement 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, 7 and 10).

a. Acute Toxicity

The registrant submitted acceptable data and information from the open technical literature (Refs. 2 and 3) to justify the data waiver requests 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 categorized both the manufacturing-use and end-use octanoate esters products as Toxicity Category IV for acute oral toxicity, acute dermal toxicity and acute inhalation toxicity. On the strength of reports showing no hypersensitivity responses or incidents among workers regularly exposed for up to seven years to octanoate esters, BPPD has determined that octanoate esters are not a sensitizer, and has waived the hypersensitivity study (Ref. 5 and MRID 455973-01).

Sucrose Octanoate Esters: 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 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 [40.0%] as Toxicity Category II.

Sorbitol Octanoate: The manufacturing use product (MUP) caused corneal opacity in six of six rabbits tested at 24 hours after dosing with the MUP at 0.1 ml/eye. Corneal opacity resolved by day 14 after dosing. Eyes of all six rabbits also showed conjunctival irritation, and five of six rabbits showed iritis at one hour post-dosing. These signs also resolved by day 14. Sorbitol octanoate MUP is severely irritating to the eye and is Toxicity Category II (MRID 455974-03).

Sucrose Octanoate Esters: 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 the Agency determined 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 octanoate esters are not genotoxic and/or mutagenic, nor 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 and sorbitol octanoate constituent, octanoic acid, to be negative for genotoxicity/mutagenicity (Ref. 4).

c. Other Subdivision M Toxicity Data Requirements

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 and sorbitol octanoate 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 sucrose octanoate esters, derived from edible 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).

Sorbitol octanoate also rapidly hydrolyzes to sorbitol and octanoic acid, both of which are common human dietary components of no toxicological concern. Both sorbitol and octanoic acid are included in the Agency=s List 4 inert ingredients, and thus are of minimal concern.

Mammalian toxicity data for sucrose octanoate esters and sorbitol octanoate are summarized in Tables 2A and 2B, respectively.

Table 2A. Sucrose Octanoate Esters - 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) Toxicity Category II (EP)	446101-05 446101-06
152-14, 870.2500	Primary dermal irritation in rabbits	Toxicity Category IV (MUP) Toxicity Category IV (EP)	446101-03 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,	Mammalian	Data waiver granted	

GUIDELINE NO.	STUDY	RESULTS	MRID NO.
870.5195	mutagenicity test	(see text for details)	NA
152-20, 870.3100	90-Day Feeding	Data waiver granted (see text for details)	NA
152-23, 870.3700	Teratogenicity	Data waiver granted (see text for details)	NA

Table 2B. Sorbitol Octanoate - 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	Not Applicable
152-11, 870.1200	Acute dermal toxicity	Data waiver granted (see text for details) Toxicity Category IV	Not Applicable
152-12, 870.1300	Acute inhalation toxicity	Data waiver granted (see text for details) Toxicity Category IV	Not Applicable
152-13, 870.2400	Primary eye irritation in rabbits	Toxicity Category II (MUP)	455974-03
152-14, 870.2500	Primary dermal irritation in rabbits	Data waiver granted (see text for details) Toxicity Category IV	Not Applicable
152-15, 870.2600	Dermal sensitization	Data waiver granted (see text for details) Not a sensitizer	455973-01
152-17,	Studies to detect	Data waiver granted	Not Applicable

GUIDELINE NO.	STUDY	RESULTS	MRID NO.
870.5300	genotoxicity	(see text for details)	
152-18, 870.8700	Cellular immune response	Data waiver granted (see text for details)	Not Applicable
152-19, 870.5195	Mammalian mutagenicity test	Data waiver granted (see text for details)	Not Applicable
152-20, 870.3100	90-Day Feeding	Data waiver granted (see text for details)	Not Applicable
152-23, 870.3700	Teratogenicity	Data waiver granted (see text for details)	Not Applicable

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) Amay 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 octanoate esters.

2. Dose Response Assessment

No toxicological endpoints are expected.

3. Dietary Exposure and Risk Characterization

a. Dietary

ii. Food

Sucrose octaonate esters: Because sucrose octanoate 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 octanoate 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 octanoate esters per day for a 176 lb person. In studies with rats ands humans, it was demonstrated that sucrose octanoate 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 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.

Sorbitol octanoate: Sorbitol octanoate rapidly hydrolyzes to sorbitol and octanoic acid, naturally-occurring compounds both of which are common human dietary components of no toxicological concern. Sorbitol octanoate is chemically similar to certain sorbitan esters and other sugar fatty acid esters that have been approved by FDA for direct food use as food emulsifiers and postharvest protective fruit coatings (see 21 CFR 172.836; 172,838; 172.840; and 172.842). Sorbitan esters are different from sorbitol octanoate only in that sorbitol has one more water molecule than sorbitan. Sorbitol octanoate is chemically similar to sucrose octanoate (EPA Registration Number 70950-2) and its effects on target pests are virtually identical (Puterka et al., 2003). Furthermore, sorbitol is a naturally-occurring carbohydrate found in apples, plums, pears, cherries, dates, peaches, apricots, and other fruits (Lawson, 1997). Octanoic acid (caprylic acid) is a naturally-occurring fatty acid found in plants, coconut oil, meat, and milk (Hall, 1995; Rogge et al., 1991; Tatsuka et al., 1993). Both sorbitol and octanoic acid are included in the Agency=s List 4 inert ingredients, and thus are of minimal concern. Sorbitol is cleared for food use in unlimited quantities as an anti-dusting agent under 40 CFR ' 180.910.

iii. 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 20-27EC, 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.

No drinking water exposure is expected; and as noted above, sorbitol octanoate rapidly hydrolyzes to sorbitol and octanoic acid, naturally-occurring compounds (found in foods) both of which are common human dietary components of no toxicological concern.

b. Other Non-occupational Exposure

The potential for non-dietary exposure to octanoate esters residues for the general population, including infants and children, is unlikely because potential use sites are commercial, agricultural, and large-scale horticultural. 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

Significant additional human exposure to 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 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 product-specific 24 or 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 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. Sucrose octanoate esters= and sorbitol octanoate=s chemical 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 octanoate esters.

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

Aggregate exposure to 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 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 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 and sorbitol octanoate 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 and sorbitol octanoate by the consumer would include other sources in addition to the limited amount on treated agricultural/horticultural products. The 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.

7. Cumulative Effects

Except through ocular exposure, 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).

8. Risk Characterization

The Agency has considered 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 [40.0%] or Avachem Sorbitol Octanoate [90.0%] when label instructions are followed.

C. ENVIRONMENTAL ASSESSMENT

1. Ecological Effects Hazard Assessment

The end-use products Avachem Sucrose Octanoate [40.0%] and Avachem Sorbitol Octanoate [90.0%] are 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 octanoate esters is expected to occur.

Sucrose Octanoate Esters: 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).

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 Φg 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).

Sorbitol Octanoate: Waivers were requested for sorbitol octanoate based on evidence that it degrades in the environment to sorbitol and octanoic acid, which already are natural components of plants. Further, sorbitol and octanoic acid are Agency List 4 inerts and thus are of minimal concern. Field trials with sorbitol octanoate have not shown any phytotoxicity.

As a result of BPPD=s assessment of the information and data described above, organism/ecological effects studies were waived for the particular uses of Avachem Sucrose Octanoate [40.0%] and Avachem Sorbitol Octanoate [90.0%]. However, standard precautionary label statements under AEnvironmental 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

Octanoate esters do not persist in the environment. Sucrose octanoate esters biodegrade within approximately five days at approximately 20-27EC, in both aerobic and anaerobic conditions. The approximate half-life for sorbitol octanaote is seven to ten days post-application in the field. Minimal potential for exposure exists to insects, fish and other non-target wildlife as a result of Avachem Sucrose Octanoate [40.0%] or Avachem Sorbitol Octanoate [90.0%] use.

D. EFFICACY DATA

No efficacy data are required, because no public health uses are involved. However, acceptable product performance data were submitted for sucrose octanoate esters, demonstrating activity against aphids, pear pyslla and whitefly. Product performance data for sorbitol octanoate demonstrated a range of activities against pear psylla nymphs, tobacco aphid, tobacco hornworm, and the two-spotted spider mite.

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 criterion AA@ above, the fatty acid composition of octanoate esters accounts for the 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. Criterion AB@ is satisfied by the product -specific current labels and by the data presented in this document. It is believed that octanoate esters (sucrose octanoate esters and sorbitol octanoate) will not cause any unreasonable adverse effects, and will act as a pesticide to control soft-bodied insects, satisfying Criterion AC.@ Criterion AD@ is satisfied by the data/information submitted and the products= low toxicity when used according to the label directions.

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

B. REGULATORY POSITION

1. Unconditional Registration

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 octanoate esters as their sole active ingredient.

2. Tolerances for Food Uses and/or Exemptions

Sucrose Octanaote Esters: 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 Asucrose 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 Asucrose octanoate esters [(α-D-glucopyranosyl-β-D-fructofuranosyl-octanoate), mono-, di-, and triesters of sucrose octanoate].@

Sorbitol Octanoate: EPA received a pesticide petition (2E6389) 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, sorbitol octanoate, in or on all food commodities.

3. CODEX Harmonization

There are no CODEX Maximum Residue Levels (MRLs)established for residues of sucrose octanoate esters or sorbitol octanoate.

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

There exits a risk from ocular exposure. Risks to workers are mitigated by label language requiring protective clothing, and a product-specific 24 or 48 hour re-entry interval.

6. Endangered Species Statement

Sucrose Octanaote Esters: 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.

Sorbitol Octanoate: The Agency has determined there will be No Adverse Effect (NAE) on endangered species or other non-target organisms following the use of Avachem Sorbitol Octanoate [90.0%] containing sorbitol octanoate as its active ingredient. There is no evidence of toxicity to any non-target organisms or effects on critical habitat based on data obtained from a review of the available literature. Exposure to non-target organisms is mitigated by the rapid degradation of the active ingredient in the environment. The constituent molecules, sorbitol and octanoic acid, are simple molecules that are common in the environment and rapidly metabolized by animal systems.

C. LABELING RATIONALE

The Agency=s position is that the labeling of Avachem Sucrose Octanoate [40.0%] and Avachem Sorbitol Octanoate [90.0%], as well as that of the technical grade active ingredient/manufacturing-use products, Avachem Sucrose Octanoate Manufacturing Use Product (85.43%)(cancelled) and Avachem Sorbitol Octanoate Manufacturing Use Product (90.00%), complies with current pesticide labeling requirements.

1. Human Health Hazard

a. Worker Protection Standard

This end-use products come under the provisions of the Worker Protection Standards (WPS). PPE (long-sleeved shirt and long pants, shoes plus socks, and protective eyewear) and REI (product-specific 24 or 48-hour) required.

b. Non-Worker Protection Standard

There are no non-WPS human health hazard issues.

c. Precautionary Labeling

The Agency has examined the toxicological data base for Avachem Sucrose Octanoate Manufacturing Use Product (cancelled), Avachem Sucrose Octanoate [40.0%], Avachem Sorbitol Octanoate Manufacturing Use Product and Avachem Sorbitol Octanoate [90.0%] 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 (cancelled)B ADANGER.@ For Avachem Sorbitol Octanoate Manufacturing Use Product B AWARNING.@

Hazards to Humans and Domestic Animals:

Sucrose Octanoate Esters: 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.

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

End-Use Product Precautionary Labeling:

For Avachem Sucrose Octanoate [40.0%] B AWARNING@
For Avachem Sorbitol Octanoate [90.0%] -- AWARNING@

Hazards to Humans and Domestic Animals:

For Avachem Sucrose Octanoate [40.0%]: 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.

For Avachem Sorbitol Octanoate [90.0%]: 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 and wash contaminated 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 octanoate esters are considered non-toxic to the environment, the environmental hazards statement is nevertheless required on the end-use products= label.

3. Application Rate

The Agency's position is that the labeling for the pesticide products containing octanoate esters complies with current pesticide labeling requirements.

Sucrose Octanoate Esters: The Agency has not stipulated a maximum number of applications for the active ingredient. The 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.

Sorbitol Octanoate: The proposed label specifies application by ground spray equipment (e.g. ground boom, air blast). Shake or stir before use, adding the appropriate quantity to water, with agitation. Maintain gentle agitation during application. The proposed label specifies an application rate of 0.5% volume/volume (v/v) for all use sites. Initiate applications as soon as pest pressure is observed. Repeat applications, as necessary, at intervals of 7 to 10 days.

D. LABELING

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

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
- Registration Number
- Signal Word (DANGER)

(2) Product name: **Avachem Sucrose Octanoate [40.0%]**

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)

(3) Product name: **Avachem Sorbitol Octanoate Manufacturing Use Product**

Active Ingredient:
Sorbitol Octanoate..... .....90.00%
Other Ingredients:..... .10.00%

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)

(4) Product name: **Avachem Sorbitol Octanoate [90.0%]**

Active Ingredient:
Sorbitol Octanoate..... .....90.0%
Other Ingredients:..... .10.0%

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

Avachem Sucrose Octanoate [40.0%] Use Sites: Field Crops (including certain non-food ornamentals), Mushroom Growing Media, Adult Honey Bees	Official date registered: **September 16, 2002**

Table 5 lists the use sites for the sorbitol octanoate end-use product. The label for it and the manufacturing-use product are also attached.

Table 5. End-Use Product Name, Use Sites, Registration/Reregistration

Avachem Sorbitol Octanoate [90.0%] Use Sites: Field, greenhouse, and nursery use on any type of agricultural commodity (including certain non-food ornamentals).	Official date registered: JAN 17 2006

VII. References

A. References for Sucrose Octanoate

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

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

7. 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.

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

B. References for Sorbitol Octanoate

9. USEPA; Endangered Species Risk Assessment for Sorbitol Octanoate, A New Active Ingredient in Two Proposed Products. R. S. Jones to D. Greenway, September 13, 2005.

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Effect of Sucrose Octanoate on Survival of Nymphal and Adult *Diaphorina citri* (Homoptera: Psyllidae)

C. L. McKENZIE[1] AND GARY J. PUTERKA[2]

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ABSTRACT Asian citrus psylla, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae) was detected for the first time in the United States near Delray Beach, FL, on 2 June 1998 and is continuing to spread and multiply throughout southern Florida. This psyllid is the vector of *Liberobacter asiaticum*, a phloem-limited bacterium that causes citrus greening disease. This pathogen has not been found in the Western Hemisphere to date. Furthermore, high infestation levels of *D. citri* can impact citrus plant health, fruit quality, or yield. Replicated laboratory and spray booth bioassays were conducted to determine the insecticidal activity of a synthetic analog of natural sugar esters found in leaf trichomes of wild tobacco, *Nicotiana gossei* Domin, to nymphal and adult *D. citri*. Field trials were initiated in Fort Pierce, FL, in 2000 to determine activity of the sugar ester formulation (sucrose octanoate) on *D. citri* and other citrus pests, including immature Asian citrus leafminer, *Phyllocnistis citrella* Stainton and mites. Sucrose octanoate rates tested ranged from 400 to 8,000 ppm (0.1–2% formulated product). Our data suggest that both nymphal and adult *D. citri* as well as the mite complex tested would be equally controlled to levels of >90% at the higher concentrations of sucrose octanoate and that good coverage is key to efficacy.

KEY WORDS Asian citrus psylla, Asian citrus leafminer, mites, sucrose octanoate, sugar ester

THE ASIAN CITRUS PSYLLA *Diaphorina citri* Kuwayama, is the primary vector of the plant pathogen, *Liberobacter asiaticum*. This phloem-limited, Gram-negative bacterium-like organism causes citrus greening disease (also known as Huanglungbin or Likubin) (Catling 1970, Bové and Garnier 1994). The greening pathogen is also readily transmitted by grafting and propagating with infected plant material and is lethal to most commercial citrus cultivars (Gottwald et al. 1989). Greening is one of the most devastating citrus diseases in Asia and Africa (Aubert 1993), but the pathogen has not been found in the Western Hemisphere to date. However, *D. citri* was detected for the first time in the United States near Delray Beach, FL, in the summer of 1998 and has continued to spread and multiply throughout southern Florida (Knapp et al. 1998). *D. citri* is a serious threat to the Florida citrus industry if the pathogen that causes citrus greening disease becomes introduced or should *D. citri* be found to vector other diseases of citrus. Moreover, high infestation levels of *D. citri* can impact citrus plant health, fruit quality, or yield.

Florida has instituted successful biological control programs in citrus to control various mites and scale insects (Muma 1955; Clancy et al. 1963; Selhime et al. 1969; Debach and Rose 1976; Hart et al. 1978; Dowell et al. 1979; Nguyen and Sailor 1979; Nguyen 1986, 1987, 1988; Sailor et al. 1984; Glenn and Baranowski 1987; Thompson 1989; Browning 1990; Caceres and Childers 1991; Tefertiller et al. 1991), due in part to the stabilization of the grove. Pest control strategies that use harsh insecticides as the first line of defense against *D. citri* and other citrus pests could lead to the disruption of their natural enemies. In addition, indiscriminate use of pesticides often results in the development of resistance and control failure. Alternative chemistry that is safe for the beneficial insect complex, yet is selectively effective against the target pest, is a desirable component in citrus integrated pest management programs where most pests have been successfully controlled by their natural enemies. Sugar esters are of great interest because they have been found to be relatively nontoxic to key predators and parasitoids of many citrus pests (Michaud and McKenzie 2004).

Sugar esters are a new class of insecticidal compounds that seem to fit these criteria. Sucrose esters naturally occur in plants, are benign to the environment, and are being commercially synthesized for use in the food industry (Chortyk et al. 1996). Natural and synthetic sugar esters have been shown to be effective biorationals with insecticidal activity against a range of insect species. Soft-bodied arthropods, including

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Table 1. Toxicity of sucrose octanoate 1 DAT to immature and adult *D. citri* by using a petri dish bioassay with a detached leaf substrate

Developmental stage	*n*	Slope ± SE	LC_{50}[a] (95% FL)	LC_{90}[a] (95% FL)
Small nymph	250	1.48 ± 0.37	120a (24–240)	920a (640–1,640)
Large nymph	190	4.01 ± 0.43	920a (120–1,640)	1,960a (960–5,120)
Adult	387	3.71 ± 0.42	2,720b (2,480–3,040)	6,080b (5,160–7,800)

Means within columns followed by the same letter are not significantly different (95% fiducial limit [FL]).
[a] LC values expressed in ppm active ingredient of commercially formulated sucrose octanoate; five replications with seven concentrations.

mites, lepidopteran larvae, aphids, whiteflies, and psyllids experience rapid knock down after contact (Parr and Thurston 1968, Neal et al. 1994, Puterka and Severson 1995, Liu et al. 1996). In addition, mites (Neal et al. 1994), whiteflies (Liu and Stansly 1995), and leafminers (Hawthorne et al. 1992) have demonstrated ovipositional and feeding deterrence to sugar esters. Although the mode of action is unknown, it has been suggested that two primary modes of action against pear psylla, *Cacopsylla pyricola* Foerster, are possible: desiccation by alterations in the insect cuticle or suffocation by the sugar ester solution (Puterka et al. 2003).

The primary objective of this study was to determine the dosage–mortality relationships of sucrose octanoate (α-D, glucopyranoside, β-D-fructofuranosyl octanoate), a synthetic analog of natural sugar esters found in leaf trichomes of wild tobacco, *Nicotiana gossei* Domin, to nymphal and adult *D. citri* via laboratory bioassays and spray booth experiments. Additionally we wanted to establish sucrose octanoate efficacy against this and other key mite and insect pests in field trials on citrus.

Materials and Methods

Insect Source and Rearing. *D. citri* were collected in 1999 from field populations infesting citrus at the United States Horticultural Research Laboratory experimental farm at Fort Pierce, FL, and used to start a laboratory colony. All stages of pysllid (egg, nymph, and adult) were reared on orange jasmine, *Murraya paniculata* (L.) Jack, and housed in large screened Plexiglas cages located in air-conditioned greenhouses with ambient light and humidity. Temperatures fluctuated between day and night highs of 29.4 and 26.7°C, respectively, with an overall low of 23.9°C.

Biorationals. Sucrose octanoate, a synthetic analog of natural sugar esters, was provided by AVACHEM (AVA Chemical Ventures, L.L.C., Portsmouth, NH) as a 40% (AI) formulation and was used in all bioassays, spray booth experiments, and field trials. M-Pede (Dow AgroSciences, L.L.C., San Diego, CA), a common insecticidal soap, and AGRI-50 (Cal-Agri Products, L.L.C., Los Angeles, CA), a nontoxic, food-safe insecticide, were used for efficacy comparison to sucrose octanoate in field trials.

Laboratory Bioassays. Sucrose octanoate solutions were prepared in seven concentrations of 400–8,000 ppm (0.1–2.0% formulated product) in double distilled H_2O (ddH_2O) plus a ddH_2O control. The bioassay consisted of one sterilized *Citrus sinensis* (L.) Osbeck leaf 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. Concentrations were applied to *D. citri* nymphs or adults (n = 15–20) by using a petri dish spray device according to Puterka and Severson (1995). Each concentration was replicated five times, and bioassays were repeated three times.

Whole-plant bioassays were also conducted on *D. citri* nymphs to obtain dosage–mortality relationships under more natural conditions. Six-month-old orange jasmine seedlings in cone tubes were exposed to *D. citri* adults for 6 d. Plants were removed and held for an additional 5 d to allow nymphs to hatch and develop. On average, each plant was infested with 75 nymphs. Sucrose octanoate solutions were prepared in six concentrations of 800–8,000 ppm (0.2–2.0% formulated product) in H_2O plus a H_2O control and applied to infested plants with a spray booth (R&D Sprayers, Opelousas, LA). The spray booth was equipped with a single TJ nozzle operated at 61 psi to deliver 795 L/ha. Each concentration was replicated four times, and experiments were repeated three times. Mortalities were determined 24 h after treatment by examining each leaf of the treated plants for number of alive and dead small (first and second instar) and large (third and fourth instar) *D. citri* nymphs.

Field Trials. Three field trials were conducted at USHRL experimental farm at Fort Pierce, FL, to determine the effectiveness of sucrose octanoate against natural populations of *D. citri* under field conditions. Three-year-old 'Ortanique' Tangor, *Citrus sinensis* (L.) Osbeck × *Citrus reticulata* Blanco, trees were used in trials 1 and 2, and 2-yr-old 'Sun shu sha' *Citrus sinensis* (L.) Osbeck rootstock trees were used in trial 3. All treatments were arranged in a randomized complete block design with eight replications in trial 1, five replications in trial 2, and four replications in trial 3. Each replication contained three trees in trials 1 and 2, and five trees in trial 3. In trial 1, treatments were applied with a CO_2 backpack sprayer equipped with 50.8-cm boom and two TX18 hollow cone nozzles calibrated at 38 psi to deliver 125 ml per tree, and treatments were evaluated 1, 4, and 7 days after treatment (DAT). In an effort to obtain more complete tree coverage, trials 2 and 3 were applied with a CO_2 backpack sprayer equipped with a single TX18 hollow cone nozzle calibrated at 50 psi to deliver 200 ml (trial 2) and 250 ml (trial 3) per tree. Trial 2 was designed to evaluate Asian citrus leafminer, *Phyllocnistis citrella* Stainton mortality that compared a higher rate of su-

Table 2. Toxicity of sucrose octanoate 1 DAT to immature *D. citri* by using a spray booth with a whole-plant substrate

Nymph	*n*	Slope ± SE	LC_{50}[a] (95% FL)	LC_{90}[a] (95% FL)
Small	349	1.99 ± 0.35	1,040a (720–1,280)	4,600a (3,440–7,800)
Large	163	2.22 ± 0.56	1,520a (800–2,040)	5,760a (4,000–14,200)
Total nymph	530	1.97 ± 0.26	1,160a (880–1,400)	5,120a (4,080–7,360)

Means within columns followed by the same letter are not significantly different (95% fiducial limit [FL]).
[a] LC values expressed in ppm active ingredient of commercially formulated sucrose octanoate; four replications with seven concentrations.

crose octanoate (8,000 ppm) to the labeled use rate of M-Pede soap [2% (vol:vol) solution = 9,800 ppm], although *D. citri* and mite data were also taken. Treatments were evaluated 1 and 4 DAT in trial 2. Trial three was designed to evaluate the impact of two applications of sucrose octanoate in comparison to standard label rates of two biorational soap materials, 2% M-Pede (9,800 ppm), and 0.5% Agri-50 (50 ppm), for the control of *D. citri*. Treatments were evaluated 1, 4, and 7 d after the first application (DAT1) and 1, 4, 7, 10, 14, and 20 d after the second application (DAT2). Other citrus pests that were evaluated included *P. citrella* and a mite complex (common Texas citrus mite, red spider mite, and rust mite). Data taken from these studies included the number of *D. citri* adults per tree visible in a 2-min period and counts of *D. citri* eggs, small and large *D. citri* nymphs, mites and citrus leafminer larvae and pupae (trial 2) per 7.6-cm fresh flush terminal. Leaves infested with citrus leafminer larvae and pupae from the 7.6-cm fresh flush terminals were probed to determine whether they were dead or alive.

Statistics. Dosage–mortality relationships of sucrose octanoate to *D. citri* were evaluated by probit analysis (Sparks and Sparks 1987). Differences between LC_{50} values among life stages of *D. citri* were determined by failure of fudicial limits to overlap. Mortality data from the spray booth experiments were subjected to an analysis of variance (ANOVA). Mortality comparisons among different nymphal sizes in response to sucrose octanoate concentration were made using ANOVA and Ryan–Einot–Gabriel–Welsch multiple-range test (REGWQ) at $\alpha = 0.05$ (SAS Institute 2000). Field data were analyzed by the General Linear Models (GLM) procedure, and differences among treatment means were determined by Ryan–Einot–Gabriel–Welsch multiple range test (REGWQ) at $\alpha = 0.05$ (SAS Institute 2000).

Results and Discussion

Laboratory Bioassays. Sucrose octanoate showed higher toxicity to small and large *D. citri* nymphs, compared with adult *D. citri* by using the detached leaf petri dish bioassay (Table 1). Lethal concentration (LC) values were not significantly different between small and large nymphs. The LC_{50} and LC_{90} values for adult *D. citri* were ≈3 times greater than for nymphs, indicating that adults are less susceptible to sucrose octanoate than nymphs.

When sucrose octanoate was evaluated using a whole-plant bioassay and applied within a spray booth, LC values for small and large nymphs did not significantly differ (Table 2). Furthermore, LC_{50} and LC_{90} values were considerably higher for nymphs using the whole-plant bioassay. The higher sugar ester concentrations that were required to produce lethal concentration values for nymphs in the whole-plant bioassay may be due to less spreading on leaf surfaces, which is a factor that can affect sugar ester performance on plants (Puterka et al. 2003). The whole-plant bioassay produced LC_{50} and LC_{90} values for *D. citri* that were comparable with those that resulted in detached leaf bioassays for *Cacopsylla pyricola* Foerster (Puterka et al. 2003). Mortalities were significantly different among sucrose octanoate concentrations one DAT for small ($F = 15.43$; df = 6, 16; $P < 0.0001$) and large ($F = 25.58$; df = 6,18; $P < 0.0001$) nymphs, ranging from 51 to 100% and from 30 to 96% for small and large nymphs, respectively (Table 3).

Field Trials. *D. citri* populations were moderately high when the first field trial was initiated in the spring of 2000 with averages of 24 adults per 2-min tree count, six small nymphs, and four large nymphs per 7.6-cm fresh flush terminal. *D. citri* eggs averaged four per 7.6-cm fresh flush terminal. The number of *D. citri* life stages within each treatment did not differ over time ($P > 0.05$), thus psylla numbers were averaged over the 1-, 3-, and 7-d sample periods (Table 4). All treatments significantly reduced citrus psylla nymphs and adults compared with the untreated control ($P > 0.05$). However, there were no significant differences ($P > 0.05$) in egg and small and large nymph numbers in response to increasing sucrose octanoate concentrations from 1,600 to 4,000 ppm. Egg counts reveal a slight reduction in *D. citri* oviposition for the lower

Table 3. *D. citri* nymphal mortality for sucrose octanoate 1 DAT by using a spray booth with a whole-plant substrate

Sucrose octanoate conc.[a]	% Small nymph Mortality ± SE	% Large nymph Mortality ± SE
8,000	100.0 ± 0.00a	96.4 ± 3.57a
4,000	88.2 ± 11.76ab	90.1 ± 5.76a
3,200	86.8 ± 4.35ab	76.0 ± 5.49ab
2,400	71.7 ± 5.26ab	60.1 ± 5.14b
1,600	71.0 ± 7.21ab	51.6 ± 7.62bc
800	50.9 ± 17.16b	29.8 ± 11.72c
Control	0.0 ± 0.00c	0.0 ± 0.00d

Means within columns followed by the same lowercase letter are not significantly different ($P > 0.05$, REGWQ).
Spray booth was equipped with a single TJ nozzle operated at 61 psi to deliver 85 gal/acre.
[a] Concentration expressed in ppm active ingredient of commercially formulated sucrose octanoate.

Table 4. Field trial 1. Mean number of *D. citri* and citrus mite averaged over sample dates 1, 3, and 7 d after treatment (Trt) in treated and untreated Tangor citrus, St. Lucie County, Fort Pierce, FL

Treatment	Rate[a] (ppm)	Egg ± SE (% reduction)	Small nymph ± SE (% reduction)	Large nymph ± SE (% reduction)	Adult ± SE (% reduction)	Mites ± SE (% reduction)
Sucrose octanaote	1600	1.5 ± 0.5b (91)	0.8 ± 0.2b (95)	1.1 ± 0.3b (84)	12.2 ± 1.1bc (55)	0.6 ± 0.1b (95)
Sucrose octanoate	2400	1.9 ± 1.0b (89)	1.5 ± 0.6b (90)	0.8 ± 0.2b (88)	8.5 ± 0.9c (69)	1.0 ± 0.3b (91)
Sucrose octanaote	3200	5.0 ± 2.5ab (71)	2.0 ± 0.6b (88)	0.9 ± 0.4b (87)	7.8 ± 0.7c (71)	0.6 ± 0.3b (95)
Sucrose octanoate	4000	9.1 ± 2.9ab (47)	2.7 ± 0.8b (82)	1.7 ± 0.3b (75)	9.4 ± 0.9bc (65)	1.8 ± 0.7b (84)
M-Pede	9800	10.0 ± 4.1ab (42)	3.8 ± 1.4b (75)	1.4 ± 0.3b (80)	13.8 ± 1.1b (49)	1.4 ± 0.4b (88)
Untreated		17.1 ± 5.6a	15.2 ± 2.7a	6.9 ± 1.1a	27.2 ± 2.5a	11.2 ± 1.8a
Trt* date		$F = 1.87$	$F = 0.62$	$F = 0.16$	$F = 0.99$	$F = 1.18$
		df = 5,410	df = 5,410	df = 5,410	df = 5,315	df = 5,410
		$P = <.0987$	$P = <.6842$	$P = <.9773$	$P = <.4246$	$P = <.3197$
Trt		$F = 3.29$	$F = 16.19$	$F = 20.05$	$F = 34.95$	$F = 27.46$
		df = 5,410	df = 5,410	df = 5,410	df = 5,315	df = 5,410
		$P = <.0064$	$P = <.0001$	$P = <.0001$	$P = <.0001$	$P = <.0001$

Means within columns followed by the same lowercase letter are not significantly different ($P > 0.05$, REGWQ).
[a] Rate expressed in ppm active ingredient of commercially formulated product.

sucrose octanoate concentrations in comparison with the control ($F = 3.29$; df = 5, 410; $P < 0.006$), but overall sugar esters and insecticidal soaps had little effect on oviposition. Higher reductions of nymphs surviving sugar ester treatments occurred in the field trial than in the whole-plant bioassay; however, the field trial corresponded well to the results from the petri dish bioassay. Adults were less affected by the sucrose octanoate treatments than the nymphs, but field data indicated that adults were either reduced or repelled and resulted in 55–71% fewer adults in the sucrose octanoate treatments. Mite numbers were also greatly reduced by the sugar ester and insecticidal soap treatments in comparison with the untreated control ($F = 27.46$; df = 5, 410; $P < 0.0001$). These data support the high degree of miticidal activity for sugar esters found in previous research (Puterka et al. 2003).

During the first field trial, unpublished data of *D. citri* mortality in sucrose octanoate treatments led to the increased concentration of sucrose octanoate tested in the second field trial. Day by treatment interactions were not significant for larval ($F = 0.89$; df = 2, 23; $P < 0.4241$) or pupal mortalities ($F = 1.28$; df = 2, 15; $P < 0.3065$), indicating no response to treatment over time; thus, citrus leafminer mortalities were averaged over time (Table 5). Citrus leafminer mortality was substantially higher ($P > 0.05$) for sucrose octanoate (72%) than for M-Pede (4%). However, neither material produced pupal mortalities.

In trial 2, *D. citri* populations were very high, with a mean of 29 adults per 2-min count, 34 small nymphs, and six large nymphs per 7.6-cm terminal. Day by treatment interactions were not significant, indicating no response to treatment over time; thus, we averaged *D. citri* mortality over time (Table 6). Number of small and large nymphs did not significantly differ for each treatment ($F = 1.5$; df = 6, 29; $P < 0.2136$); therefore, we combined for total nymphal presentation. No significant difference in the number of eggs was detected between treatments during trial 2, and eggs averaged 25 per 7.6-cm terminal. In trial 2, the treatments performed similarly against *D. citri*, producing 89 and 65% nymphal mortalities, 90 and 74% adult reductions, and 91 and 86% mite mortalities for sucrose octanoate and M-Pede, respectively. However, the 8,000 ppm rate of sucrose octanoate used in trial 2 increased adult *D. citri* mortality by ≈20% compared with the lower rates used in trial 1. We found that sucrose octanoate was not phytotoxic at much higher rates (8,000 ppm) than needed to be effective against mites and immature *D. citri*, which is of concern with insecticidal soaps.

Table 5. Field trial 2. Mean number of citrus leafminer (CLM) averaged over sample dates 1 and 4 d after treatment (Trt) in treated and untreated Tangor citrus, St. Lucie County, Fort Pierce, FL

Treatment	Rate[a] (ppm)	Alive CLM larvae	Total CLM larvae	% CLM larval mortality	Alive CLM pupae	Total CLM pupae	% CLM pupae mortality
Sucrose Octanoate	8,000	0.43a	1.57a	72.61b	0.63a	0.63a	0a
M-Pede	9,800	0.93a	0.97a	4.12a	0.59a	0.59a	0a
Untreated		1.53a	1.53a	0a	0.70a	0.70a	0a
Trt* Date		$F = 0.03$	$F = 0.08$	$F = 0.89$	$F = 0.33$	$F = 0.33$	$F = 1.28$
		df = 2, 78	df = 2, 78	df = 2, 23	df = 2, 78	df = 2, 78	df = 2, 15
		$P < 0.9700$	$P < 0.9266$	$P < 0.4241$	$P < 0.7194$	$P < 0.7194$	$P < 0.3065$
Trt		$F = 2.46$	$F = 0.65$	$F = 52.79$	$F = 0.05$	$F = 0.26$	$F = 1.12$
		df = 2, 78	df = 2, 78	df = 2, 23	df = 2, 78	df = 2, 78	df = 2, 15
		$P < 0.0922$	$P < 0.5263$	$P < 0.0001$	$P < 0.9535$	$P < 0.7755$	$P < 0.3522$

Means within columns followed by the same lowercase letter are not significantly different ($P > 0.05$, REGWQ).
Leaves infested with citrus leafminer larvae and pupae from 7.6-cm fresh flush citrus terminals were probed to determine whether insects were dead or alive; total (alive plus dead) reflects all CLM larvae or pupae present in the terminal.
[a] Rate expressed in ppm active ingredient of commercially formulated product.

Table 6. Field trial 2. Mean number of *D. citri* and citrus mite averaged over 1 and 4 d after treatment (Trt) in treated and untreated Tangor citrus, St. Lucie County, Ft. Pierce, FL

Treatment	Rate[a] (ppm)	Egg ± SE[b]	Total nymph ± SE[b] (% mortality)	Adult ± SE[c] (% mortality)	Mites ± SE[b] (% mortality)
Sucrose Octanoate	8000	29.8 ± 12.4a	8.7 ± 2.9b (89)	4.3 ± 0.7b (90)	1.1 ± 0.7b (91)
M-Pede	9800	21.7 ± 7.6a	26.8 ± 6.9b (65)	11.4 ± 1.4b (74)	1.8 ± 0.9b (86)
Untreated		22.1 ± 6.3a	77.6 ± 22.3a	43.6 ± 7.3a	12.7 ± 5.1a
Trt* date		$F = 4.87$	$F = 2.45$	$F = 1.2$	$F = 0.08$
		df = 2, 78	df = 2, 78	df = 2, 78	df = 7, 78
		$P < 0.0102$	$P < 0.0930$	$P < 0.3082$	$P < 0.9240$
Trt		$F = .28$	$F = 7.86$	$F = 33.46$	$F = 4.63$
		df = 2, 78	df = 2, 78	df = 2, 78	df = 2, 78
		$P < 0.7559$	$P < 0.0008$	$P < 0.0001$	$P < 0.0126$

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

[a] Rate expressed in ppm active ingredient of commercially formulated product.

[b] Mean number of *D. citri* eggs, total small and large nymphs, and mites per 3-in. fresh flush citrus terminal counted with a dissecting microscope.

[c] Mean number of *D. citri* adults per tree visible in a 2-min period.

In trial 3, *D. citri* populations were also very high, which presented a good opportunity to study the repeated application of sucrose octanoate in comparison with insecticidal soaps, M-Pede, and Agri-50 for control of *D. citri*. *D. citri* eggs and total nymphs per 7.6-cm fresh flush terminal averaged 32 and 22, respectively. Adult *D. citri* averaged 34 per 2-min visual tree inspection. Number of small and large nymphs did not significantly differ for each treatment ($F = 1.08$; df = 179, 703; $P < 0.2505$); therefore, size categories for nymphs were combined for total nymph presentation. There were significant day by treatment interactions for *D. citri* nymphs ($F = 10.58$; df = 4, 884; $P < 0.0001$) and adults ($F = 5.08$; df = 4, 533; $P < 0.0005$); thus, data are presented over time (Fig. 1). No significant day by treatment interaction was observed for citrus psylla eggs ($F = 1.22$; df = 4, 884; $P < 0.3027$). Means averaged over sample dates for percentage of egg reduction were highest with M-Pede (56%) followed by Agri-50 (49%) and the high sucrose octanoate rate (43%). The lower rate of sucrose octanoate (3,200 ppm) was significantly different ($P > 0.05$) from the untreated and the other treatments and provided 24% egg reduction. All treatments performed similarly in reducing *D. citri* nymphal and adult populations during the duration of the study ($P > 0.05$). Adult numbers were significantly reduced ($P > 0.05$) by all of the treatments to ≈50% from 6 d after the first treatment applications to 12 d after the second treatment applications. The effect of treatments on adult presence and oviposition was essentially lost 5 d after the second treatment applications. However, *D. citri* nymphs were reduced by 60–80% by all treatments beginning 3 d after initial treatment applications, and this effect lasted for the duration of the study.

These results indicate that sucrose octanoate or the other insecticidal soaps can achieve reasonable control of *D. citri* on citrus. Furthermore, these studies demonstrate that sucrose octanoate and insecticidal soaps can obtain a high level of mite control. Sucrose octanoate may be especially useful in citrus due to its activity against another important pest, *P. citrella*, and the low toxicity of this material to beneficial predators and parasitoids in citrus. Lack of sucrose octanoate rate effect in the field indicates complete coverage is crucial for good efficacy and this is especially true when dealing with contact poisons that lack residual. Our data suggest that both nymphal and adult *D. citri* as well as the mite complex tested would be equally controlled to levels of >90% at the higher concentrations of sucrose octanoate tested and that good cov-

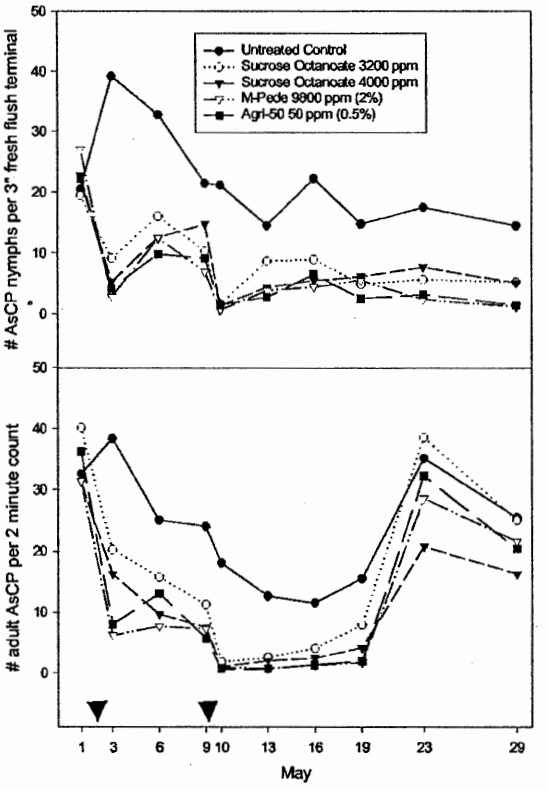

Fig. 1. Field trial 3. Mean number of Asian citrus psylla (AsCP) nymphs and adults in treated and untreated 'Sun shu sha' rootstock citrus trees 1, 4, and 7 DAT1 and 1, 4, 7, 10, 14, and 20 DAT2, St. Lucie County, Fort Pierce, FL. ▼ denotes spray treatment applications 1 (2 May 2003) and 2 (9 May 2003 after insect evaluations).

erage is key to efficacy. Sucrose octanoate has also been found to be relatively nontoxic to a host of beneficial insects typically found in citrus groves (Michaud and McKenzie, 2004) and could prove to be a valuable addition to the list of dwindling products available to the commercial grower.

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Structure-Function Relationships Affecting the Insecticidal and Miticidal Activity of Sugar Esters

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ABSTRACT Synthetic sugar esters are a relatively new class of insecticidal compounds that are produced by reacting sugars with fatty acids. The objective of this research was to determine how systematic alterations in sugar or fatty acid components of sugar ester compounds influenced their insecticidal properties. Sucrose octanoate, sorbitol octanoate, sorbitol decanoate, sorbitol caproate, xylitol octanoate, xylitol decanoate and xylitol dodecanoate were synthesized and evaluated against a range of arthropod pests. Dosage-mortality studies were conducted on pear psylla (*Cacopsylla pyricola* Foerster) on pear, tobacco aphid (*Myzus nicotianae*) Blackman and tobacco hornworm (*Manduca sexta* [Johannson]) on tobacco, and twospotted spider mite (*Tetranychus urticae* Koch) on apple in laboratory bioassays. These sugar esters were compared with insecticidal soap (M-Pede, Dow AgroSciences L.L.C., San Diego, CA), to determine how toxicologically similar these materials were against the arthropod pests. Substitutions in either the sugar or fatty acid component led to significant changes in the physical properties and insecticidal activity of these compounds. The sugar esters varied in their solubility in water and in emulsion stability, yet, droplet spread upon pear leaves occurred at low concentrations of 80–160 ppm and was strongly correlated with psylla mortalities ($R^2 = 0.73$). Sequentially altering the sugar or fatty acid components from lower to higher numbers of carbon chains, or whether the sugar was a monosaccharide or disaccharide did not follow a predictable relationship to insecticidal activity. Intuitively, changing the hydrophile from sorbitol (C_6) to xylitol (C_5) would require a decrease in lipophile chain length to maintain hydrophilic-lipophilic balance (HLB) relationships, yet an increase in lipophile chain length was unexpectedly needed for increasing insecticidal activity. Thus, the HLB of these materials did not correlate with pear psylla mortalities. Initial insect bioassays and dosage-mortality data found significant differences among sugar ester compounds' toxicity to the range of arthropod species. Sucrose octanoate high in monoester content had the highest activity against the range of arthropod pests at low concentrations of 1200–2400 ppm. No single chemical structure for the xylitol or sorbitol esters were optimally effective against the range of arthropods we tested and sorbitol octanoate and xylitol decanoate had the highest insecticidal activity of this group. All of the sugar ester materials produced high *T. urticae* mortalities on apple at very low concentrations of 400 ppm. Overall, most of the sugar esters that were examined had superior insecticidal activity compared with insecticidal soap. Sugar ester chemistry offers a unique opportunity to design an insecticide or miticide specific to certain arthropod pests which would be valuable in crop integrated pest management (IPM) programs. Sucrose esters are currently used as additives in the food industry which makes them especially attractive as safe and effective insecticides.

KEY WORDS biopesticide, biorational insecticide, polyol esters, surfactant, acyl sugar, sucrose octanoate

SUGAR ESTERS, ALSO KNOWN as acyl sugars or polyol esters, are a class of compounds produced by reacting sugars, including reduced sugars, with aliphatic or aromatic acids. Sucrose esters occur naturally in plants and are being commercially synthesized for use in the food industry (Chortyk et al. 1996). The glandular trichomes of wild tobacco, *Nicotiana gossei* Domin, have been known to possess insecticidal materials for some time (Thurston and Webster 1962). Yet, it was not until the early 1990s that sucrose esters were determined to be the primary insecticidal compounds within these glandular trichomes (Buta et al. 1993, Pittarelli et al. 1993). Synthetic sucrose esters that are similar in structure to those that naturally occur in *N. gossei* have comparable insecticidal activity (Chortyk et al. 1996). Both natural and synthetic sucrose esters have been shown to have contact toxicity with very

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rapid knock-down ability to soft-bodied arthropods including mites, aphids, whitefly and psyllids (Neal et al. 1994, Puterka and Severson 1995, Liu et al. 1996). Feeding and ovipositional deterrence to mites (Neal et al. 1994) and whiteflies (Liu and Stansly 1995) have also been demonstrated with sucrose esters.

There are eight free hydroxyl groups on a sucrose molecule that can be esterified during sucrose ester synthesis. Differing methods of synthesis can influence whether the resulting sucrose ester product is comprised primarily of mono-acyl sucrose (Farone et al. 2002) or a mixture of di- and tri-acyl sucroses (Chortyk et al. 1996). Previous research has established that di-acyl sucrose esters have the greatest insecticidal activity while mono- or tri-acyl sucrose esters had little activity (Chortyk et al. 1996). Furthermore, these researchers found that the sugar esters containing C_9, C_{10}, and C_{12} fatty acids produced low aphid mortalities compared with those containing C_7 and C_8 fatty acids. Yet, field evaluations found no differences in whitefly control when comparing sucrose esters comprised of 7, 8, 9, or 10 chain fatty acids (Liu et al.1996).

Further research is needed for a better understanding of the structure-function relationships that influence the insecticidal activity of sugar esters. Our study examines how varying the sugar and fatty acid components of sugar esters influences their insecticidal activity against a range of insect species. The performance of these compounds were compared with a common insecticidal soap,

M-Pede (Dow AgroSciences, L.L.C., Indianapolis, IN), to determine how toxicologically similar these materials were to arthropod pests.

Materials and Methods

Physical Properties of Sugar Esters. The sugar ester compounds, henceforth called sugar esters, that we examined were sucrose octanoate, sorbitol octanoate, sorbitol caproate, sorbitol decanoate, xylitol octanoate, xylitol dodecanoate, and xylitol decanoate. These compounds were produced by Applied Power Concepts, Inc., Anaheim, CA, that used the manufacturing processes described by Farone et al. (2002) to synthesize the xylitol and sorbitol esters or by Farone and Serfass (1998) to produce sucrose octanoate high in monoester content (>60%). The purity of these compounds ranged from 88 to 94% active ingredient (AI) with the inert ingredients being unreacted sugar. The insecticidal soap, M-Pede, was commercially available and contained 49% (AI) potassium salts of fatty acids. Stock solutions of all the experimental materials were prepared at 40% (AI) (vol:vol) before the series of dilutions were made. The sugar ester compounds were selected to provide the sugar-fatty acid combinations that enabled comparisons to be made between sugar esters containing reduced sugars of different molecule size (xylitol = C_5, sorbitol = C_6) and disaccharide sugars (sucrose = C_{12}), and between sugar esters containing C_6 (caproate), C_8 (octanoate), C_{10} (decanoate) and C_{12} (dodecanoate) fatty acids.

The physical properties assessed for each sugar ester were color, physical state of the technical material (80–94% [AI]), solubility in water, stability of a 2,400 ppm (AI) sugar ester solution in water, and sugar ester concentration at which droplets spread freely on pear leaves at a temperature of 25 ± 2°C. Sugar ester solubility in water was determined by adding 0.6 g of sugar ester material (40% [AI]) in 100 ml of water (25°C) to make a 2,400-ppm solution and classifying each material by the ease of making an emulsion. The classifications were: excellent = readily mixed with water and hand shaking 30 s made a uniform emulsion; good = mixed with difficulty and vigorous hand shaking for 1 min produced a uniform emulsion; poor = did not mix easily with water, warm water (30°C), and vigorous hand shaking for 1 min was required and produced a nonuniform emulsion with visible oil-like globules present. Emulsion stability was estimated by preparing 2,400 ppm (AI) sugar ester solutions in 25°C water (30°C for xylitol dodecanoate) and classifying the ability to remain an emulsion: excellent = no separation into layers, uniform emulsion; good = layers beginning to form, but emulsifies readily upon light agitation; poor = rapidly separates into layers or oil-like globules appear, constant agitation is needed to keep the sugar ester in solution. Sugar ester concentration at which droplets freely spread upon mature pear leaves was quantified by applying 10-μl droplets with a micropipette at concentrations of 40, 200, 400, 800, 1,200, 1,800, 2,400, and 3,000 ppm (AI) (Cowles et al. 2000). Four replicates were performed for each physical characteristic. Concentration at which droplets spread on leaves were subjected to analysis of variance (ANOVA) procedures and means were separated using the Ryan-Einot-Gabriel-Welsch multiple comparison (REGW) test (SAS Institute 2000).

Initial Screening for Insecticidal Activity. The sugar esters were initially screened for insecticidal activity against second- to third- (n = 40–60) instar pear psylla, *Cacopsylla pyricola* Foerster, nymphs on pear leaves to compare relative insecticidal activities of these compounds. The sugar ester compounds were tested at a concentration of 1,200 ppm (AI) sugar ester in water and compared with a water only control. Twenty to thirty second- to third-instar pear psylla nymphs were established 24 h before treatment on individual pear leaves held in 10 dram glass vials filled with tap water. Sugar ester solutions were applied to run-off by an atomizer sprayer that delivered 25 μl solution/cm^2 leaf area. Psylla mortalities were recorded for a short 2-min and 15-min time intervals to differentiate the quick knock-down activity of the sugar esters. The experiment was designed as a randomized complete block design with four replications which was conducted in an environmental chamber with temperature and light conditions of 25 ± 2°C, 14:10 L:D with 40% RH.

Mortality data were analyzed by ANOVA and differences among treatments were determined using the REGW multiple comparison test (SAS Institute 2000). The results of each sugar ester compound's insecticidal activity was classified into none = 0%,

rapid knock-down ability to soft-bodied arthropods including mites, aphids, whitefly and psyllids (Neal et al. 1994, Puterka and Severson 1995, Liu et al. 1996). Feeding and ovipositional deterrence to mites (Neal et al. 1994) and whiteflies (Liu and Stansly 1995) have also been demonstrated with sucrose esters.

There are eight free hydroxyl groups on a sucrose molecule that can be esterified during sucrose ester synthesis. Differing methods of synthesis can influence whether the resulting sucrose ester product is comprised primarily of mono-acyl sucrose (Farone et al. 2002) or a mixture of di- and tri-acyl sucroses (Chortyk et al. 1996). Previous research has established that di-acyl sucrose esters have the greatest insecticidal activity while mono- or tri-acyl sucrose esters had little activity (Chortyk et al. 1996). Furthermore, these researchers found that the sugar esters containing C_9, C_{10}, and C_{12} fatty acids produced low aphid mortalities compared with those containing C_7 and C_8 fatty acids. Yet, field evaluations found no differences in whitefly control when comparing sucrose esters comprised of 7, 8, 9, or 10 chain fatty acids (Liu et al.1996).

Further research is needed for a better understanding of the structure-function relationships that influence the insecticidal activity of sugar esters. Our study examines how varying the sugar and fatty acid components of sugar esters influences their insecticidal activity against a range of insect species. The performance of these compounds were compared with a common insecticidal soap,

M-Pede (Dow AgroSciences, L.L.C., Indianapolis, IN), to determine how toxicologically similar these materials were to arthropod pests.

Materials and Methods

Physical Properties of Sugar Esters. The sugar ester compounds, henceforth called sugar esters, that we examined were sucrose octanoate, sorbitol octanoate, sorbitol caproate, sorbitol decanoate, xylitol octanoate, xylitol dodecanoate, and xylitol decanoate. These compounds were produced by Applied Power Concepts, Inc., Anaheim, CA, that used the manufacturing processes described by Farone et al. (2002) to synthesize the xylitol and sorbitol esters or by Farone and Serfass (1998) to produce sucrose octanoate high in monoester content (>60%). The purity of these compounds ranged from 88 to 94% active ingredient (AI) with the inert ingredients being unreacted sugar. The insecticidal soap, M-Pede, was commercially available and contained 49% (AI) potassium salts of fatty acids. Stock solutions of all the experimental materials were prepared at 40% (AI) (vol:vol) before the series of dilutions were made. The sugar ester compounds were selected to provide the sugar-fatty acid combinations that enabled comparisons to be made between sugar esters containing reduced sugars of different molecule size (xylitol = C_5, sorbitol = C_6) and disaccharide sugars (sucrose = C_{12}), and between sugar esters containing C_6 (caproate), C_8 (octanoate), C_{10} (decanoate) and C_{12} (dodecanoate) fatty acids.

The physical properties assessed for each sugar ester were color, physical state of the technical material (80–94% [AI]), solubility in water, stability of a 2,400 ppm (AI) sugar ester solution in water, and sugar ester concentration at which droplets spread freely on pear leaves at a temperature of 25 ± 2°C. Sugar ester solubility in water was determined by adding 0.6 g of sugar ester material (40% [AI]) in 100 ml of water (25°C) to make a 2,400-ppm solution and classifying each material by the ease of making an emulsion. The classifications were: excellent = readily mixed with water and hand shaking 30 s made a uniform emulsion; good = mixed with difficulty and vigorous hand shaking for 1 min produced a uniform emulsion; poor = did not mix easily with water, warm water (30°C), and vigorous hand shaking for 1 min was required and produced a nonuniform emulsion with visible oil-like globules present. Emulsion stability was estimated by preparing 2,400 ppm (AI) sugar ester solutions in 25°C water (30°C for xylitol dodecanoate) and classifying the ability to remain an emulsion: excellent = no separation into layers, uniform emulsion; good = layers beginning to form, but emulsifies readily upon light agitation; poor = rapidly separates into layers or oil-like globules appear, constant agitation is needed to keep the sugar ester in solution. Sugar ester concentration at which droplets freely spread upon mature pear leaves was quantified by applying 10-μl droplets with a micropipette at concentrations of 40, 200, 400, 800, 1,200, 1,800, 2,400, and 3,000 ppm (AI) (Cowles et al. 2000). Four replicates were performed for each physical characteristic. Concentration at which droplets spread on leaves were subjected to analysis of variance (ANOVA) procedures and means were separated using the Ryan-Einot-Gabriel-Welsch multiple comparison (REGW) test (SAS Institute 2000).

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Mortality data were analyzed by ANOVA and differences among treatments were determined using the REGW multiple comparison test (SAS Institute 2000). The results of each sugar ester compound's insecticidal activity was classified into none = 0%,

low = 1–25%, moderate = 26–60%, or high = 61–75% to demonstrate how insecticidal patterns changed with substitutions of sugar or fatty acid components. These classifications reflected the statistical differences between treatment means.

Detailed Dosage-Mortality Relationships. After the initial screening and results on nymphal mortality were analyzed, dosage-mortality relationships of five sugar ester compounds with the highest insecticidal activity were compared. These esters were sucrose octanoate, sorbitol octanoate, sorbitol decanoate, xylitol dodecanoate, xylitol decanoate, and M-Pede insecticidal soap, evaluated at 11 concentrations (0, 40, 200, 400, 600, 1,200, 2,400, 3,200, 4,000, 8,000, and 12,000 ppm [AI] in water). The 0 ppm (AI) concentration represented a water only control that enabled insecticidal mortalities to be adjusted using Abbott's correction formula (Abbott 1925) if control mortalities exceeded 2%. The commercial insecticidal soap, M-Pede, was included to determine how the dosage-mortality relationships of sugar esters differed from insecticidal soaps. The bioassays were conducted in the same manner as the previous experiment with each treatment concentration replicated six times over time with each replicate containing 40–60 pear psylla nymphs. Nymphal mortalities were determined 15 min after treatment.

Data on dosage-mortality relationships were analyzed using ANOVA and treatment comparisons were made using the REGW test (SAS Institute 2000). Data on dosage-mortality relationships for each sugar ester were analyzed by probit analyses to determine LD_{50} and LD_{90} values (SAS Institute 2000). The probit analysis used data from each material that provided nymphal mortalities of 10–90% to obtain more accurate estimates of LD_{50} and LD_{90} values (Robertson and Preisler 1992). The confidence limits of the LD values were adjusted by the heterogeneity factor (H) if the Pearson's χ^2 indicated a significant departure from the probit model.

Insecticidal Activity to a Range of Arthropod Species. The five sugar ester compounds that were evaluated in the dosage-mortality experiment, plus M-Pede, were tested against three divergent arthropod pests: the tobacco aphid, *Myzus nicotianae* Blackman and tobacco hornworm, *Manduca sexta* (Johannson) on tobacco; and the twospotted spider mite, *Tetranychus urticae* Koch on apple. The insecticidal and miticidal activity of the sugar esters were tested at low concentrations of 400, 1,200, and 2,400 ppm (AI) in water and a water only control. In addition, sorbitol decanoate and xylitol dodecanoate, which showed little activity against *M. sexta* and *M. nicotianae* at the low concentrations, were compared with insecticidal soap at its recommended labeled rates and higher concentrations (4,000, 8,000, 12,000 ppm [AI]) for activity against the three arthropod species. The experimental design and bioassays for both experiments were conducted in the same manner as the previous experiments, except there were three replications and an *M. sexta* bioassay was developed. The *M. sexta* bioassay consisted of placing second-instar larvae (n = 20) on a 4.0 cm diameter disc of tobacco leaf within a 100-mm diameter Petri dish before treatment. Arthropod mortalities were recorded at 2-min and 15-min time intervals.

The experiment was designed as a randomized complete block design with three replications. The data were analyzed by ANOVA and differences among treatments were determined using the REGW test (SAS Institute 2000).

Results

Physical Properties of Sugar Esters. The sugar ester compounds we synthesized differed in physical properties (Table 1). Only sucrose octanoate and sorbitol octanoate mixed readily with water although those materials rated as having good solubility mixed easily with water after vigorous shaking. Xylitol dodecanoate existed as a soft solid material that did not easily emulsify with water, but once emulsified, had surprisingly good leaf wetting properties. Analysis of leaf wetting ability showed significant differences among sugar esters ($F = 118.8$, df = 6, 18; $P = 0.0001$). There was no obvious relationship between a material's solubility in water, emulsion stability and droplet spread on pear leaves and hydrophilic-lipophilic balance (HLB). Sucrose octanoate and sorbitol octanoate were unique in that they readily mixed with water and formed stable emulsions. These two compounds also exhibited good surfactant properties by having droplets spread at low concentrations of 40–200 ppm. Xylitol dodecanoate could also wet leaves at a concentration of 40 ppm, yet this material was difficult to keep in solution and quickly separated from water. The remaining compounds had good water solubility, but needed constant agitation in order for serial dilutions to be made and for solutions to be applied.

Initial Screening. The effects of sugar ester treatments on psylla mortality (Table 2) were significantly affected by treatment ($F = 33.5$, df = 7, 45; $P < 0.0001$), time ($F = 74.2$, df = 1, 45; $P < 0.0001$), and time by treatment interaction ($F = 5.7$, df = 7, 45; $P < 0.0001$). Although most nymphs appeared to become paralyzed or killed immediately after treatment, some nymphs curved the tips of their abdomens upward in an apparent attempt to keep the spiracles above the film of solution on the leaf. Many nymphs died with their abdomens extended upward when sprayed with toxic concentrations of sugar ester, whereas, nymphs did not display this behavior when they were sprayed with an ineffective sugar ester concentration. Treated leaves dried within the 2-min time period after treatment and approximately half of the mortality occurred at that time in comparison to the 15-min time period except for materials that showed no insecticidal activity. In our preliminary bioassays, psylla nymphal mortality did not change significantly between 15 min and 24 h after application of sugar esters (G.J.P., unpublished data).

Sucrose octanoate, sorbitol octanoate, and xylitol decanoate applied at 1,200 ppm produced relatively high psylla mortalities in comparison to the other

Table 1. Physical characteristics of the sugar ester compounds

Sugar ester compound	CAS registry no.[a]	Chemical index name	Color	Physical state	Solubility with Water[b]	Stability of a 2,400 ppm emulsion[c]	Conc. (ppm ± SE) for droplet spread[d]	HLB[e]
Sucrose octanoate	42922-74-7, 58064-47-4	α-D, Glucopyranoside β-D-fructofuranosyl octanoate	Dark amber	Liquid	Excellent	Excellent	80.0 ± 40.0 d	22.0
Sorbitol octanoate	108175-15-1	D-Glucitol octanoate	Dark amber	Liquid	Excellent	Excellent	160.0 ± 40.0 d	15.8
Xylitol octanoate	-	-	Dark amber	Liquid	Good	Poor	2550.0 ± 150.0 a	11.8
Sorbitol hexanoate	50809-54-6	D-Glucitol hexanoate	Dark amber	Liquid	Good	Poor	2100.0 ± 300.0 b	16.8
Xylitol dodecanoate	211867-92-5	Xylitol dodecanoate	White	Solid	Poor	Poor	80.0 ± 40.0 d	9.9
Sorbitol decanoate	108175-14-0	D-Glucitol decanoate	Dark amber	Liquid	Good	Good	1500.0 ± 300.0 c	14.9
Xylitol decanoate	-	-	Dark amber	Liquid	Good	Poor	160.0 ± 40.0 d	10.8

[a] Chemical Abstracts Service (CAS) registry number for compounds that have been synthesized at least once. Those compounds without a CAS number represent a newly synthesized compound.
[b] Ability to dissolve readily with 25°C water when making a concentration of 2,400 ppm.
[c] Ability to remain an emulsion without separating into layers for 1 min after mixing a concentration of 2,400 ppm in 25°C water (30°C) for xylitol dodecanoate.
[d] Concentration at which a 10-μl droplet spread when applied directly to a mature pear leaf. Means within a column followed by the same letter are not significantly different, Ryan-Einot-Gabriel-Welsch multiple comparison (REGW) test, $P > 0.05$ (SAS Institute, 2000).
[e] Hydrophilic/lipophilic balance.

Table 2. Comparison of *C. pyricola* nymphal mortalities to different sugar ester-fatty acid compositions 2 and 15 minutes after applying 1,200 ppm AI solutions

Treatment	Percent Kill (±SE)	
	2 min	15 min
Untreated control	0.0 ± 0.0bA	0.0 ± 0.0fA
Sucrose octanoate	25.3 ± 9.3aA	71.8 ± 7.7aB
Sorbitol octanoate	25.6 ± 2.7aA	58.3 ± 4.8bB
Xylitol octanoate	0.0 ± 0.0bA	15.9 ± 2.8eB
Sorbitol hexanoate	0.0 ± 0.0bA	0.0 ± 0.0fA
Xylitol dodecanoate	11.5 ± 3.0abA	34.4 ± 6.0cB
Sorbitol decanoate	0.0 ± 0.0bA	25.0 ± 4.8dB
Xylitol decanoate	32.1 ± 5.1aA	73.1 ± 8.1aB

Means within columns followed by the same lowercase letter or means within rows followed by the same uppercase letter are not significantly different, $P > 0.05$, REGW test (SAS Institute 2000).

sugar esters at both the 2-min ($F = 6.7$, df = 7, 21; $P < 0.0003$) and 15-min time intervals ($F = 32.4$, df = 7, 21; $P < 0.0001$ (Table 2). Xylitol dodecanoate and sorbitol decanoate expressed lower psylla mortalities (25.0–34.4%), whereas xylitol octanoate and sorbitol caproate had little or no activity. Percent nymphal mortalities (Table 2) were negatively correlated with droplet spread on pear leaves which is directly associated with leaf wetting ($R^2 = 0.73$; y = 60.56x - 0.022; $F = 13.7$; df = 1, 5; $P = 0.014$). Furthermore, we found no correlation ($R^2 = 0.03$; y = 21.44x - 1.25; $F = 0.18$; df = 1, 5; $P = 0.69$) between HLB (Table 1) and psylla mortalities (Table 2).

The structure-function relationships of how sugar and fatty acid substitutions affect sugar ester insecticidal activity to psylla nymphs is summarized in Table 3. No definitive pattern of insecticidal activity of sugar ester compounds was evident when sequentially altering the sugar or fatty acid components from lower to higher numbers of carbon atoms (molecule size), or when altering the size of the sugar molecule. In sugar ester compounds containing a C_8 fatty acid (octanoate), insecticidal activity progressively increased when the sugar component increased from a C_5 (xy-

Table 3. Summary of how alterations sugar ester components affected insecticidal activity of materials applied at 1,200 ppm AI to *C. pyricola* nymphs

Constituent change	Sugar (carbon atoms)	Fatty acid (carbon chain length)	Insecticidal activity (% nymphal mortality)
Carbohydrate	xylitol (5)	octanoate (8)	low (15.9)
	sorbitol (6)	octanoate (8)	moderate (58.3)
	sucrose (12)	octanoate (8)	high (71.8)
	xylitol (5)	decanoate (10)	high (73.1)
	sorbitol (6)	decanoate (10)	low (25.0)
Fatty Acid	xylitol (5)	octanoate (8)	low (15.9)
	xylitol (5)	decanoate (10)	high (73.1)
	xylitol (5)	dodecanoate (12)	moderate (34.4)
	sorbitol (6)	hexanoate (6)	none (0.0)
	sorbitol (6)	octanoate (8)	high (58.3)
	sorbitol (6)	decanoate (10)	low (25.0)

Categories of none = 0%, low = 1–25%, moderate = 26–57%, or high = 58–75% nymphal mortalities based on significant statistical differences among sugar ester materials as shown in Table 2.

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[b] Ability to dissolve readily with 25°C water when making a concentration of 2,400 ppm.
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	xylitol (5)	dodecanoate (12)	moderate (34.4)
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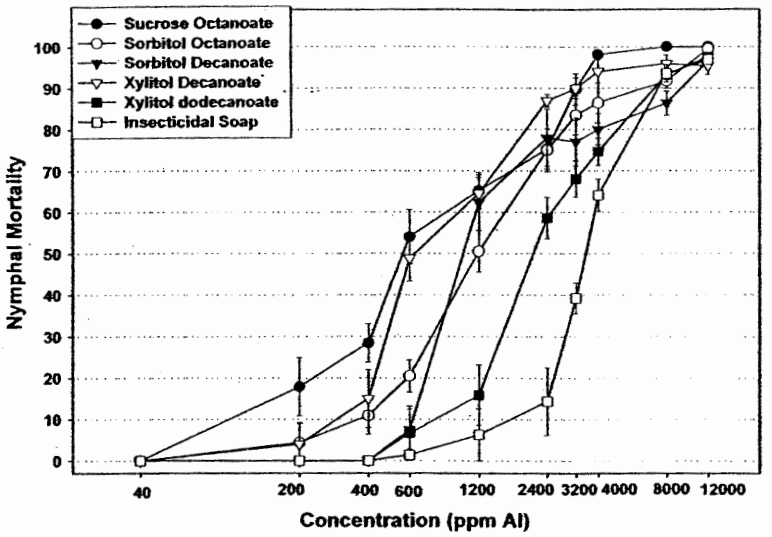

Fig. 1. Dosage-mortality curves for sugar ester compounds and insecticidal soap (M-Pede) applied at various concentrations to pear psylla nymphs on pear leaves 15 min after application.

litol) to a C_6 (sorbitol) reduced sugar to a C_{12} disaccaride (sucrose). Quite the opposite occurred for the C_{10} fatty acid esters (decanoate), which showed a decrease in insecticidal activity when the sugar component increased from C_5 to C_6 reduced sugar.

More of a discernible pattern for insecticidal activity was evident when the sugar component was held constant and the fatty-acid component was sequentially increased (Table 3). Xylitol or sorbitol based sugar esters produced insecticidal patterns that went from low activity with lower carbon chain fatty acids, to peak activity with mid-ranged carbon chained fatty acids, and then declined with higher carbon chain fatty acid substitutions. For example, insecticidal activity for xylitol was lowest for octanoate (C_8), high for decanoate (C_{10}), and moderate for dodecanoate (C_{12}) fatty acid substitutions.

Dosage-Mortality Relationships. Pear psylla nymphal mortalities (Fig. 1) were significantly affected by sugar ester treatment ($F = 7.6$, df = 6, 269; $P < 0.0001$), concentration ($F = 15.7$, df = 9, 296; $P < 0.0001$), and concentration by treatment interaction ($F = 9.0$, df = 39, 269; $P < 0.0001$). Sucrose octanoate had higher insecticidal activity than the other materials at concentrations of 200 ($F = 59.7$, df = 5, 25; $P < 0.0001$) and 400 ppm ($F = 569.7$, df = 5, 25; $P < 0.0001$). Most of the sugar esters performed similarly at concentrations between 600 and 2,400 ppm except for xylitol dodecanoate and insecticidal soap which had much lower levels of insecticidal activity.

The slopes and LD values from the probit analyses differed among the sugar ester and insecticidal soap materials as determined by overlapping confidence limits (Table 4). All of the sugar esters and insecticidal soap had high Pearson's χ^2 values which indicated a significant departure from the probit model. Examination of the residuals revealed that these departures were mainly because of the lack of psylla mortality at lower concentrations followed by rapidly increasing morality at higher concentrations (e.g., xylitol dodecanoate, insecticidal soap), a poor mortality response to increasing concentration at the high end of the dosages (xylitol decanoate, sorbitol octanoate) or both (e.g., sorbitol decanoate). Sucrose octanoate, sorbitol octanoate, and xylitol decanoate produced significantly higher psylla mortalities than the other

Table 4. Comparisons among sugar ester and insecticidal soap toxicities to *C. pyricola* nymphs on pear leaves based on a probit analysis

Treatment	*n*	Slope (95% CL)	LC_{50} (95% CL)[a]	LC_{90} (95% CL)[a]	Pearson's χ^2 (df = 4)[b]
Sucrose octanoate	48	3.13 (2.44–3.81)ABC	2124 (1691–2576)B	10680 (7973–16386)B	166.6*
Sorbitol octanoate	54	3.41 (2.71–4.12)AB	3107 (2449–3786)AB	13655 (10591–17659)B	298.6*
Sorbitol decanoate	54	1.83 (1.04–2.62)C	3523 (2730–4302)A	32189 (19407–62929)A	445.7*
Xylitol decanoate	48	4.39 (3.62–5.15)A	2103 (1800–2419)B	6650 (5509–8544)B	152.2*
Xylitol dodecanoate	48	2.43 (1.25–3.60)BC	5926 (2820–6719)A	39436 (21250–22132)A	387.0*
Insecticidal soap	48	2.72 (1.26–4.17)BC	7828 (5360–14177)A	50269 (22440–70052)A	783.3*

Means within columns followed by the same letter had overlapping confidence limits and are not significantly different (probit analyses, SAS Institute 2000).

[a] Concentrations are given in ppm AI material in water.

[b] Pearson's χ^2 followed by an asterisk are significant at $P = 0.10$, df = $n - 1$ (SAS Institute 2000).

Table 5. Arthropod mortalities 15 min after application of sugar esters at a low range of concentrations

Treatment	% Conc. (ppm AI)	Percent mortality (±SE)		
		M. sexta	*M. nicotianae*	*T. urticae*
Untreated control	0.00	0.0 ± 0.0cA	0.0 ± 0.0gA	0.0 ± 0.0cA
Sucrose octanoate	400	3.3 ± 1.7cC	71.6 ± 5.0bB	97.4 ± 1.9aA
	1,200	26.7 ± 4.4bC	79.7 ± 3.9bB	100.0 ± 0.0aA
	2,400	41.7 ± 10.1aB	98.5 ± 1.1aA	100.0 ± 0.0aA
Sorbitol octanoate	400	3.3 ± 3.3cB	4.2 ± 1.2gB	84.9 ± 7.3bA
	1,200	0.0 ± 0.0cC	21.6 ± 1.2defB	96.2 ± 4.8aA
	2,400	3.3 ± 3.3cC	51.4 ± 9.0cB	100.0 ± 0.0aA
Xylitol decanoate	400	1.7 ± 1.7cB	8.5 ± 1.5fgB	81.6 ± 4.7bA
	1,200	0.0 ± 0.0cC	25.8 ± 4.0deB	100.0 ± 0.0aA
	2,400	0.0 ± 0.0cC	29.9 ± 1.6dB	100.0 ± 0.0aA
Xylitol dodecanoate	400	0.0 ± 0.0cC	29.1 ± 3.7deB	100.0 ± 0.0aA
	1,200	0.0 ± 0.0cC	35.2 ± 6.2dB	100.0 ± 0.0aA
	2,400	0.0 ± 0.0cC	34.9 ± 2.6dB	100.0 ± 0.0aA
Sorbitol decanoate	400	0.0 ± 0.0cC	9.8 ± 2.1efgB	100.0 ± 0.0aA
	1,200	0.0 ± 0.0cC	32.6 ± 8.5dB	100.0 ± 0.0aA
	2,400	0.0 ± 0.0cC	71.6 ± 5.0bB	100.0 ± 0.0aA

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sugar ester compounds or insecticidal soap, based on LD_{90} values. The probit analyses determined that the dosage-mortality curves for sorbitol decanoate and xylitol dodecanoate were similar to insecticidal soap in reference to psylla mortalities.

Toxicity to a Range of Arthropod Species. Insect mortalities for the low range of sugar ester concentrations (Table 5) were significantly affected by arthropod species ($F = 1{,}716.3$, df = 2, 166; $P < 0.0001$), sugar ester treatment ($F = 55.6$, df = 5, 166; $P < 0.001$), and treatment concentration ($F = 134.4$, df = 3, 166; $P < 0.0001$). All interactions between arthropod species, sugar ester treatment, and treatment concentration were significant ($P < 0.0001$). *M. sexta* mortalities were lowest of the insect species and only sucrose octanoate at the higher concentrations of 1,200 and 2,400 ppm produced significantly higher mortalities than the other materials ($F = 19.0$, df = 15, 44; $P < 0.0001$). *M. nicotianae* mortalities on tobacco (Table 5) closely followed those of *C. pyricola* on pear (Table 2). Sucrose octanoate and sorbitol decanoate yielded significantly higher *M. nicotianae* mortalities than the other materials ($F = 77.4$, df = 15, 44; $P < 0.0001$). *T. urticae* was the most susceptible arthropod species to the sugar ester materials which yielded mortalities ranging from 88.8 to 100%.

Higher concentrations of 4,000, 8,000, and 12,000 ppm were explored for xylitol dodecanoate and sorbitol decanoate to determine their toxicities to the three arthropod species relative to insecticidal soap, which is typically applied at rates of 4,900–9,800 ppm for soft bodied insect control (Table 6). Xylitol dodecanoate and sorbitol decanoate were selected because they had no toxicity to *M. sexta* and low or moderate toxicity, respectively, to *M. nicotianae* (Table 5). Arthropod mortalities were significantly affected by concentration ($F = 26.9$, df = 3, 66; $P < 0.0001$), arthropod species ($F = 305.4$, df = 2, 66; $P < 0.0001$), and treatment by insect interaction ($F = 45.8$, df = 4, 66; $P < 0.0001$). However, the treatment by concentration interaction was not significant for the range of concentrations tested ($F = 0.0$, df = 3, 66; $P < 0.0001$), which indicated a lack of response in mortality as concentrations increased. Therefore, arthropod mortalities were averaged across concentrations for each insect by treatment combination. Low *M. sexta* mortalities were obtained from both sugar esters and insecticidal soap when applied at these high concentrations, although xylitol dodecanoate and insecticidal soap produced significantly higher mortalities than sorbitol decanoate, which essentially had no activity against this insect ($F = 15.0$, df = 3, 24; $P < 0.0001$). Both sugar esters provided high *M. nicotianae* mortalities compared with insecticidal soap ($F = 17.3$, df = 3, 24; $P < 0.0001$), whereas all three materials produced >94% *T. urticae* mortalities ($F = 1$, 141.3, df = 3, 24; $P < 0.0001$).

Discussion

We have demonstrated that altering the sugar or fatty acid component of sugar ester compounds will alter the insecticidal activity of a sugar ester compound (Tables 2–4). Little information is available on how the sugar component actually influences the insecticidal activity of sugar ester, even though it serves as the backbone of this compound. Sugar structures contain free hydroxyl groups that serve as sites for fatty acid esterification. Disaccharides (e.g., sucrose) have more free hydroxyl sites that give rise to a mixture of sugar ester isomers, but differing methods of

Table 6. Arthropod mortalities averaged over treatment concentrations of 4,000, 8,000, and 12,000 ppm AI, 15 minutes after application

Treatment	Percent mortality (±SE)		
	M. sexta	*M. nicotianae*	*T. urticae*
Untreated control	0.0 ± 0.0bA	0.0 ± 0.0cA	0.0 ± 0.0bA
Xylitol dodecanoate	16.1 ± 1.4aC	70.0 ± 6.4aB	100.0 ± 0.0aA
Sorbitol decanoate	1.6 ± 1.2bB	94.8 ± 2.9aA	100.0 ± 0.0aA
M-Pede soap	11.7 ± 2.5aC	37.4 ± 6.8bB	97.2 ± 1.6aA

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synthesis can produce sucrose esters high in monoester (Farone and Serfass 1998) or diester content (Chortyk et al. 1996). Natural sugar ester extracts from certain *Nicotiana* spp. contain a mixture of both sucrose and glucose esters (Matsuzaki et al. 1992, Severson et al. 1994), which have excellent insecticidal properties (Puterka and Severson 1995, Liu et al. 1996), yet sucrose esters and their synthesis have been the primary focus of insecticidal study (Buta et al. 1993, Chortyk et al. 1996). Our study indicated that changing the sugar component greatly influenced insecticidal activity and that high insecticidal activity could be achieved with sugars by modulating the fatty acid chain length (Table 3). Previous research established that the fatty acid component also has profound effects on the insecticidal properties of sugar esters. Chortyk et al. (1996) determined that sucrose esters containing C_7 and C_8 fatty acids produced high *M. nicotiana* mortalities, whereas C_6 and C_{9-12} fatty acids produced much lower mortalities. We discovered that different sugar bases (xylitol, sorbitol) influenced which fatty acid carbon chain length produced the greatest insecticidal activity. Insecticidal activity peaked at fatty acid chain lengths of C_8 or C_{10}, depending upon the sugar backbone (Table 2 and 3). If insecticidal activity could be simply explained as a surfactant relationship one would expect a certain HLB would hold. Thus, decreasing the size of the hydrophile from sorbitol (C_6) to xylitol (C_5) would require a decrease in size of the lipophile to maintain the same HLB relationship (Adamson 1976). Quite the opposite was the case for sugar esters in which increasing the size of the hydrophile as indicated above required an increase in lipophile size to maintain an HLB of 10.8–11.8 (Table 1). With regard to the degree of esterification of sucrose esters, it has been established that monoacyl sucrose ester produced low *M. nicotiana* mortalities on tobacco leaves, whereas diacyl sucrose esters of C_7 and C_8 fatty acids resulted in high mortalities (Chortyk et al. 1996). In contrast, our studies demonstrated that C_8 sucrose esters high in monoester content provided high levels of mortality against *C. pyricola*, *M. nicotiana*, and *T. urticae*, regardless of leaf type (Tables 2 and 5). Moreover, several of the sorbitol and xylitol esters (sorbitol octanoate, xylitol decanoate) had relatively high levels of activity against the aforementioned arthropods. Our results indicate that changes in either the sugar or fatty acid component of sugar esters lead to unpredictable effects on insecticidal activity that can only be determined by empirical tests.

Sugar esters have excellent surfactant and emulsifier properties that are desirable to the food and cosmetics industry, but also share a number of insecticidal properties with other surfactants. Initial insect bioassays (Table 1) and dosage-mortality data (Table 4) revealed that sucrose octanoate, xylitol decanoate, and sorbitol octanoate were most toxic to *C. pyricola* nymphs. These compounds varied in their solubility in water and in emulsion stability, yet droplet spread on pear leaves was achieved at low concentrations of 80–160 ppm (Table 1) and was strongly correlated with psylla mortalities (Table 2). Droplet spread is related to leaf wetting, which is closely associated with surface tension values. It is of importance to note that the droplet spread for the sugar ester solutions on pear leaves occurred at far lower concentrations than the concentration needed to uniformly wet the leaf and kill arthropod pests. Sugar ester materials applied at concentrations that produced >70% psylla mortalities also uniformly wet the leaf, whereas lower concentrations produced only partial coverage because the droplets failed to coalesce. We lacked the equipment needed to determine surface tension and other properties of our sugar ester compounds in solution. However, studies have shown that the insecticidal and miticidal activity of surfactants are correlated with surface tension values <30 mN/m (Imai et al. 1994) and leaf wetting at low concentrations of 50–300 ppm (Cowles et al. 2000). Sugar ester HLB did not correlate with insect mortalities, which was also found to be true for a large range of surfactants tested by Imai et al. (1994). Sugar esters (Xia et al. 1997) share another property with surfactants, including insecticidal soaps (Imai et al. 1995), in that higher humidities increase insecticidal activity by increasing the duration of wetting. Imai et al. (1995) proposed that insecticidal surfactants require low surface tension to spread readily upon insect cuticles and that increasing wetting time increases the probability that the insect will suffocate. Although surfactants must readily spread over an insect cuticle, their interaction with the host plant cuticle also plays a key role in the suffocation process. Cowles et al. (2000) has demonstrated that miticidal activity of Silwet L-77 on strawberry was significantly less than on kidney bean and suggested this may have resulted from differences in the ability of Silwet L-77 to wet these leaf surfaces. Surfactant performance is influenced by plant surfaces through complex interrelated physiochemical factors that include cuticle roughness, presence of trichomes, trichome type, and cuticular hydrocarbons (Halloway 1970). It is not known how hydrophobic insect cuticles are or how closely related the hydrophobicity or wettability of insect cuticles are to their respective host plants. Surface active materials that wet leaves readily at one concentration may require higher concentrations to wet the target insect. Clearly, leaf wetting is only one factor affecting the insecticidal activity of sugar esters. Our data on two insect species, *M. nicotiana* and *M. sexta*, on tobacco (Table 5) demonstrates that even with thorough wetting of the leaf *M. nicotiana* mortalities were high but *M. sexta* mortalities were very low, suggesting that other factors specific to the insect are in play. Certainly, more research is needed on characterizing the surface-active properties of sugar esters and their relationship to the physiochemical interactions between the leaf and insect before we can arrive at an understanding of the mechanisms of insecticidal action and how sugar ester performance can be improved.

The underlying mechanisms of insecticidal action of sugar esters, or surfactants and soaps for that matter, is subject to much speculation but little study. Sugar

esters contain lipophilic acyl chains and free hydroxyls, which give them soaplike properties (Neal et al. 1994). Two main theories of insecticidal action for soaps that were presented in the early 1900s and still remain controversial today include (1) insects suffocate when soap produces mechanical occlusions of the body openings; or (2) the insects become desiccated by the caustic properties of the free alkaline constituents of soaps upon the insect's cuticle. These theories were challenged by Siegler and Popenoe (1925) who presented data showing that the free fatty acids are themselves the toxic agents and they believed that the fatty acids were absorbed through the insects cuticle and act upon the insects cellular membranes. Soaps that are alkali salts of fatty acids undergo partial hydrolysis when mixed with water, which results in free alkali and free fatty acids. Research by Puritch (1975) further supports this idea. He found fatty acids of soaps can disrupt cellular membrane integrity in insects. Certainly, fatty acids themselves are known to be insecticidal and their activity is greatly increased through saponification or esterification (Kabara 1987). Yet, others maintain that suffocation is the likely mechanism because of the highly wettable nature of soaps and other surfactant solutions (Imai et al. 1994). Sugar ester structure does not lend itself to hydrolysis during the short time-frame when it is applied and kills insects. Therefore, it is doubtful that direct toxicity to insects from free fatty acids is an plausible mechanism for sugar esters. From our data we agree that suffocation is possible because wetting of the leaf is needed before insect mortalities occur. However, as we already stated, leaf wetting does not always lead to insect mortality, as was the case for *M. sexta*. Furthermore, in an earlier study (Puterka and Severson 1995) dry sugar ester residues on pear leaves caused high mortalities in newly eclosed *C. pyricola* nymphs that contacted treated leaf surfaces. The dead nymphs appeared to be swollen with water, which supported the idea that disruption of the cuticle or cellular membranes occurred. Sugar esters will naturally break down as a result of microbial action hours after application, in which case free fatty acid may affect the insect cuticle. From the current body of information on mechanisms and from our study, it seems likely that more than one mechanism can be involved, depending on the insect's surface chemistry, surface structure, and other specialized structural and physiological adaptations that have evolved to enable a particular insect species to occupy a specific niche or environment.

Studies on natural sugar esters obtained from trichome exudates or leaf extractions from *N. gossei* have established they are effective against *M. sexta* (Parr and Thurston 1968) *C. pyricola* (Puterka and Severson 1995), mites, and a variety of aphids and whiteflies (Buta et al. 1993, Neal et al. 1994, Severson et al. 1994, Liu et al. 1996). Neal et al. (1994) reported that natural sugar esters extracts were not effective against the western flower thrips *Frankliniella occidentalis* (Pergande) or the Colorado potato beetle *Leptinotarsa decemlineata* (Say). Synthetic diesters of sucrose octanoate have been less tested and efficacy reports are limited to aphids and whiteflies (Chortyk et al. 1996, Xia et al. 1997, Liu et al. 1996). The monoacyl sugar ester compounds we examined varied considerably in toxicity to the range of arthropod species we examined. Sucrose octanoate was the only material that was effective against the range of arthropod pests on different leaf types at concentrations of 1,200–2,400 ppm (Table 2 and 5). No single chemical structure for the xylitol or sorbitol esters were optimally effective against the range of arthropods at these concentrations and sorbitol octanoate and xylitol decanoate showed the greatest insecticidal activity of this group. Xylitol decanoate produced high *C. pyricola* mortalities on pear at 1,200–2,400 ppm, but showed low activity against *M. nicotiana* and no activity against *M. sexta* on tobacco. In contrast, sorbitol decanoate at these concentrations produced low *C. pyricola* mortalities on pear, yet ranked second in *M. nicotiana* mortality and was not active against *M. sexta* on tobacco. All of the sugar ester materials produced high *T. urticae* mortalities on pear at very low concentrations of 400 ppm. Those sugar esters with no insecticidal activity to *M. sexta* (xylitol dodecanoate, sorbitol decanoate) at concentrations of 2,400 ppm (Table 5) still produced very low mortalities when concentrations were increased to as high as 12,000 ppm (Table 6). Yet, even these two sugar ester compounds with the lowest insecticidal activity of those we synthesized outperformed the insecticidal soap (Fig. 1; Table 6). These results expand the list of insects that are susceptible to sucrose octanoate and establishes one new compound, xylitol decanoate, as having notable insecticidal and miticidal activity.

Further characterization of the physical and chemical properties of sugar ester compounds, testing sugar esters against a broader range of arthropod species, and in-depth research into the mechanisms-of-action for sugar esters against arthropods and how these mechanisms are affected by different arthropod and plant surfaces are needed to better understand how sugar ester chemistry can be improved. We have established that sucrose octanoate high in monoester content has the broadest insecticidal activity of the sugar esters we produced. Our study has shown that changes in the fatty acid and sugar components of sugar esters produces unpredictable levels of insecticidal activity and also affects the range of arthropods that sugar ester compounds are effective against. We produced sugar ester compounds with toxicity to a broad range of arthropod species (sucrose octanoate) or narrow range of species (sorbitol decanoate) and synthesized a new sugar ester compound that is effective against psylla and mites. Sugar ester chemistry presents a special opportunity in which compounds could be designed to fit particular crop pest needs. This chemistry may be of particular interest today where safety to humans and the environment are an issue. Another important quality of sugar esters is that to date we have not observed phytotoxicity to many types of plant species at concentrations that are effective against mites and aphids, which can be of

esters contain lipophilic acyl chains and free hydroxyls, which give them soaplike properties (Neal et al. 1994). Two main theories of insecticidal action for soaps that were presented in the early 1900s and still remain controversial today include (1) insects suffocate when soap produces mechanical occlusions of the body openings; or (2) the insects become desiccated by the caustic properties of the free alkaline constituents of soaps upon the insect's cuticle. These theories were challenged by Siegler and Popenoe (1925) who presented data showing that the free fatty acids are themselves the toxic agents and they believed that the fatty acids were absorbed through the insects cuticle and act upon the insects cellular membranes. Soaps that are alkali salts of fatty acids undergo partial hydrolysis when mixed with water, which results in free alkali and free fatty acids. Research by Puritch (1975) further supports this idea. He found fatty acids of soaps can disrupt cellular membrane integrity in insects. Certainly, fatty acids themselves are known to be insecticidal and their activity is greatly increased through saponification or esterification (Kabara 1987). Yet, others maintain that suffocation is the likely mechanism because of the highly wettable nature of soaps and other surfactant solutions (Imai et al. 1994). Sugar ester structure does not lend itself to hydrolysis during the short time-frame when it is applied and kills insects. Therefore, it is doubtful that direct toxicity to insects from free fatty acids is an plausible mechanism for sugar esters. From our data we agree that suffocation is possible because wetting of the leaf is needed before insect mortalities occur. However, as we already stated, leaf wetting does not always lead to insect mortality, as was the case for *M. sexta*. Furthermore, in an earlier study (Puterka and Severson 1995) dry sugar ester residues on pear leaves caused high mortalities in newly eclosed *C. pyricola* nymphs that contacted treated leaf surfaces. The dead nymphs appeared to be swollen with water, which supported the idea that disruption of the cuticle or cellular membranes occurred. Sugar esters will naturally break down as a result of microbial action hours after application, in which case free fatty acid may affect the insect cuticle. From the current body of information on mechanisms and from our study, it seems likely that more than one mechanism can be involved, depending on the insect's surface chemistry, surface structure, and other specialized structural and physiological adaptations that have evolved to enable a particular insect species to occupy a specific niche or environment.

Studies on natural sugar esters obtained from trichome exudates or leaf extractions from *N. gossei* have established they are effective against *M. sexta* (Parr and Thurston 1968) *C. pyricola* (Puterka and Severson 1995), mites, and a variety of aphids and whiteflies (Buta et al. 1993, Neal et al. 1994, Severson et al. 1994, Liu et al. 1996). Neal et al. (1994) reported that natural sugar esters extracts were not effective against the western flower thrips *Frankliniella occidentalis* (Pergande) or the Colorado potato beetle *Leptinotarsa decemlineata* (Say). Synthetic diesters of sucrose octanoate have been less tested and efficacy reports are limited to aphids and whiteflies (Chortyk et al. 1996, Xia et al. 1997, Liu et al. 1996). The monoacyl sugar ester compounds we examined varied considerably in toxicity to the range of arthropod species we examined. Sucrose octanoate was the only material that was effective against the range of arthropod pests on different leaf types at concentrations of 1,200–2,400 ppm (Table 2 and 5). No single chemical structure for the xylitol or sorbitol esters were optimally effective against the range of arthropods at these concentrations and sorbitol octanoate and xylitol decanoate showed the greatest insecticidal activity of this group. Xylitol decanoate produced high *C. pyricola* mortalities on pear at 1,200–2,400 ppm, but showed low activity against *M. nicotiana* and no activity against *M. sexta* on tobacco. In contrast, sorbitol decanoate at these concentrations produced low *C. pyricola* mortalities on pear, yet ranked second in *M. nicotiana* mortality and was not active against *M. sexta* on tobacco. All of the sugar ester materials produced high *T. urticae* mortalities on pear at very low concentrations of 400 ppm. Those sugar esters with no insecticidal activity to *M. sexta* (xylitol dodecanoate, sorbitol decanoate) at concentrations of 2,400 ppm (Table 5) still produced very low mortalities when concentrations were increased to as high as 12,000 ppm (Table 6). Yet, even these two sugar ester compounds with the lowest insecticidal activity of those we synthesized outperformed the insecticidal soap (Fig. 1; Table 6). These results expand the list of insects that are susceptible to sucrose octanoate and establishes one new compound, xylitol decanoate, as having notable insecticidal and miticidal activity.

Further characterization of the physical and chemical properties of sugar ester compounds, testing sugar esters against a broader range of arthropod species, and in-depth research into the mechanisms-of-action for sugar esters against arthropods and how these mechanisms are affected by different arthropod and plant surfaces are needed to better understand how sugar ester chemistry can be improved. We have established that sucrose octanoate high in monoester content has the broadest insecticidal activity of the sugar esters we produced. Our study has shown that changes in the fatty acid and sugar components of sugar esters produces unpredictable levels of insecticidal activity and also affects the range of arthropods that sugar ester compounds are effective against. We produced sugar ester compounds with toxicity to a broad range of arthropod species (sucrose octanoate) or narrow range of species (sorbitol decanoate) and synthesized a new sugar ester compound that is effective against psylla and mites. Sugar ester chemistry presents a special opportunity in which compounds could be designed to fit particular crop pest needs. This chemistry may be of particular interest today where safety to humans and the environment are an issue. Another important quality of sugar esters is that to date we have not observed phytotoxicity to many types of plant species at concentrations that are effective against mites and aphids, which can be of

concern when using insecticidal soaps. Sugar ester residues break down rapidly through microbial action in the environment into their base components, are already internationally approved as a food ingredient, and a new sugar ester manufacturing process has been developed that produces no toxic by-products (Farone et al. 2002). Recently, sucrose octanoate was registered as AVACHEM Sucrose Octanoate (AVA Chemical Ventures, L.L.C., Portsmouth, NH) with the United States Environmental Protection Agency for control of insect and mite pests on all agricultural crops and ornamentals, and for greenhouse use. This registration will aid in determining the practical utility of this pesticide chemistry by enabling diverse testing against arthropod pests in numerous crops and environmental conditions.

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

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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.

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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

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).

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*.

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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.) (Parr & 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

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(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-

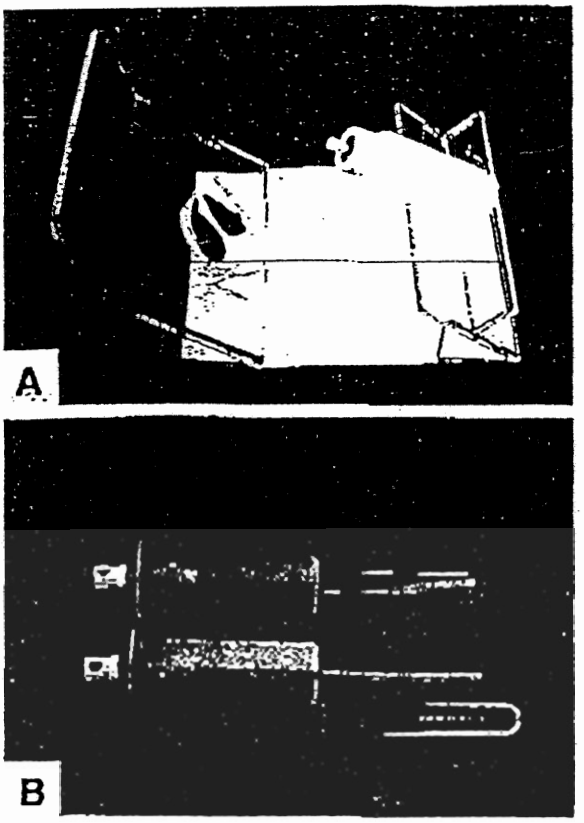

Fig. 1. Components of the ultra-low-volume spray device. (A) Spray platform with pressurizable spray bottle and petri dish with infested leaves held at the proper distance and angle to ensure consistent repeatable applications. (B) Spray bottle nozzle-pump body fitted with test tube (12 by 75 mm) that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

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).

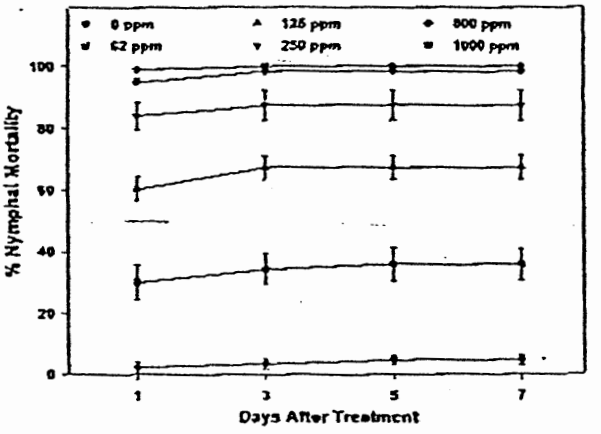

Fig. 2. Time–mortality relationships for pear psylla nymphal mortality when nymphs were treated with various concentrations of sugar ester in aqueous solutions.

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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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.) (Parr & Thurston 1968, Thurston 1970), and the greenhouse whitefly, *Trialeurodes vaporariarum* (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

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(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-

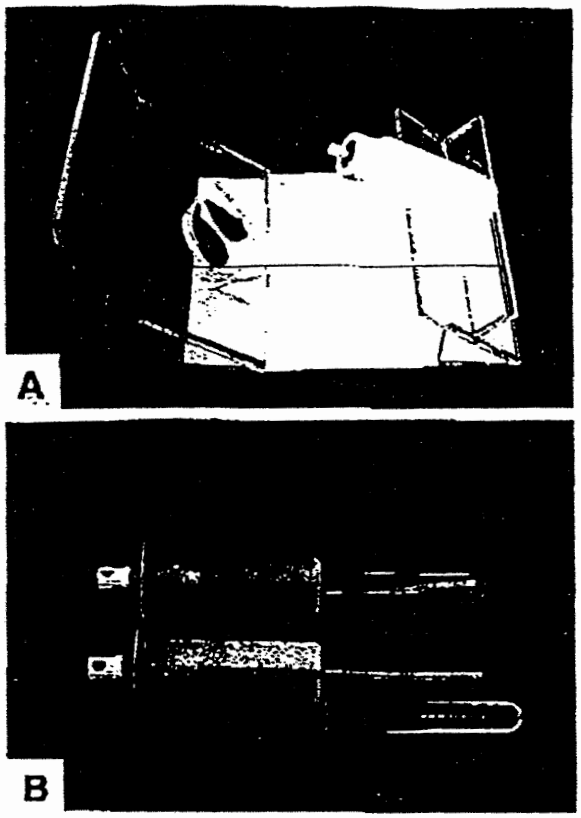

Fig. 1. Components of the ultra-low-volume spray device. (A) Spray platform with pressurizable spray bottle and petri dish with infested leaves held at the proper distance and angle to ensure consistent repeatable applications. (B) Spray bottle nozzle-pump body fitted with test tube (12 by 75 mm) that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

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).

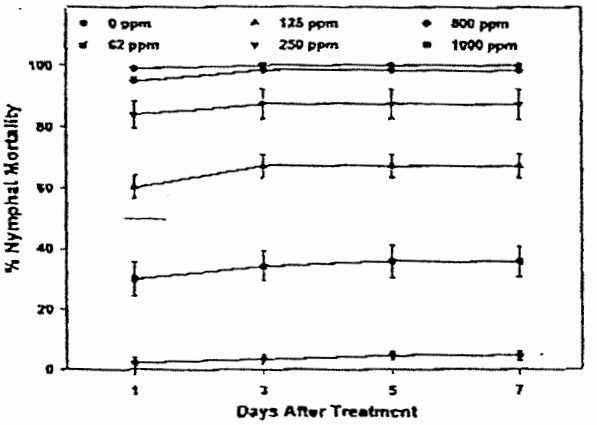

Fig. 2. Time–mortality relationships for pear psylla nymphal mortality when nymphs were treated with various concentrations of sugar ester in aqueous solutions.

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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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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51/75

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.

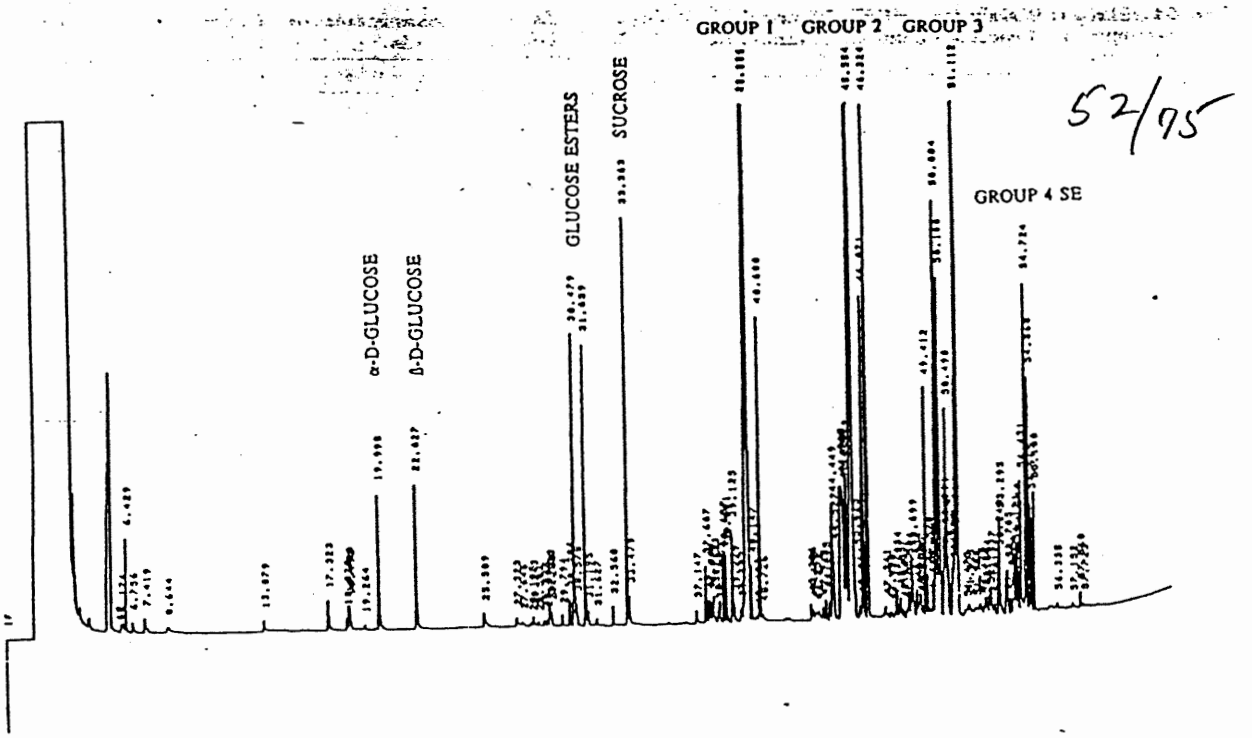

Figure 1. Gas chromatogram of total octanoyl sucrose ester reaction products (TMS derivatives).

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

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

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
mononononanoyl 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%.

method and then separated into individual groups by SA column chromatography, as was done for the C_8SE.

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

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

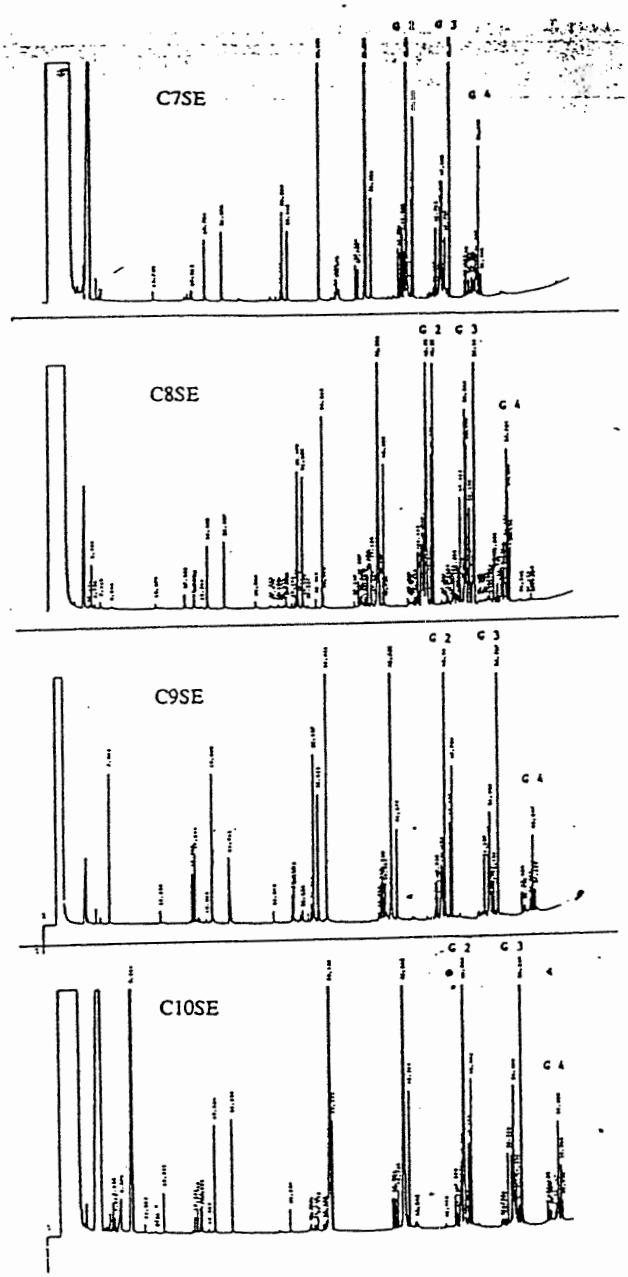

Figure 2. Gas chromatograms of heptanoyl, octanoyl, nonanoyl, and decanoyl sucrose esters products (TMS derivatives).

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 simplicity and reproducibility of the developed synthesis and the large quantity of toxic SE that can now be produced in a rapid manner indicate that this synthetic method should be readily adapted for commercial production of environmentally-friendly insecticides against the highly destructive whiteflies and aphids. The future of these sucrose esters appears to be bright. A patent application has been filed on the use of these SE for the control of soft-bodied arthropods. Three commercial companies have signed cooperative research and development agreements and are committing extensive funds and expertise for the testing of these compounds, and over a dozen cooperators are field testing these SE against various insects on various agricultural and ornamental crops.

55/75

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56/75

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 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, monanoic, 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 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/cm² 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) (OASIS 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/cm² 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/cm²) (≈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 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.

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).

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).

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.

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/cm²; ≈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 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).

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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 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	Hydrolyxed 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 07054–1698.

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-

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 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 ± 3.4i,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

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 ± 17.3bc,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).

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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The United States of America

The Director of the United States Patent and Trademark Office

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.

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Director of the United States Patent and Trademark Office

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Farone et al.

(10) **Patent No.: US 6,419,941 B1**
(45) **Date of Patent: Jul. 16, 2002**

(54) **POLYOL ESTER INSECTICIDES AND METHOD OF SYNTHESIS**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/504,016**

(22) Filed: **Feb. 18, 2000**

(51) **Int. Cl.**[7] **A01N 25/02**

(52) **U.S. Cl.** **424/405**; 424/406; 514/25; 514/53; 514/546; 514/549

(58) **Field of Search** 424/405; 514/25, 514/53, 738, 546, 552, 547, 549

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Siegler et al., *J. Econ. Entomol.* 18, 292–299 (1925).

Chortyk et al., *J. Agric. Food Chem.* 44, 1551–1557 (1996).

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Primary Examiner—Neil S. Levy
(74) *Attorney, Agent, or Firm*—Cynthia H. O'Donohue

(57) **ABSTRACT**

The present invention relates to an environmentally acceptable synthesis method of polyol esters that produces no toxic by-products methods during the synthesis. The present invention also provides for the use of these esters as safe and effective insecticides.

1 Claim, 12 Drawing Sheets

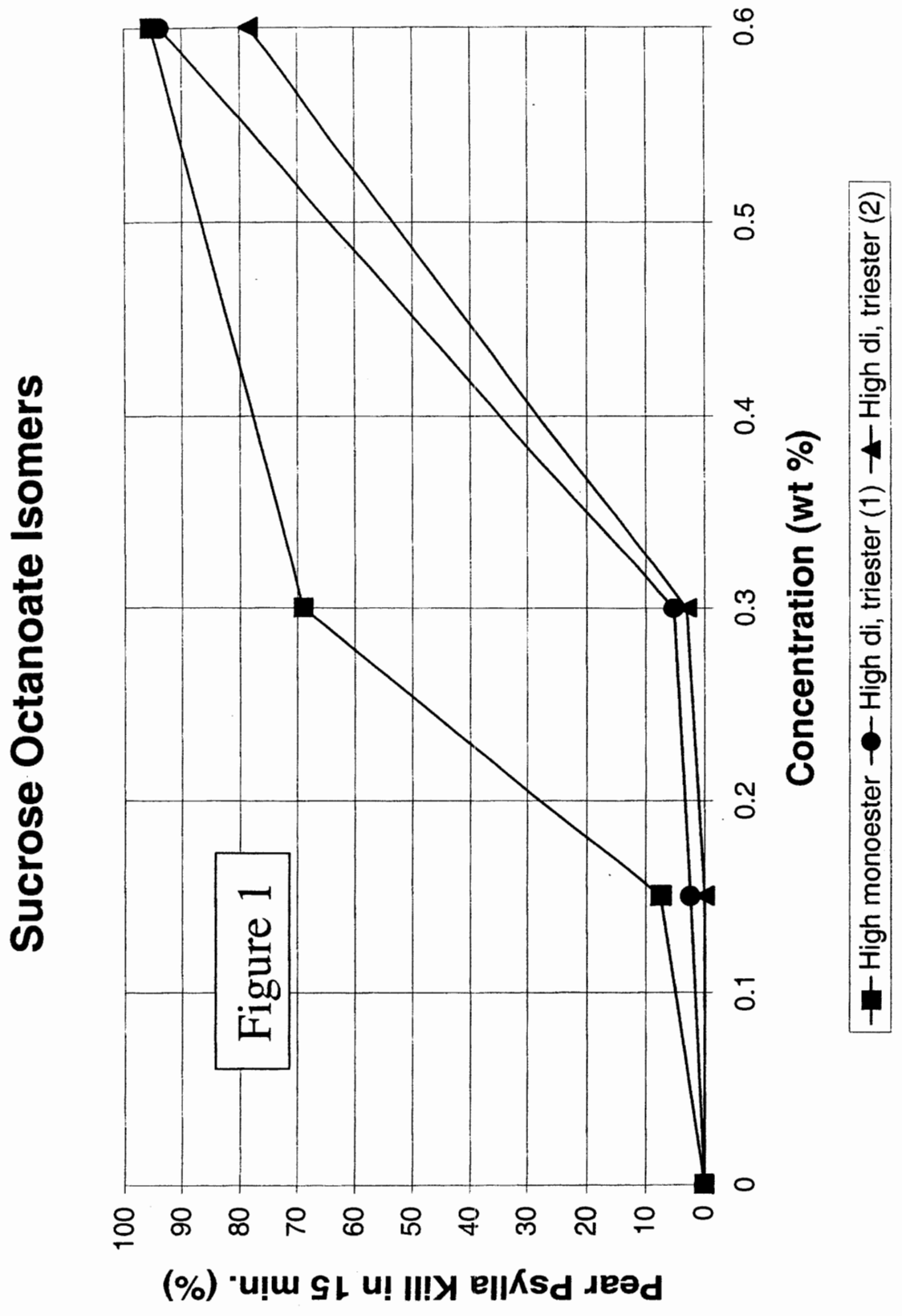

Sucrose Octanoate Isomers
Pear Psylla Kill in 15 min. (%)
100
90
80
70
60
50
40
30
20
10
0
0
0.1
0.2
0.3
0.4
0.5
0.6
Concentration (wt %)
High monoester
High di, triester (1)
High di, triester (2)

Figure 1

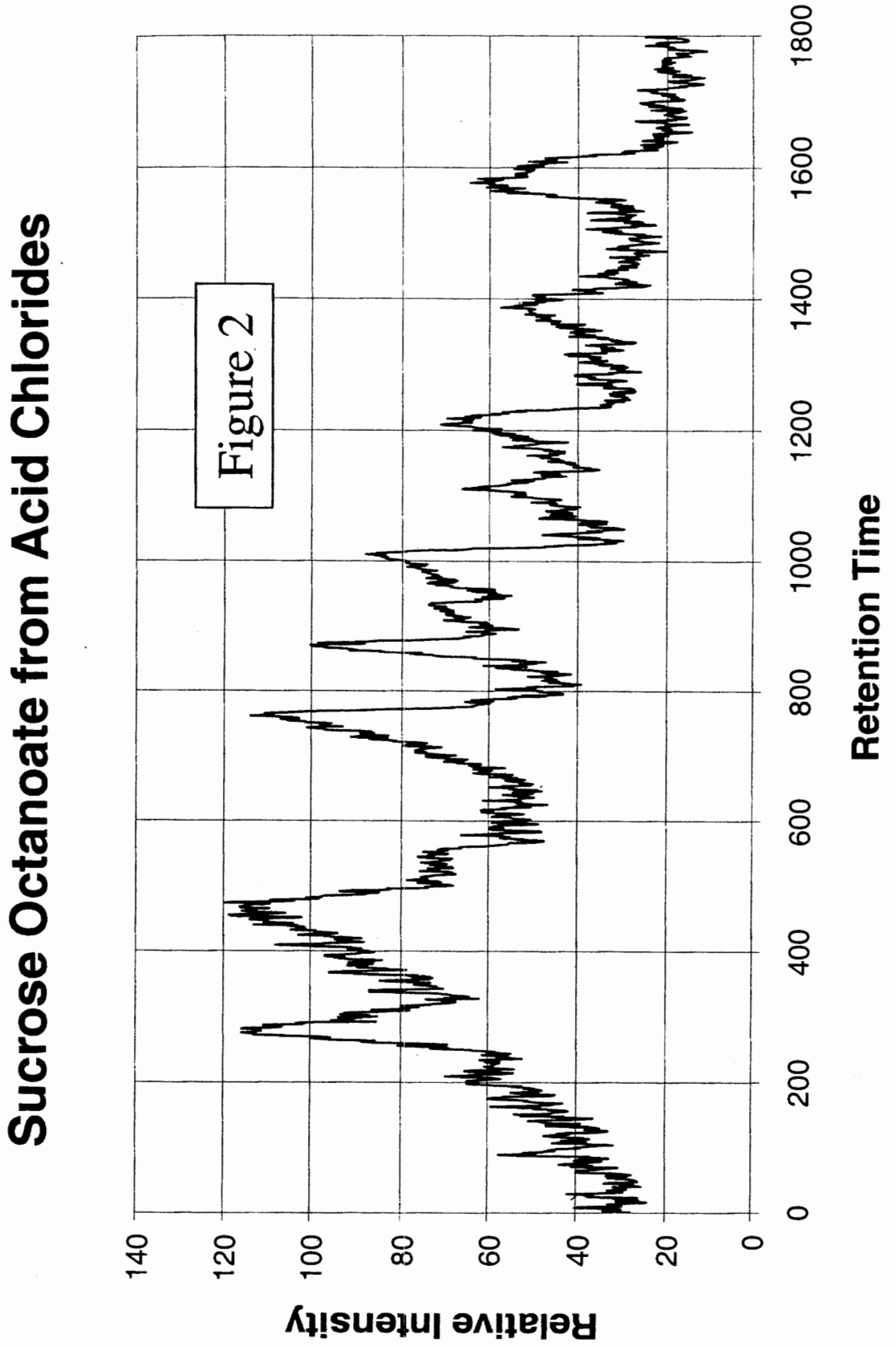
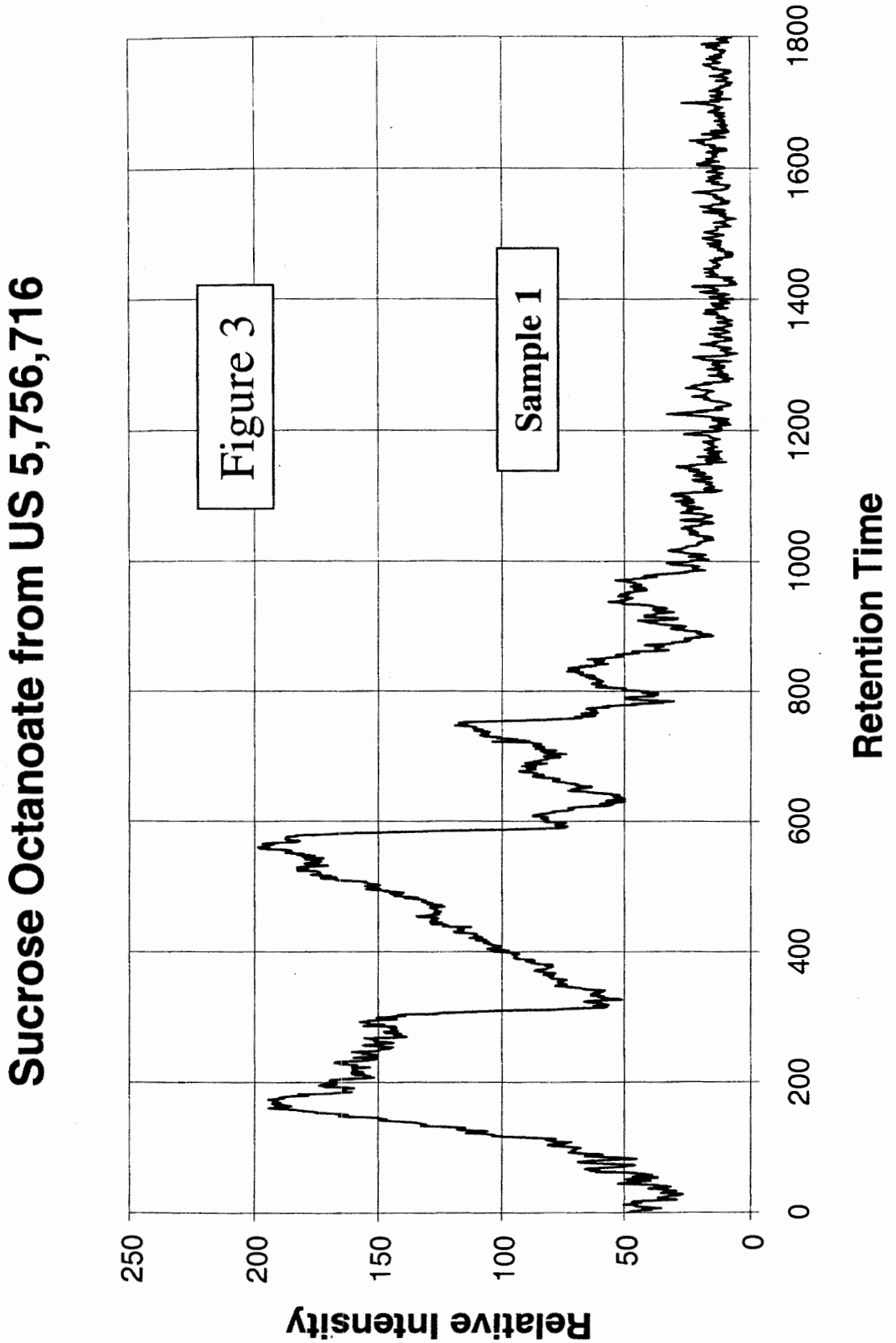
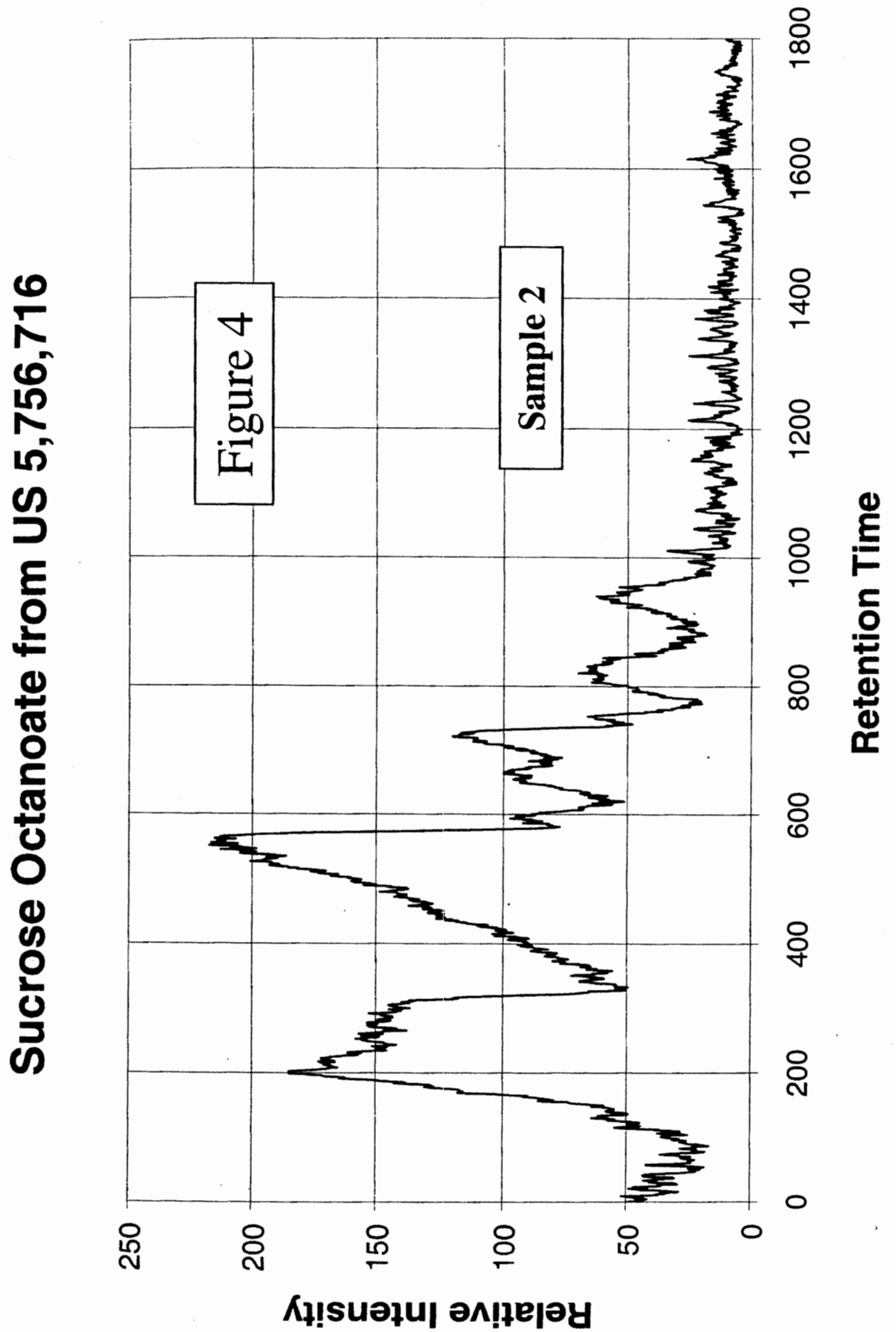

Sucrose Octanoate from Acid Chlorides
Relative Intensity
0
20
40
60
80
100
120
140
Retention Time
0
200
400
600
800
1000
1200
1400
1600
1800

Figure 2


Sucrose Octanoate from US 5,756,716
Figure 3
Sample 1
Relative Intensity
Retention Time
250
200
150
100
50
0
0
200
400
600
800
1000
1200
1400
1600
1800


Sucrose Octanoate from US 5,756,716
Figure 4
Sample 2
Relative Intensity
Retention Time
250
200
150
100
50
0
0
200
400
600
800
1000
1200
1400
1600
1800

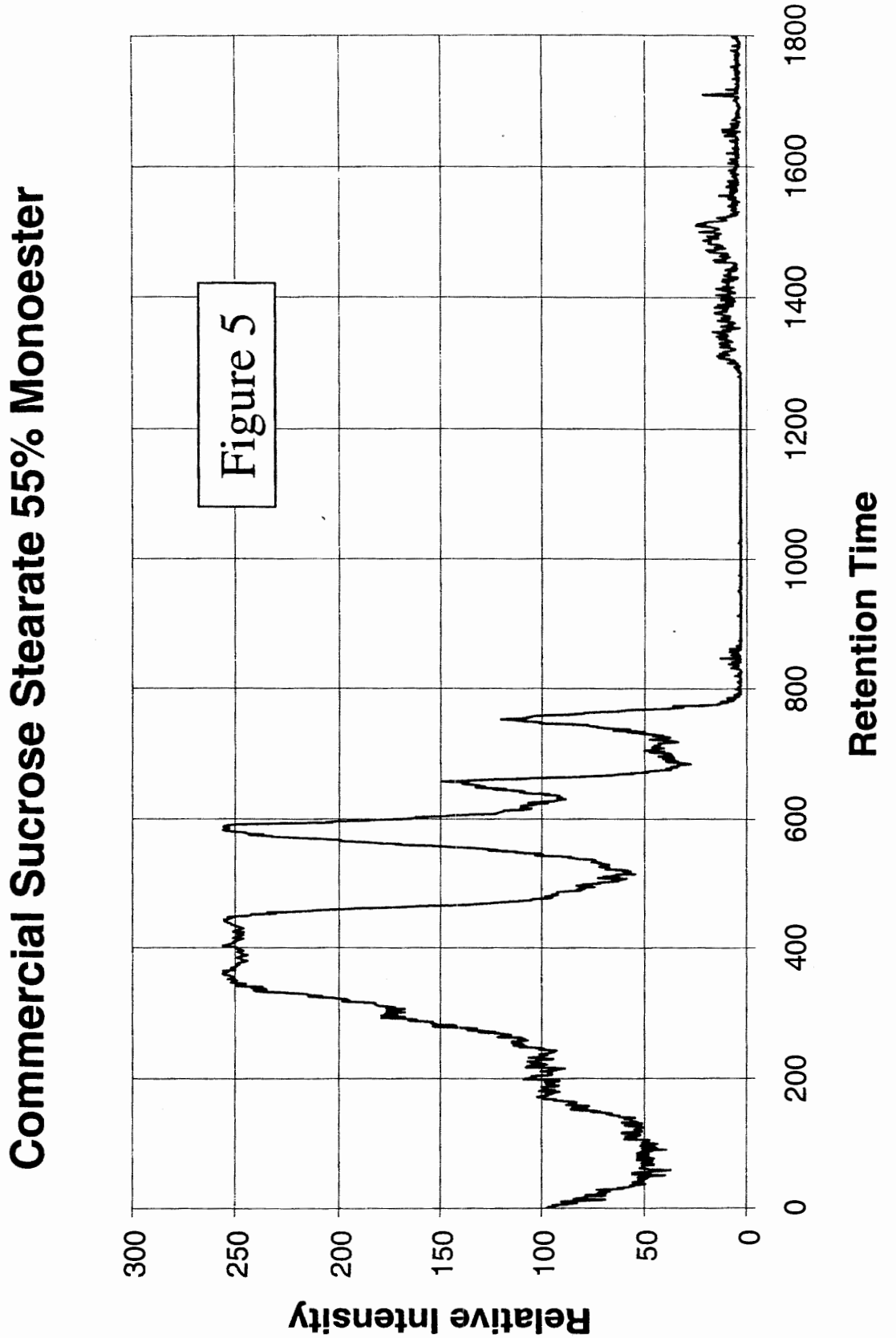

Commercial Sucrose Stearate 55% Monoester
Relative Intensity
300
250
200
150
100
50
0
0
200
400
600
800
1000
1200
1400
1600
1800
Retention Time

Figure 5

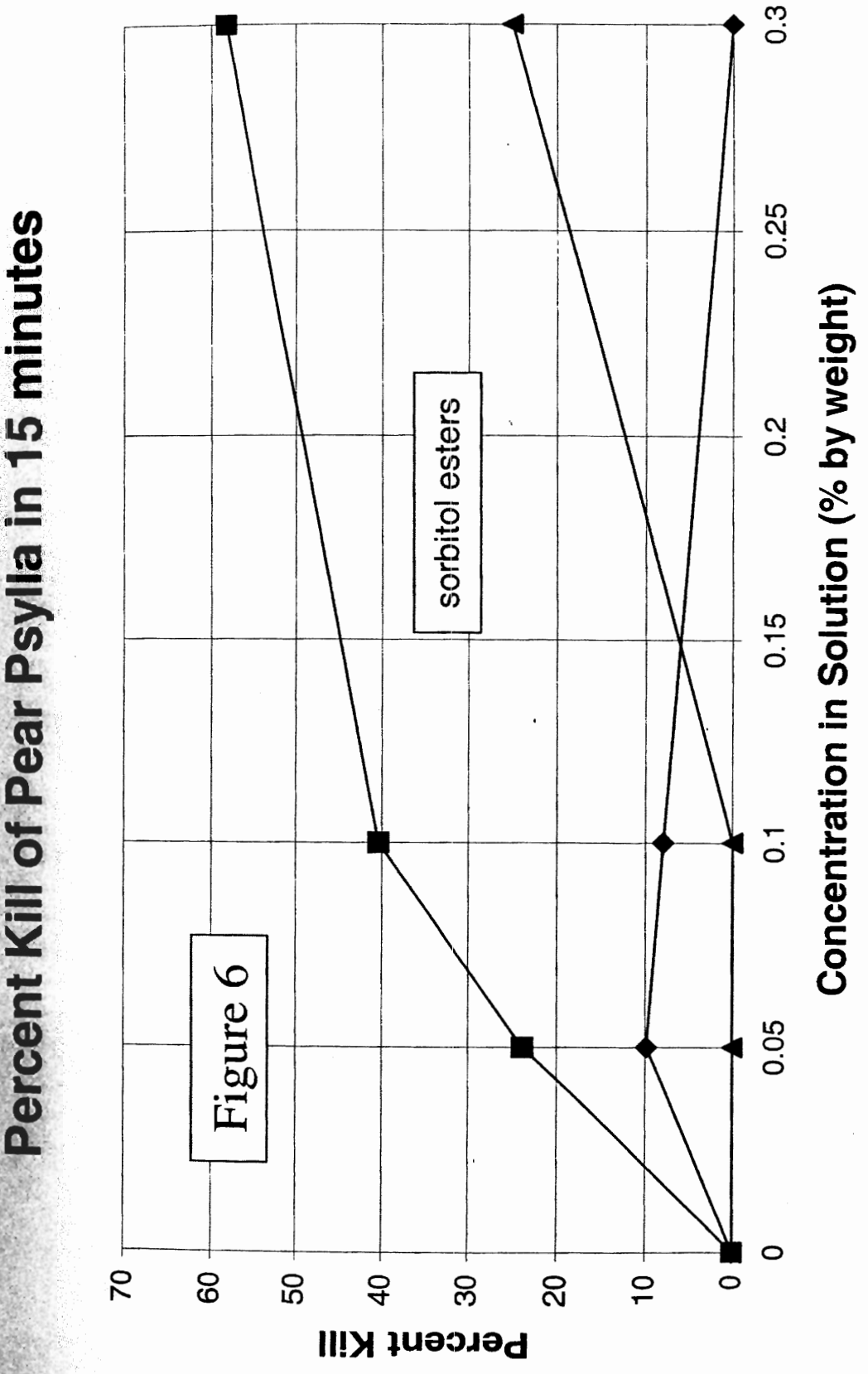

Percent Kill of Pear Psylla in 15 minutes
Percent Kill
0
10
20
30
40
50
60
70
0
0.05
0.1
0.15
0.2
0.25
0.3
Concentration in Solution (% by weight)
C6
C8
C10
sorbitol esters

Figure 6

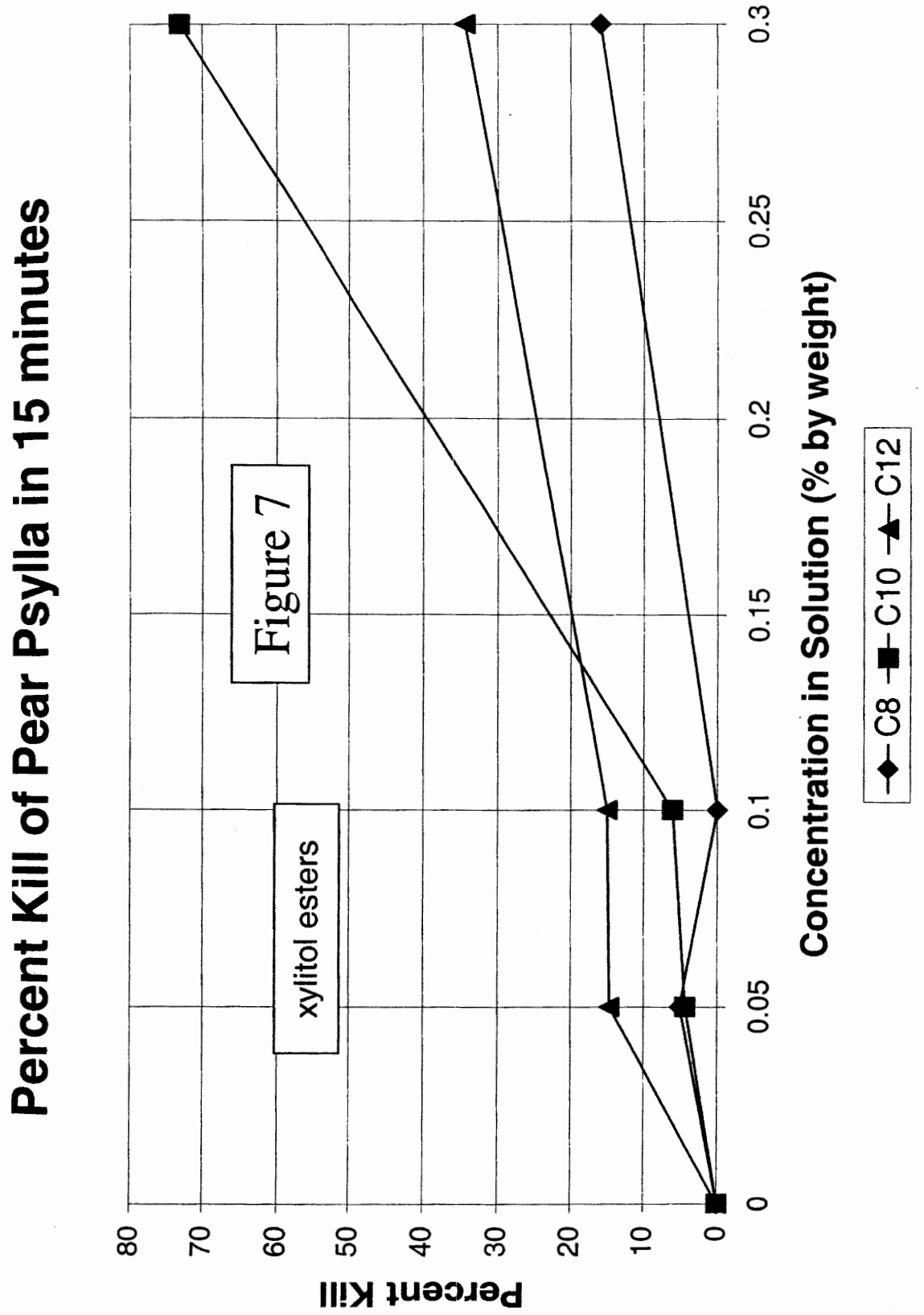

Percent Kill of Pear Psylla in 15 minutes
Percent Kill
0
10
20
30
40
50
60
70
80
0
0.05
0.1
0.15
0.2
0.25
0.3
Concentration in Solution (% by weight)
C8
C10
C12
xylitol esters

Figure 7

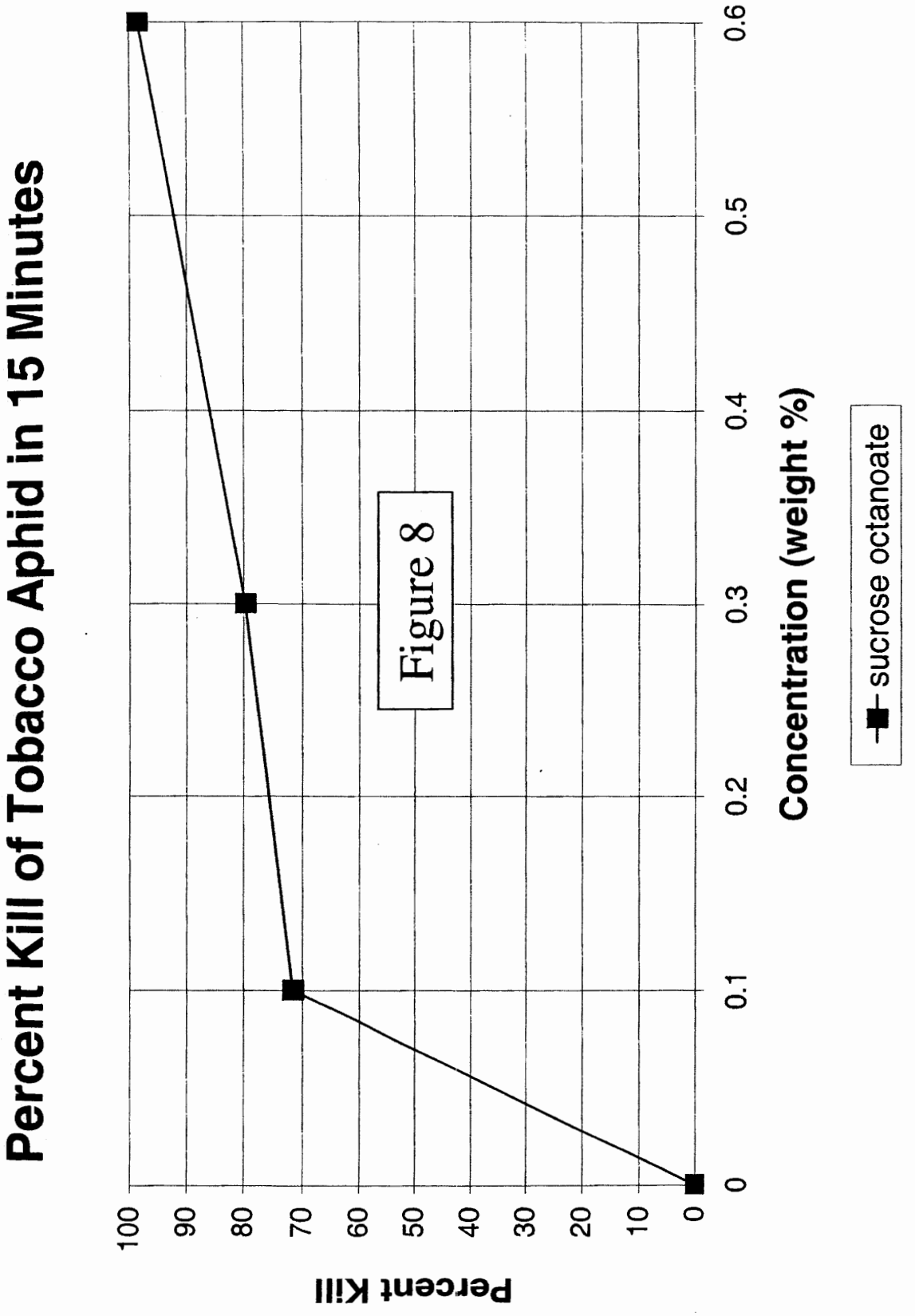

Percent Kill of Tobacco Aphid in 15 Minutes
Percent Kill
100
90
80
70
60
50
40
30
20
10
0
0
0.1
0.2
0.3
0.4
0.5
0.6
Concentration (weight %)
sucrose octanoate

Figure 8

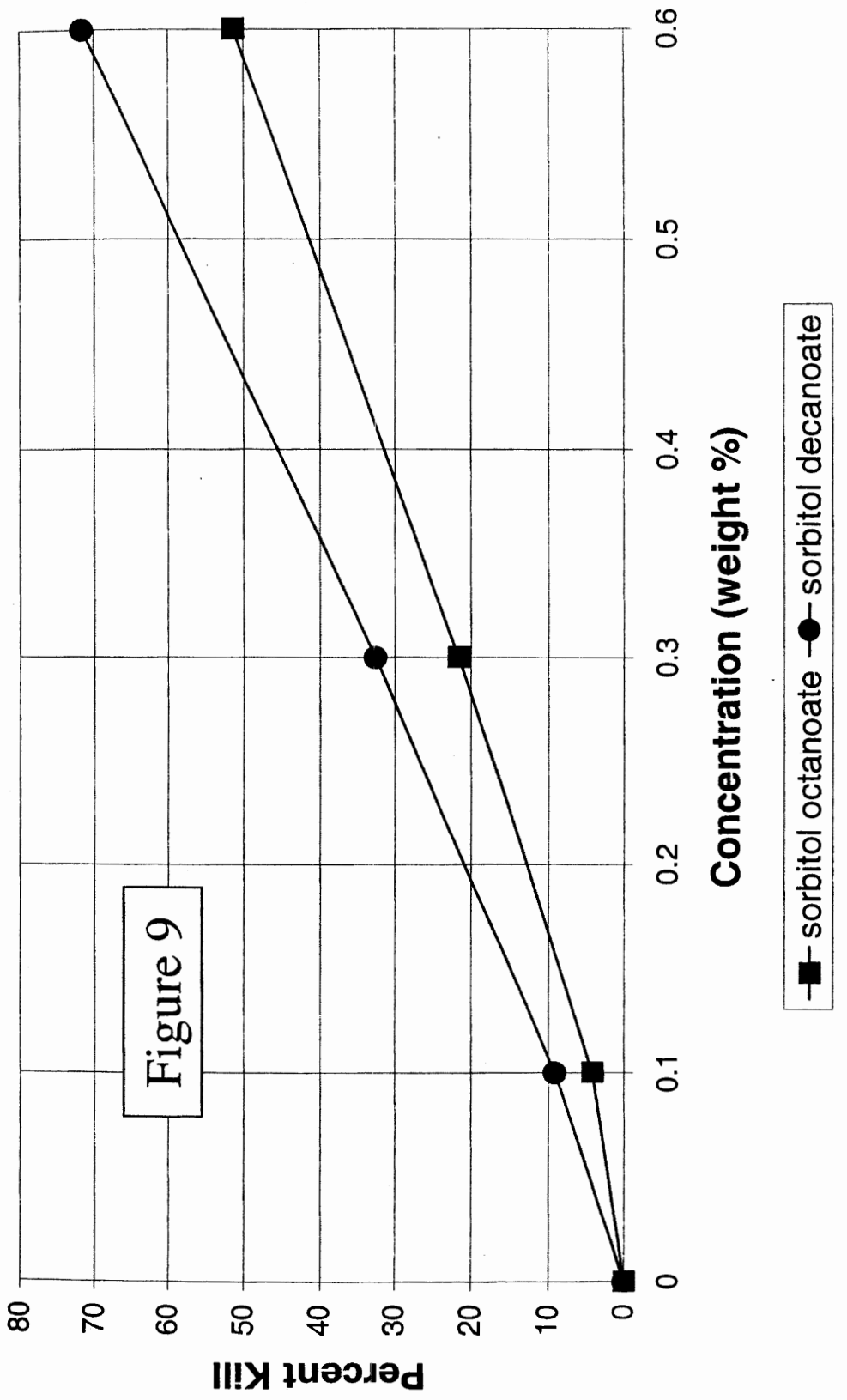

Percent Kill of Tobacco Aphid in 15 Minutes
Percent Kill
0
10
20
30
40
50
60
70
80
0
0.1
0.2
0.3
0.4
0.5
0.6
Concentration (weight %)
sorbitol octanoate
sorbitol decanoate

Figure 9

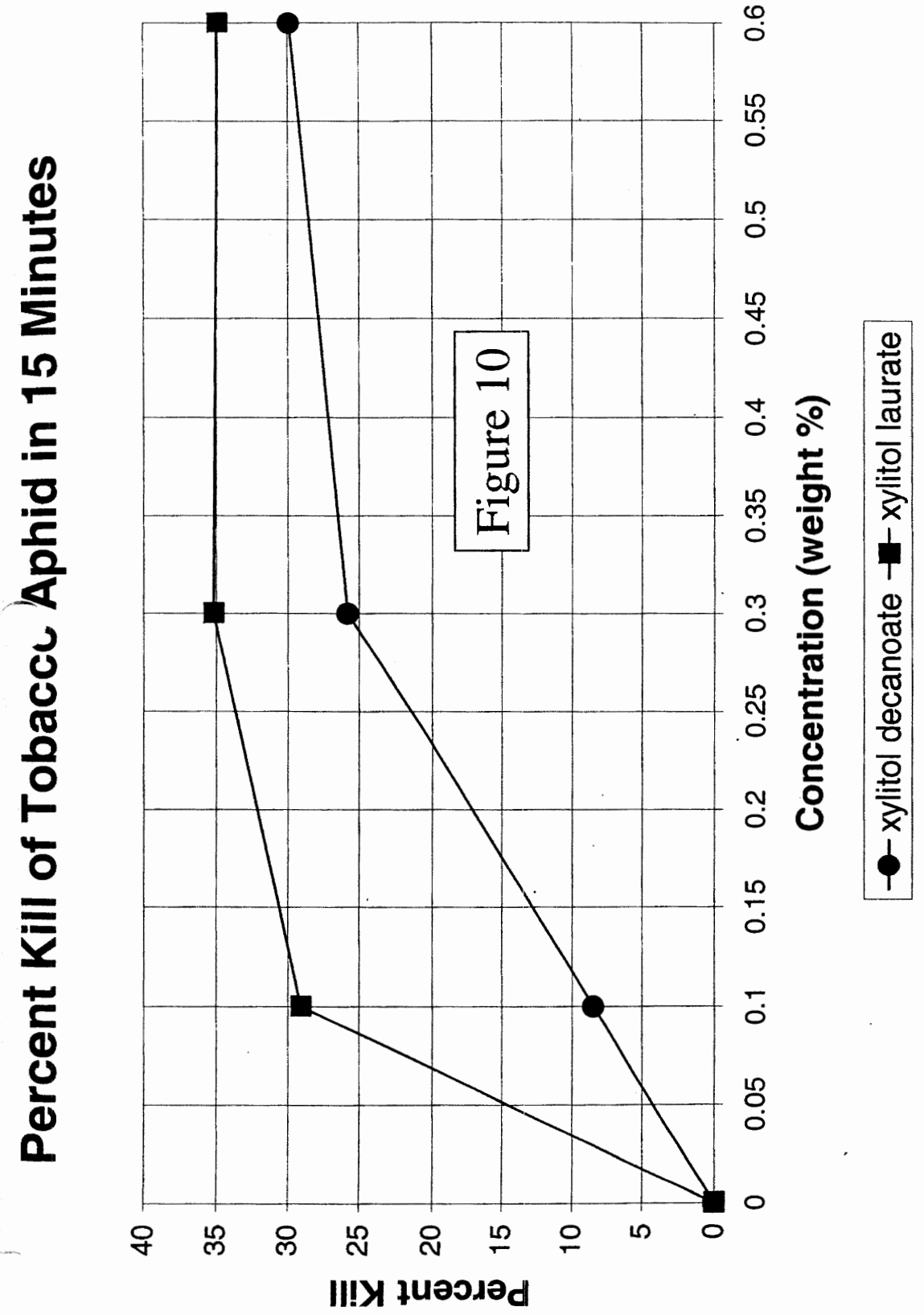

Percent Kill of Tobacco Aphid in 15 Minutes
Percent Kill
0
5
10
15
20
25
30
35
40
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5
0.55
0.6
Concentration (weight %)
xylitol decanoate
xylitol laurate

Figure 10

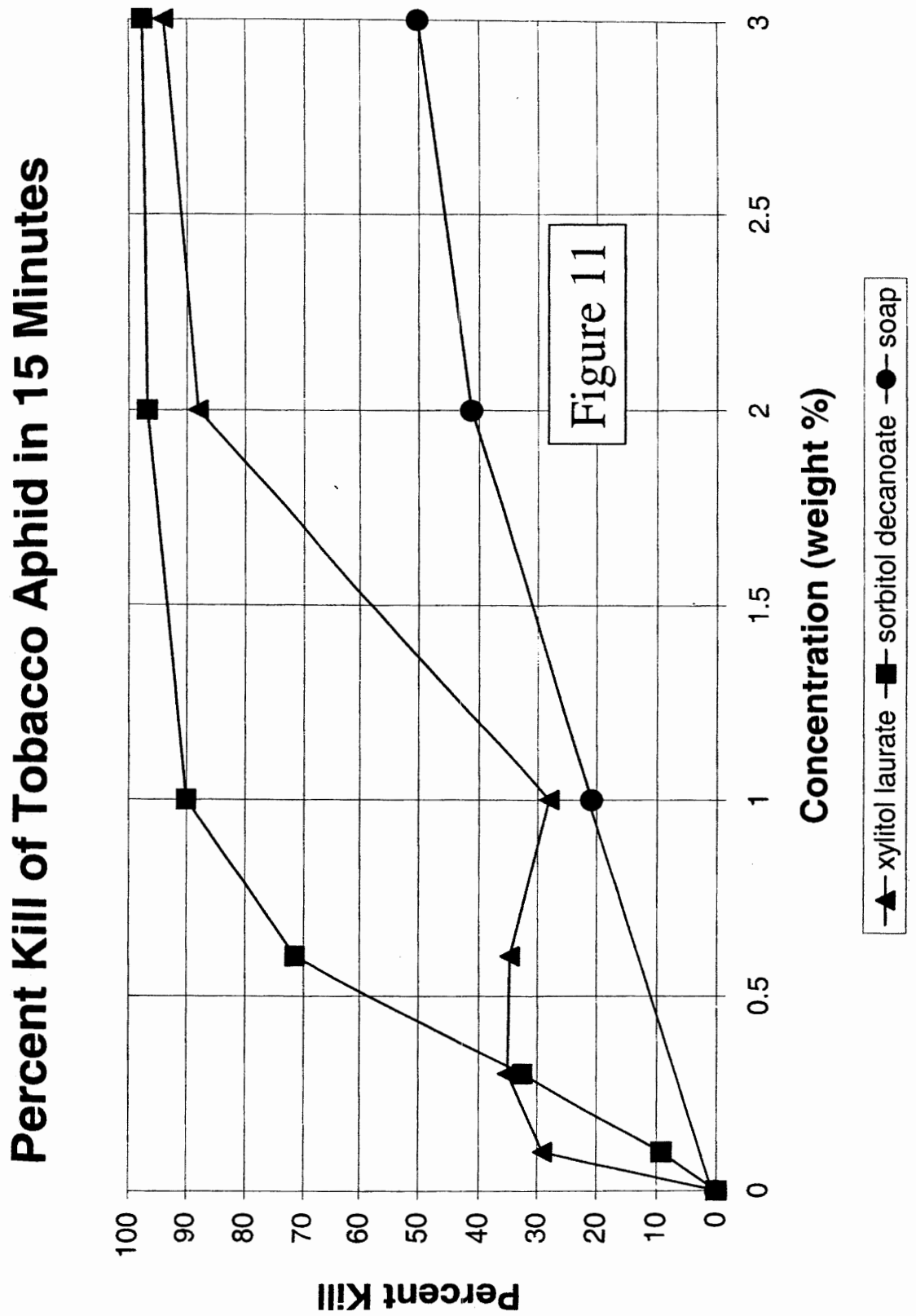

Percent Kill of Tobacco Aphid in 15 Minutes
Percent Kill
100
90
80
70
60
50
40
30
20
10
0
0
0.5
1
1.5
2
2.5
3
Concentration (weight %)
xylitol laurate
sorbitol decanoate
soap

Figure 11

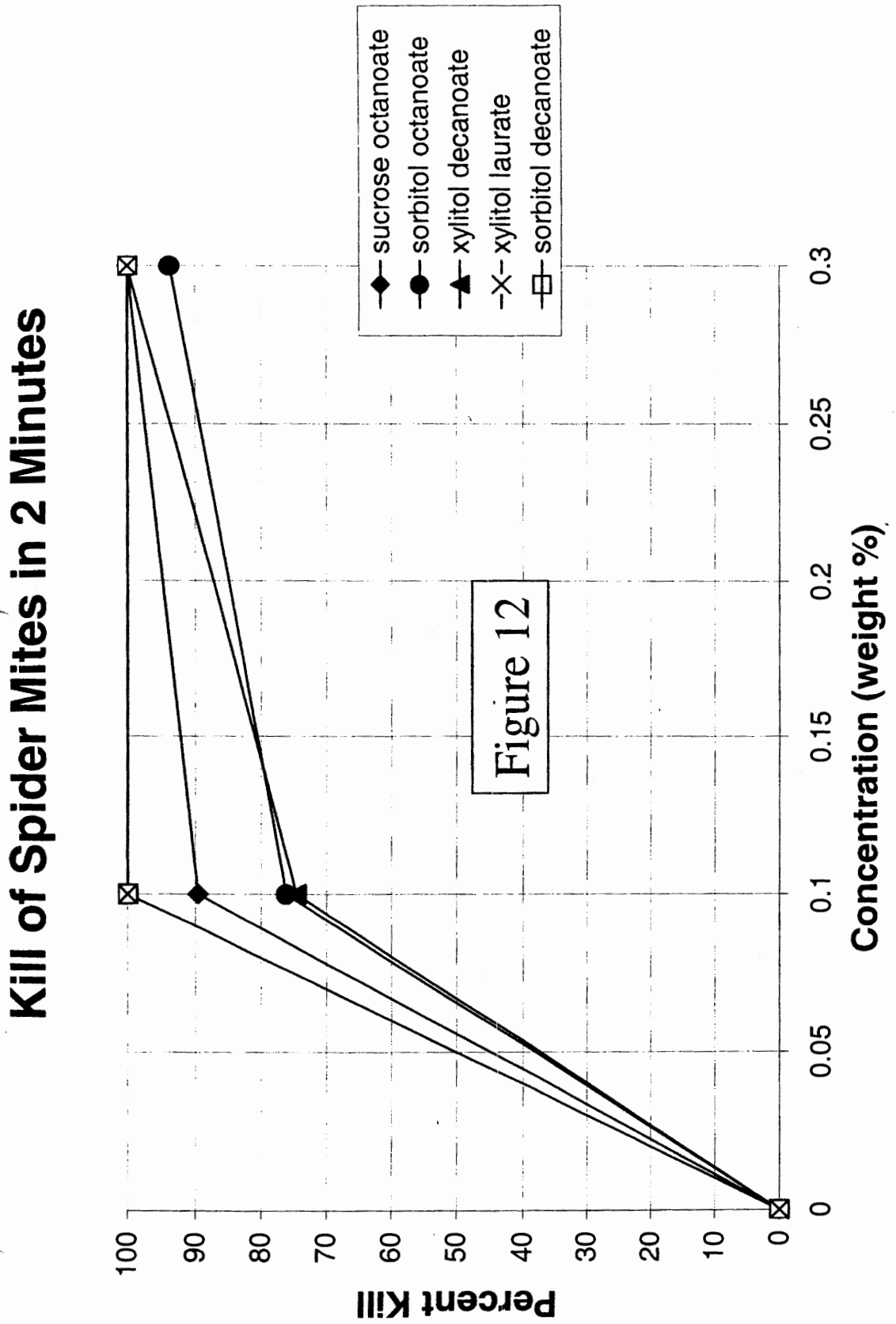

Kill of Spider Mites in 2 Minutes
Percent Kill
100
90
80
70
60
50
40
30
20
10
0
0
0.05
0.1
0.15
0.2
0.25
0.3
Concentration (weight %)
sucrose octanoate
sorbitol octanoate
xylitol decanoate
xylitol laurate
sorbitol decanoate

Figure 12

POLYOL ESTER INSECTICIDES AND METHOD OF SYNTHESIS

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

The U.S. Government has a paid-up license in this invention and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided for by the terms of Grant 99-33610-7466 awarded by U.S. Department of Agriculture.

FIELD OF THE INVENTION

The present invention relates to methods of synthesis of polyol ester insecticides. More particularly, this invention concerns a method of synthesis of sugar esters which ensures that the resulting ester's chemical structure has insecticidal activity.

BACKGROUND OF THE INVENTION

Sucrose octanoate has proven to be a useful insecticide compound. Varieties of sucrose esters are contained in the natural wax of leaves. Discussions of these esters may be found, for example, in Neal, J. W. Jr. et al, J. Econ. Entomol. 87, 1600–1607(1994); Puterka, G. J., et al, J. Econ. Entomol. 88, 615–619(1995), and Lui, T. X. et al, J. Econ. Entomol. 89, 1233–1239 (1996). Sucrose octanoate is contained in the mixture of sucrose esters made when coconut fatty acids are used to make sucrose esters. The sucrose esters are readily biodegradable and hydrolyze to readily metabolizable sucrose and fatty acid. Sucrose esters can be made by the methods disclosed in U.S. Pat. No. 5,756,716, William A. Farone and Robert Serfass, "Method for Production of Sugar Esters", May 26, 1998. Other methods for making these compounds are also known and referenced in this patent.

The efficient production of sucrose octanoate involves several steps, including an esterification, a transesterification and then a purification step. It would be extremely useful to have compounds with similar insecticidal activity, similar environmental acceptability, made from similar natural products, that could be synthesized in fewer steps. Unfortunately there is no means of predicting the chemical structures that will have insecticidal activity. There is no general agreement as to exactly how the sugar ester compounds obtain their insecticidal activity.

One hypothesis is that the compounds like sucrose laurate or sucrose octanoate act as surfactants to dewax the insect's protective coating. The insect then either dehydrates or is readily attacked by microbes. This hypothesis is supported by the observation that the compounds are "contact" insecticides. Since the sucrose esters are constituents of plant leaves, there is another hypothesis that the compounds somehow interfere with the metabolism of the insect to prevent them from eating the tissue that the esters protect. This hypothesis requires ingestion of the material by the insect and cannot be ruled out since "contact" can also result in ingestion.

It is also known that the short chain sucrose esters that are effective as insecticides have certain properties that seem to enhance that activity. Chortyk and co-workers at the United States Department of Agriculture [see Chortyk, O. T., Pomonis, J. G., and Johnson, A. W., J. Agric. Food Chem., 44, 1551–1557 (1996)] concluded that the sucrose esters with fatty acid chain lengths below 12 were more effective especially when there were 2 or 3 side chains on the sucrose.

The fact that there are eight hydroxyl groups that can be esterified in sucrose means that, in principal, one can make 8 sucrose monoester, 28 diester and 56 triester isomers. It is unpredictable if all esters of one type (e.g. monoesters, diesters, etc.) are equally effective. Molecular orbital calculations performed in the inventors' laboratory suggest that not all esters are equally likely to be produced during synthesis.

SUMMARY OF THE INVENTION

In one aspect the present invention relates to a new environmentally friendly method of synthesis of polyol esters. The inventors found that the synthesis method is important in defining the distribution of isomers in complex molecules with the subsequent result that one must either specify the exact nature of the isomers involved and/or the method of synthesis as a mean of selecting the best insecticides.

More particularly another aspect of this instant invention is the use of these esters as safe effective insecticides. The inventors found the surprising and unexpected result that octanoic acid (C8) sorbitol esters are more effective as insecticides, and that the decanoic acid (C10) acid esters are the most effective for xylitol.

Also there was an unexpected finding that for sucrose octanoate the monoesters were more effective as insecticides than the diesters and triesters. This finding is in contradiction to the finding of Chortyk who concluded that sucrose esters with fatty acid chain lengths below 12 were more effective especially when there were 2 or 3 side chains on the sucrose. Additionally Chortyk synthesized his esters through the use of a multi-step process using acid chlorides which generated hazardous by-products. His method of synthesis generated primarily diesters and triesters while the method of U.S. Pat. No. 5,756,716 optimally can generate a high percentage of monoester.

DETAILED DESCRIPTION OF THE INVENTION

The method of preparation of the polyol esters, in particular sorbitol and xylitol, of this invention is best explained in terms of 7 steps. One of the objects of the preparation method is have an environmentally acceptable synthesis that produces no toxic by-products. Another object is to develop a method that allows the entire range of esters to be prepared using essentially the same procedure thus allowing mixed esters to be produced or the same production facility to be used to make esters that could be targeted against specific insects. Without limiting the scope of this invention as expressed by the claims which follow, the synthesis steps will be discussed briefly.

The process is basically as follows:

1. The desired organic acid (e.g. octanoic, deconoic, but not limited to these) is charged to the reactor at a temperature sufficiently high to keep it in liquid form.
2. The polyol (e.g. either xylitol or sorbitol) is added in an amount that would allow the production of the monoester stoichiometrically plus an additional 10% to drive the reaction essentially to completion.
3. An esterification catalyst is added. Any usual catalyst can be used such as sulfuric acid or phosphoric acid. Phosphoric acid is the preferred embodiment in this case since neutralization at the completion of the reaction provides a phosphate salt that can either be left in the product (since phosphorous is an essential plant

nutrient and phosphates are a known method of providing phosphorus) or removed by filtration if desired (whereupon the salt can be sold separately for fertilizer use).

4. The reactor is held at a temperature sufficiently high along with a pressure sufficiently low to allow water to be removed as the esterification reaction proceeds. For most of the esters a temperature around 150° C. and atmospheric pressure was used.
5. The reaction is allowed to proceed until the remaining organic acid reaches a low equilibrium value. This point can be determined very simply by monitoring the free acid content of the reaction mixture and comparing differing reaction times (see Example 1 and 2). When the free organic acid is reduced no further the reaction is essentially completed. The equilibrium value in weight percent depends on the molecular weight of the organic acid and the structure of the isomers formed. Once determined for a particular organic acid and polyol combination it can be used as a measure of reaction completion.
6. At the completion of the reaction (approximately 18–30 hours for the esters synthesized for the insecticidal studies) the solution is neutralized with an amount of base that is sufficient to neutralize all of the mineral acid used as a catalyst plus bring the solution to a desired pH for subsequent use. If calcium hydroxide is used as the base, calcium phosphate can be filtered out of the product. Other bases could be used depending on the desired nature of the final product. This procedure was followed to allow for a product of good water solubility with little or no residual fine solid particles.
7. The product (filtrate from Step 6) is analyzed and is ready for use.

This procedure of this present invention is deliberately made deceptively simple. Due to the fact that the insecticide nature as well as other properties of these materials change depending on the isomers it is desired to have a simple process that can be repeated with little difficulty. The only "waste product" of the reaction is the water removed during the esterification. The equipment and reaction conditions are selected in such a manner that the tendency of any of the organic acid to distill over with the water is thwarted by the use of appropriate reflux allowing the water to be removed and the acid to fall back into the reactor. Thus, in the preferred method a distillation column (tray or packed column) is used over the reactor to insure retention of the acids.

Sucrose octanoate is synthesized by the method described in U.S. Pat. No. 5,756,716, incorporated herein by reference. The resultant product, sucrose octanoate, is found to have monoesters that are more effective as insecticides than the diesters and triesters of sucrose octanoate. FIG. **1** shows the results on pear psylla for the sucrose octanoate with high monoester content prepared by the referenced method compared to the Chortyk esters, the high di and triesters. This finding is in contradiction to the finding of Chortyk. The inventors of the above described process find the method of synthesis is important in defining the distribution of isomers in complex molecules with the subsequent result that one must either specify the exact nature of the isomers involved and/or the method of synthesis as a mean of selecting the best insecticides.

To study the chemical differences between the sucrose octanoate synthesized by Chortyk and the inventors' material, the products were subjected to thin layer chromatography under identical conditions, side by side, on the same plate. The chromatograms obtained by digitization of the plates are shown in FIGS. **2–4**. A chromatogram of a commercial sucrose stearate product, obtained by the same method, is shown in FIG. **5**. Although the commercial sucrose stearate product shown in FIG. **5** does not have insecticidal activity it is shown as an independent example of the nature of a product that is high in monoester. In this analytical methodology the higher the number of free hydroxyl groups in the molecule the tighter it is bound to the silica substrate. Thus the monoesters move more slowly and appear near the origin on the chromatogram while the diesters move fast and the triesters faster, thus the di- and triesters are further from the origin on the chromatogram. The result is separation of the various isomer fractions.

FIG. **2** shows a chromatogram of the esters made by Chortyk. Note that there are many peaks resulting from the higher isomers. FIGS. **3** and **4** show two different batches of sucrose octanoate made by the method of U.S. Pat. No. 5,756,716. There are fewer peaks with lesser area than the same sucrose octanoate ester made by the acid chloride route. The commercial product is shown in FIG. **5**.

The thin layer chromatography technique is very reproducible as to the extent and number of "bands" that characterize the different isomers in the product. With liquid chromatography the individual isomer "peaks" tend to merge and overlap making resolution difficult. With the advent of computer scanners to convert the chromatogram into digital form, the thin layer technique rivals or exceeds liquid chromatographic techniques. The use of computer digitization is an improvement on the older densitometry techniques used with thin layer and gel chromatography.

In the sucrose studies the octanoate was found to be the approximately optimal chain length. Octanoic acid is a reasonably abundant fatty acid fraction of natural oils (e.g. coconut oil) after the oil is "split", i.e. hydrolyzed to glycerol and fatty acids. Nature prefers even chain fatty acids. Although the odd chain fatty acids are also likely to be reasonably effective it is the inventors' purpose to make the biodegradation products as natural as possible. It is well known that the long chain fatty acid esters of sucrose (e.g sucrose stearate) are extremely mild materials with excellen surfactant properties. These materials have been used as food emulsifiers for many years.

Finding useful analogs of sucrose octanoate is a matter of trial and error. The chemical structure can be suggestive however. The objective was to find materials that:

1. Are simpler to synthesize
2. Would have environmental fates similar to sugar and fatty acid esters
3. Would have similar insecticidal activity when used either alone or in combination.

A wide variety of compounds were synthesized. The compounds that were proven to have the best activity when compared to sucrose octanoate are sorbitol and xylitol esters of short chain fatty acids, particularly the octanoic and decanoic acid monoesters. These compounds are more easily prepared than the sucrose octanoate. They can be synthesized directly from the raw materials in a single step using only a neutralizable mineral acid as a catalyst in the process described earlier. Due to the greater ease of synthesis these materials could be less expensive even if they are slightly less effective than sucrose octanoate.

Surprisingly, there is no one chemical structure that was shown to be optimally effective for all the insects tested for the sorbitol and xylitol esters, This differs from the sucrose esters where not only is the octanoate the most effective but the distribution of isomers toward the monoesters als

ne[illegible]perior insecticide. It was found that the optimum in [illegible] for the other sugar esters differs depending on insect species. This finding seems to support a mecha- n wherein the compound is not directly toxic but rather ches some property of the insect's covering materials, provides support for the "dewaxing" hypothesis. The ering chemical make-up of the insect would be optimally ched to different "solvent" compounds.

able 1 provides a listing of the compounds of interest. of them have been reported at sometime in the past and been assigned CAS Registry numbers while three ear to be new to the literature since no CAS registry ber was found.

TABLE 1

CAS Registry Number	CA Index Name	Other Name
0809-54-6	D-Glucitol, hexanoate	Sorbitol caproate
08175-15-1	D-Glucitol, octanoate	Sorbitol octanoate
08175-14-0	D-Glucitol, decanoate	Sorbitol decanoate
3637-07-3	D-Glucitol, dodecanoate	Sorbitol laurate
	Xylitol hexanaote	Xylitol caproate
	Xylitol octanoate	Xylitol caprylate
11876-92-5	Xylitol decanoate	
	Xylitol dodecanoate	Xylitol laurate

e chain lengths were systematically varied to determine chain length was critical as to the effectiveness as ticides. The compounds were tested against pear psylla, co hornworms, tobacco aphids and two-spotted spider

G. [illegible]ws the kill data for the sorbitol esters on pear [illegible] clear that the C8 acid version is the most ive. For the xylitol data in FIG. **7**, however it appears he C10 acid ester is the most effective. This is a very ising and unexpected result because the shift in the size drophobic portion of the molecule for the xylitol esters ot kept in pace with the shift in the size of the philic portion of the molecule.

the activity was explained by a simple surfactant e, one would expect that a certain hydrophile-lipophile ce (HLB) would hold. When the size of the hydrophile ases as it has in going from sorbitol to xylitol one l expect the size of the lipophile to also decrease to ain the same HLB relationship. A discussion of the nship of the molecular structure to the HLB is given thur W. Adamson, "Physical Chemistry of Surfaces", Edition, John Wiley, 1976, pp. 505–507.

. **8** shows the effectiveness of sucrose octanoate made methods of U.S. Pat. No. 5,756,716 against tobacco FIG. **9** shows that sorbitol octanoate is more effective orbitol decanoate against tobacco aphid. Note that l decanoate could still be a commercially viable t if the cost of decanoic acid was less than octanoic y an amount that would compensate for the increased eded to maintain equal effectiveness. The simplicity of thod of preparation of the sorbitol and xylitol esters e fact that the method is essentially the same for all of rbitol and xylitol esters makes the economic choice ally only dependant upon the raw material costs.

. **10** shows that xylitol laurate is more effective than [illegible]oate against the tobacco aphid. This is in st case with pear psylla where the decanoate was ffective.

. **11** shows that sorbitol decanoate is more effective ylitol laurate against the tobacco aphid. Of greater ance, however, is the fact that both of these materials are better than a commercially available insecticidal soap product (M-Pede, Mycogen Corp., San Diego, Calif.). FIG. **12** compares several of these materials for use against the two-spotted spider mite and in this case the sorbitol decanoate is marginally more effective than the others including the sucrose octanoate.

The following are examples of making polyol esters according to the present invention. Other polyol esters as discussed above may also be made using the process of this invention.

Examples 1 and 2 were run to compare different times for the degree of conversion. This type of benchmark reaction can be performed to determine optimal conditions for other polyol esters.

EXAMPLE 1

Preparation of sorbitol octanoate: 432.44 grams of octanoic acid was put into a 2-liter round bottom, three-neck flask with a short distillation head. A mechanical stirrer was connected and 598.51 grams of sorbitol was slowly added. The catalyst in the reaction was phosphoric acid and 30.93 grams were added. A temperature controller and heating mantle were attached and the temperature of the reaction was set at 150° C. The reaction was stopped after 21 hours. The phosphoric was neutralized with 26.02 grams of calcium hydroxide. The solution was filtered to remove the calcium phosphate precipitate. The density of the product was 1.4 g/cc and the free acid was 6.40%. The degree of reaction completion was thus about 85.5%

EXAMPLE 2

Preparation of sorbitol octanoate again. 438.52 grams of octanoic acid, 600.10 grams of sorbitol and 32.122 grams of phosphoric acid was placed into a round bottom flask with a mechanical stirrer and short distillation head attached. The temperature was set at 150° C. The reaction proceeded until the free acid value was 3.88%. Total reaction time was 28 hours. The phosphoric acid was neutralized with 27.015 grams of calcium hydroxide. The product was analyzed and the density was 1.4 g/cc and the ash was 2.33%. The degree of reaction is thus 91.3%

EXAMPLE 3

Preparation of xylitol octanoate: 417.0 grams of octanoic acid was poured into a 2-liter round bottom flask with a short distillation head. A mechanical stirrer and short distillation head were attached and a heating mantle and a temperature controller was used to maintain temperature at approximately 150° C. 462.48 grams of xylitol was added slowly along with 32.422 grams of 75% phosphoric acid.

EXAMPLE 4

Sorbitol caproate was prepared by adding 348.48 grams of caproic acid and 546.51 grams of sorbitol to a 2-liter round bottom flask with a mechanical stirrer, heating mantle and temperature controller. Phosphoric acid was used as the catalyst and 39.12 grams of 75% phosphoric acid was added to the reaction. The temperature was set for 150° C. and the pressure was atmospheric pressure. The total reaction time was 24 hours. After 24 hours the phosphoric acid was neutralized with 32.90 grams of calcium hydroxide. The solution was filtered to remove the calcium phosphate precipitate. The final product was analyzed and the free acid remaining in the product was 3.97%, the density was 1.8 g/cc and the ash value was 1.42%. The degree of reaction completion was thus 89.3%.

EXAMPLE 5

Sorbitol decanoate was prepared by adding 380.80 grams of sorbitol and 302.0 grams of decanoic acid to a one-liter round bottom flask. 27.14 grams of 75% phosphoric acid were added. The agitator was turned on and the temperature was set to 150° C. The total reaction time was seven hours and ten minutes. The phosphoric acid was neutralized with 22.83 grams of calcium hydroxide and the solution was filtered to remove the calcium phosphate. The final product was analyzed and the free acid remaining was 7.50%. The density was 1.05 g/cc and the ash value was 1.00%. The degree of reaction completion was thus 76.0%.

EXAMPLE 6

Xylitol laurate was prepared by adding 466.8 grams of xylitol to 420.0 grams of lauric acid in a two liter round bottom flask. A mechanical stirrer, heating mantle and temperature controller were attached. Phosphoric acid was used as the catalyst and 35.25 grams of 75% acid was added. The temperature was set to 150° C. and the total reaction time was nine hours. At the end of nine hours the phosphoric acid was neutralized with 29.65 grams of calcium hydroxide and the solution was filtered to remove the calcium phosphate that formed upon neutralization. The xylitol laurate was analyzed and had 11.0% free acid remaining and the ash value was only 0.60%. The degree of reaction completion was thus 77.5%.

EXAMPLE 7

Sorbitol laurate was prepared in a similar manner as xylitol laurate. In this case, 600.76 grams of lauric acid and 776.01 grams of sorbitol were added to a round bottom flask. Phosphoric acid was used as the catalyst and 54.73 grams of 75% acid was added. A mechanical stirrer was used to provide the agitation and a heating mantle and temperature controller were used to control the temperature. The temperature of the reaction was set at 160° C. The reaction was stopped after 29.5 hours and the free fatty acid was high at 12.0%. The solution was neutralized with calcium hydroxide and filtered to remove the calcium phosphate precipitate. No other analysis was done on this product. The degree of reaction completion was thus 73.2%.

EXAMPLE 8

Xylitol decanoate was prepared. 602.5 grams of xylitol were placed in a two liter round bottom flask with a mechanical stirrer and heating mantle and temperature controller attached. 517.08 grams of decanoic acid were added to the flask. Phosphoric acid was used as the catalyst and 44.52 grams of 75% phosphoric acid was added. The reaction proceeded for 13 hours and 30 minutes. At the end of the reaction the phosphoric acid was neutralized with calcium hydroxide and the solution was filtered to remove the calcium phosphate precipitates. The free acid was measured and the value was 6.9%. The degree of reaction completion was thus 85.7%.

We claim:

1. A method for treating plants to reduce or eliminate insect pests utilizing monoester of an organic acid with a five to twelve carbon polyol consisting essentially of high monoester concentration obtained by the process comprising:

(a) selecting an organic acid with six to twelve carbons;

(b) selecting a polyol with five to twelve carbons;

(c) by reacting said polyol by esterification with the organic acid or transesterification with the organic acid ester,

(d) by adding said polyol in a quantity that produces a monoester stoichiometrically plus approximately an additional 10%;

(e) adding an esterification or transesterification catalyst;

(f) maintaining said temperature and pressure in said reactor to remove water until reaction is essentially complete;

(g) neutralizing solution with a base and bringing said solution to desired pH; and

(h) filtering said solution to remove solid particles;

wherein:

the monoester is sucrose octanoate, sorbitol octanoate, sorbitol decanoate, xylitol decanoate, or xylitol laurate.

* * * * *

ATTACHMENT 4

CBI CLAIM

Applied Power Concepts, Inc.
411 East Julianna Street
Anaheim, CA 92801

TEL (714) 502-1150
FAX (714) 502-2450

November 21, 2003

To: Tony Barrington

cc: Tracy Palmer
John Dvorak

CONFIDENTIAL

From: William A. Farone, Ph.D.

Subject: Degradation of Sorbitol Octanoate

Summary:

Sorbitol octanoate degradation in water systems was studied both by bacterial and enzymatic pathways. The rates of degradation and products were compared with the commonly available food and cosmetic ingredient sorbitan monoloaurate.

Sorbitol octanoate hydrolyzes quickly to sorbitol and octanoic acid which are utilized very quickly by soil bacteria. The biodegradation occurs more quickly for sorbitol octanoate than for sorbitan monolaurate.

Differences between sorbitol and sorbitan esters are based on the reactions conditions under which they are prepared. The sorbitan esters have more water removed from the sorbitol structure resulting in a ring structure that is more difficult to hydrolyze.

The findings of this analysis are consistent with those reported in earlier work on the biodegradation of sugar esters. (See, Cooper H. Wayman, "Biodegradation of Synthetic Detergents", Progress in Industrial Microbiology, 1971. Pp. 219-271, in particular pp. 258-261.

Task Background:

We have been asked to provide information on the degradation of the sorbitol octanoate into sorbitol (d-glucitol) and octanoic acid. Previous statements relative to degradation were made based on the chemical similarity to other esters of this same type such as fats and oils, i.e. triglycerides, where glycerol is the polyol. The sorbitol octanoate (SO), like the triglycerides, are not particularly soluble and this is why they make emulsions. In the case of the sorbitol esters they behave as surfactants due to the presence of the hydroxyl groups. Some previous work on lactate esters of sorbitol has also shown these esters to be completely biodegradable (see, for example, W.A. Farone, S.S. Koenigsberg and J. Hughes, "A Chemical Dynamics

Model for CAH Remediation with Polylactate Esters", in "Engineered Approaches for In Situ Bioremdiation of Chlorinated Solvent Contamination", edited by Andrea Leeson and Bruce C. Alleman, Batelle Press, 1999; S.S. Koenigsberg and W.A. Farone, "The Use of Hydrogen Release Compound (HRC™) for CAH Bioremediation", in "Engineered Approaches for In Situ Bioremediation of Chlorinated Solvent Contamination", edited by Andrea Leeson and Bruce C. Alleman, Batelle Press, 1999.)

When these compounds are mixed with water they do not dissolve readily and there are two phases, an "oil" phase that contains the degrading insoluble ester and the water phase that contains the materials that transport into the water. Since the two phases are in the form of an emulsion it is very difficult to separate these phases to measure the chemical content of the phases separately. We therefore undertook to measure the chemicals that were transported into the water phase independent of what is in the oil droplets. Our premise is that degradation products that enter the aqueous phase are the products of interest. In the sorbitol lactate esters studied in the papers cited above the lactate is readily measured and the sorbitol degrades very rapidly such that it is rarely seen in the aqueous phase even though it is highly soluble.

We know of no way to recover the ester from the emulsion to measure it separately to see what products are in that phase. For example, the emulsion will pass through a 0.1 micron filter. Further, the entire kinetics are mediated by the interface between the phases so that concentrations at that interface are the key ones for degradation.

We thus interpreted the task as one that would provide further evidence for the degradation of the sorbitol ester in water over time. The purpose was to provide evidence that degradation occurs and that the sorbitol and fatty acid or their degradation products are liberated. We are also aware that test tube studies of the type performed here are limited in utility because they do not totally mimic the field but are indicative of biodegradation mechanisms. (see, for example, William A. Farone, Stephen S. Koenigsberg, Tracy Palmer and Daniel Brooker, "Site Classification for Bioremediation of Chlorinated Compounds Using Microcosm Studies", The Second International Conference on Remediation of Chlorinated and Recalcitrant Compounds, May 22-25, 2000, published as part of Proceedings.) It is also noted that sorbitol octanoate will be applied in water and the hydrolysis reaction on leaf surfaces and inside plants would also occur in aqueous phase.

Chemical Background:

Like most simple esters the biodegradation of sorbitol octanoate (SO) begins with the hydrolysis of the ester link. In the case of triglycerides (natural fats and oils), for example, this hydrolysis produces three fatty acids and glycerol all of which are further degraded. In the case of sorbitol octanoate we expect to produce sorbitol (or some dehydrated form of sorbitol) and octanoate which are then further degraded. Due to the structural differences the rates for the hydrolysis of sorbitol octanoate are much more rapid than for triglycerides.

The scheme can be shown as follows:

$$SO + H_2O \Rightarrow S + O \quad [1]$$

$$S \Rightarrow \text{assimilated by microbes} \quad [2]$$

$$O \Rightarrow \text{assimilated by microbes} \quad [3]$$

The first step is the production of some form of sorbitol and octanoic acid. The formal degradation of sorbitol octanoate can be expressed by:

$$\frac{d[SO]}{dt} = -k[SO][H_2O] \quad [4]$$

Normally one can proceed by making the assumption that this reaction is in the aqueous phase and, since the concentrations of sorbitol octanoate are small, the concentration of water can be considered constant and combined with the reaction rate constant. Here we run into the first of several complications because the material is insoluble so the reaction [1] takes place at the interface of the "oil" and water phases and we cannot make this usual assumption. Furthermore, we do not know what the critical values of either of these concentrations are in the "reaction zone" which is the interface between the oily sorbitol octanoate phase and the water phase.

The equation for the determination of sorbitol as a function of time is more complicated than the rate for the disappearance of sorbitol octanoate. Equation [1] and [2] above are coupled so the amount of sorbitol in the solution at any time is:

$$\frac{d[S]}{dt} = k[SO][H_2O] - k_D[S] \quad [5]$$

Thus, equations [4] and [5] have to be solved simultaneously. Since the experimental data is only for the aqueous phase and one cannot readily measure the concentrations on the interface between the phases one has to rely on the experimental data for what one measured in the aqueous phase.

The situation is further complicated by the many reactions that sorbitol undergoes. Sorbitol reacts very readily to form "anhydro" sorbitol compounds where water has been lost internally to the sorbitol (dehydration). Sometimes these compounds are referred to as sorbitans. Esters of sorbitol in the anhydro form (sorbitan esters) are favored by reactions in basic conditions (See U.S. Patent 4,297,290 Oct. 27, 1981 issued to ICI Americas Inc. titled "Process for Preparing Sorbitan Esters").

The esters in our case have been prepared under acid conditions and using lower temperatures than usually used for the preparation of these esters. This is accomplished by the use of vacuum for removing the water during esterification. The reaction conditions are quite different than for the production of the sorbitan esters although the degradation is expected to be quite similar. In our case objective is to retain as much of the sorbitol polyhydroxy character as possible due to a desire to "match" the molecule to the properties of sucrose octanoate for insecticidal use. That

is, we wanted to maintain as many of the hydroxyl groups as possible and reduce dehydration. However, during subsequent reactions these compounds may still form.

Sorbitol can potentially form a wide variety of other compounds. For example, several microbes are known to form l-sorbose from sorbitol in 70-80% yield (e.g. *Acetobacter suboxydans* was used as part of the synthesis of ascorbic acid – see "Organic Chemistry" by Louis F. Fieser and Mary Fieser, D.C. Heath and Company, 1956, page 392). Sorbose, in turn, can form cyclic forms via dehydration. Dianhydroglucitol is a compound that is reported to be formed from sorbitol under certain conditions (see "Carbohydrate Building Blocks", Mikael Bols, Wiley-Interscience, 1996, page 13). The general degradation of sorbitol including some of the compounds it forms is discussed in "Biological Chemistry" by Henry R. Mahler and Eugene H. Cordes, Harper & Row, 1966, pp. 459-462. The octanoic acid is assimilated by microbes in the usual manner with the chain gradually being shortened by reaction with co-enzyme A from the carboxyl end two carbons at a time. Thus, the problem we have is that when SO is placed in water and we split the ester the two products react to form other materials very quickly.

The ability to measure the scheme outlined in equations [1] – [3] thus is related to relative rates and the ability to measure the intermediate products in water. We are somewhat assisted in this due to the fact that the fatty acids are for the most part insoluble. Octanoic acid, for example, only dissolves to the level of 68 milligrams in 100 grams of water and thus will be held in the oily phase of the emulsion until the small amount in solution is slowly metabolized. With any of these compounds that have limited access to the dissolved phase, partitioning between the undissolved phase and the dissolved phase continues to cause the compound to be transported from the oil into the water phase as the amount of the compound in solution is degraded and utilized by bacteria or fungi. This mechanism works even for recalcitrant compounds like TCE and has been the subject of the degradation work using sorbitol polylactate esters cited above.

The objective of the following experiments was to show the biodegradability of the material and the reaction scheme discussed above.

Experimental Method:

It is important to note that the material is not highly soluble and form emulsions. Thus the reactions are limited by the amount that is in the aqueous phase at any time. As the amount in the aqueous phase is reacted, more dissolves and in this manner the material is reacted and biodegraded.

Several experiments were performed. Sorbitol octanoate was placed in water and a consortium of naturally-occurring soil-borne microbes were added. The initial <u>total</u> concentration of sorbitol octanoate was 10 g/l. The amount in the aqueous phase is not known but expected to be very small. Reaction is expected to occur at the oil-water emulsion interface. The microbes were soil organisms that have been under culture for various degradation experiments. They were originally obtained from mixed soil samples from various sites in the U.S.

The amount of sorbitol and its reaction products in the solution was determined by liquid chromatography by the same method used for analysis of the finished product. In this method

glycerol is used as an internal standard and the amounts of sorbitol are measured by the peak area and then mathematically related to the known amount of glycerol added based on its peak area. The values of the mathematical relation for various ratios of glycerol and sorbitol have been previously determined during calibration of the system.

In the chromatographic system approximately 1 microliter of the aqueous phase is injected. From 180 seconds to 240 seconds after the injection, the "solvent" peak elutes from the chromatograph and is recorded. From 330 seconds to 380 seconds the glycerol internal standard peak is eluted and from 620 seconds to 700 seconds the sorbitol peak elutes. Molecules that contain hydroxyl groups that are smaller than sorbitol but larger than glycerol will elute approximately between 380 seconds and 620 seconds and those that are smaller than glycerol will either elute between 240 and 330 seconds or be "lost' (i.e. combine with) the solvent peak.

To support the experiments using microbes with the sorbitol octanoate, three other experiments were performed. In one experiment the same amount of sorbitol octanoate was placed in a mixture with lipase enzyme. The purpose of this test is to see whether the reactions of sorbitol octanoate with the bacteria are similar to those with the lipase. It is well known that lipase catalyzes hydrolysis of esters like sorbitol octanoate. The bacteria can be expected to produce the lipase that would help them degrade the esters.

In another experiment the same amount of sorbitol octanoate was subjected to hydrolysis by base. The aqueous phase was buffered to pH 10 where it is expected that the hydrolysis will occur.

Finally, a sample of sorbitan monolaurate, the closest commercial similar product, was studied under the lipase and base catalyzed conditions.

Results and Discussion:

The first experiment performed with actual bacteria showed that the degradation was so rapid that only the degradation by-products could be observed in the LC. These by-products appear at a different location in the chromatogram than either the glycerol or the sorbitol but in the controlled experiments (lipase and base catalysis) they grow gradually in keeping with equations [1]-[3]. In the case of using actual bacteria the results fluctuate since apparently the degradation products in the aqueous phase are consumed to some degree as they are produced.

The degradation products from bacterial metabolism appear predominantly in the region between the solvent peak and the glycerol internal standard implying small molecular weight fragments. However, some increase in the area between the glycerol peak and the sorbitol peak is also seen. In order to identify these degradation products several degradation experiments were performed and will be discussed further below.

The bacterial degradation data is shown in Table 1. These amounts were measured by reference to the glycerol internal standard. All these samples were taken from the same flask of 10 g/liter

sorbitol in water that had been inoculated with 1 ml of a soil bacterial culture. Note that the amounts fluctuate and these fluctuations are well within the capability of the analysis. That is, the fluctuations are most likely doe to the bacteria utilizing the materials. The emulsion itself contains the majority of the undissolved sorbitol octanoate and as time goes on the nature of the emulsion changes. The oily nature of the emulsion increases and globules tend to aggregate. This may be due to the octanoic acid being liberated during the hydrolysis since the amount of octanoate, degrading more slowly, will eventually exceed the solubility of that material due to some free octanoic acid that is present in the base sorbitol octanoate.

Table 1
Aqueous Phase Concentration of Sorbitol Bacterial Degradation Products

Time (Hours)	Weight of Products (mg/L)
0	0
2	11
3	14
6	32.7
23	11.3
25	24.4
27	15
29	29
48	33
50	18
52	22.4
150	9
170	30.4

To provide further support for the data in Table 1 the same experiment was performed but using only lipase rather than live organisms. The lipase was added to the emulsion at the rate of 1 gram per liter. These results are given in Table 2 and Figure 1.

Table 2
Aqueous Phase Concentration of Sorbitol Lipase Degradation Products

Time (hours)	Weight of products (mg/L)
0	0
4	17
22	26
28	43
95	50

Although the lipase does not give a very smooth kinetic curve the values are constantly increasing and they exceed the values from the bacteria. Neither the lipase nor the sorbitol octanoate is soluble so that the rate is dependent on the mixing and surface contact. It is most likely possible to obtain a very smooth curve by trying this experiment at high stirring rates rather than on a static basis.

Again the degradation fragments were measured in both areas of the chromatograms. The degradation products appear to contain more of the larger molecular weight degradation products..

When sorbitol itself is degraded by exposure to heat it becomes tarry and highly viscous, either in the presence of phosphoric acid or without the acid. An aqueous solution of the residue shows degradation products that appear both between the glycerol and sorbitol peaks and the glycerol and solvent peaks. Thus, the heat and acid degradation shows products of both high and low molecular weight.

To further understand this Figure 2 is a chromatogram of the reference solution, i.e. pure sorbitol and pure glycerol. Figure 3 is a chromatogram of sorbitol which has been degraded by heating with acid until it darkened or appeared "burned".

When sorbitan monolaurate is subjected to hydrolysis with base and water for 72 hours the products in the aqueous phase that are seen are located in the same location on the chromatograms as the heat degradation products of sorbitol, that is, there are two main peaks both between sorbitol and glycerol and between the solvent and glycerol. Figure 4 shows the chromatogram of this experiment. It will be seen by comparison with sorbitol octanoate that the amounts of degradation products are not the same for the same period of time, i.e. the sorbitan monolaurate is much less degradable.

Finally, the sorbitol octanoate was base hydrolyzed under similar conditions to the sorbitan monolaurate, that is, for 72 hours at pH 10 in sodium hydroxide solution. These results are shown in Figure 5. The peak heights and areas of both types of degradation products are about half the height and are of the glycerol peak compared to the sorbitan monolaurate case. With sorbitan monolaurate the peaks are only about 10-15% of the glycerol peak. Thus, the sorbitol octanoate is about 3-5 times more degradable under these conditions than the sorbitan product.

Conclusions:

The insecticidal sorbitol octanoate degrades rapidly in water due to hydrolysis of the ester bond. It is more labile than the similar sorbitan esters. Since the fatty acids are quite insoluble the primary chemicals seen in water are the degradation products of sorbitol. Sorbitol itself may exist only at the water – oil interface in the emulsion.

Figure 6 shows a typical chromatogram of the aqueous phase during the bacterial degradation of sorbitol octanoate. There are a higher proportion of lower molecular weight products than of the

larger molecular weight compounds. This is different than in chemical degradation where they are about equal.

These results are consistent with experience for sorbitol and glycerol esters that they degrade quickly in the presence of bacteria in aqueous systems. Due to the limited solubility of these products it is difficult to quantify rates. More definition of the exact products would require radiolabelled tracing along with extensive determination of the products produced on the way to carbon dioxide and water. There is no doubt, however that these compounds are microbially assimilated.

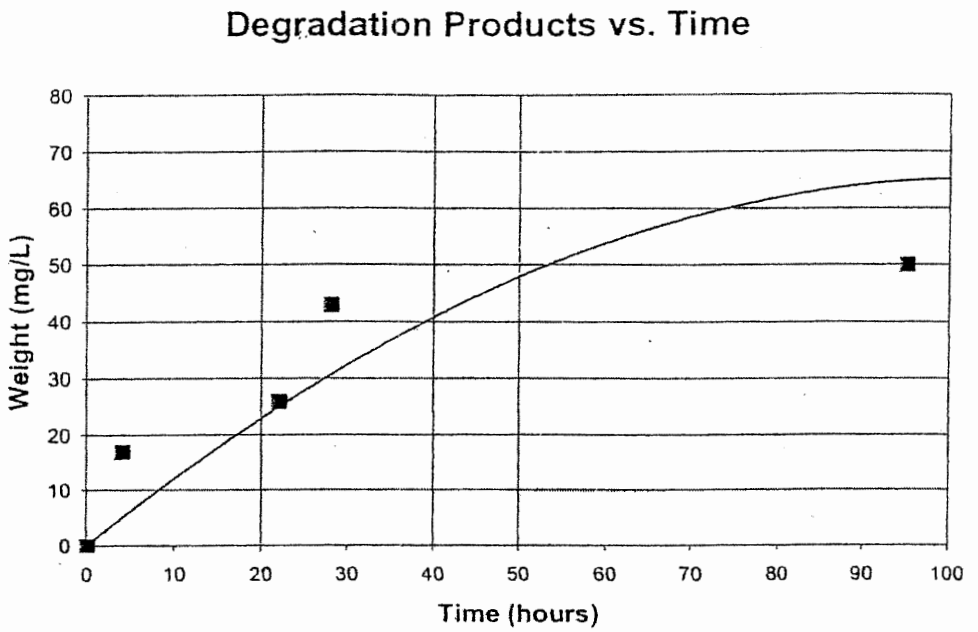


Figure 1. The weight of degradation products of emulsified sorbitol octanoate in the aqueous phase with lipase added to the emulsion.

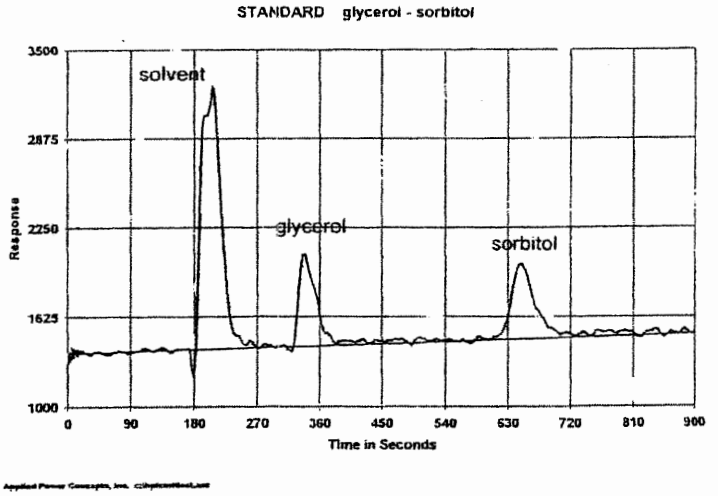


Figure 2. Sorbitol and glycerol standard.

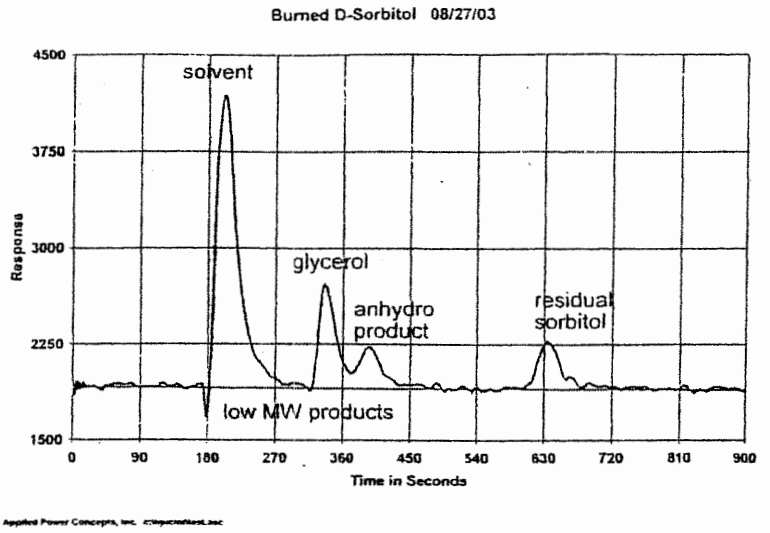


Figure 3. Heated sorbitol. Anhydro products between sorbitol and glycerol, low molecular weight degradation products between solvent peak and glycerol.

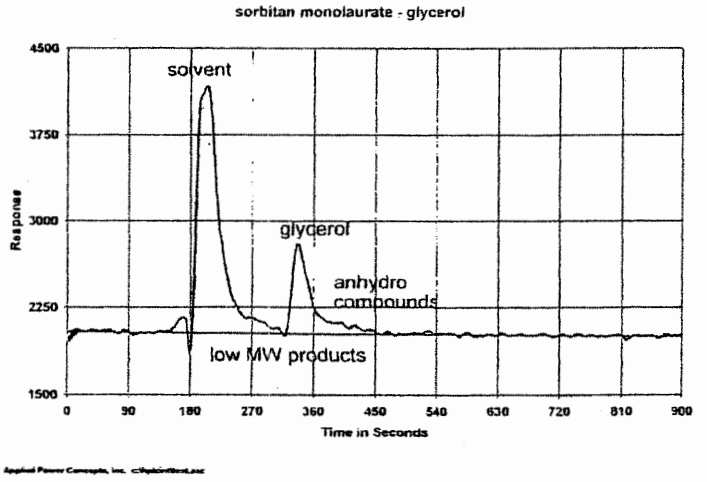


Figure 4. Base hydrolyzed commercial sorbitan monolaurate. Hydrolyzed for 72 hours at pH 10 with sodium hydroxide.

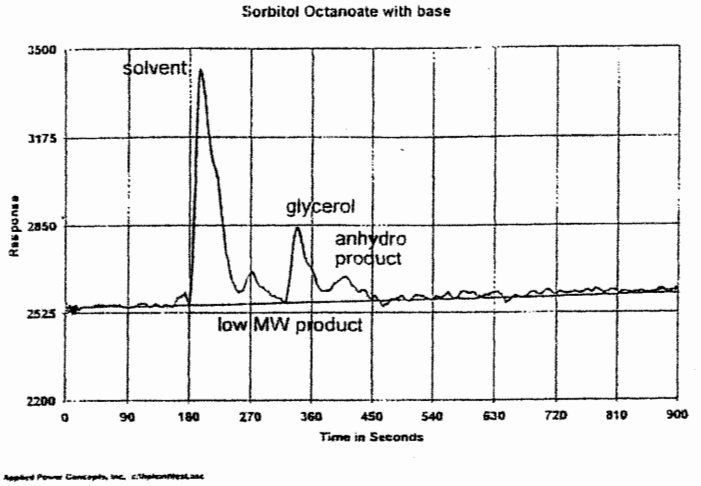


Figure 5. Base catalyzed degradation of sorbitol octanoate. Compare with Figure 4 for sorbitan monolaurate.

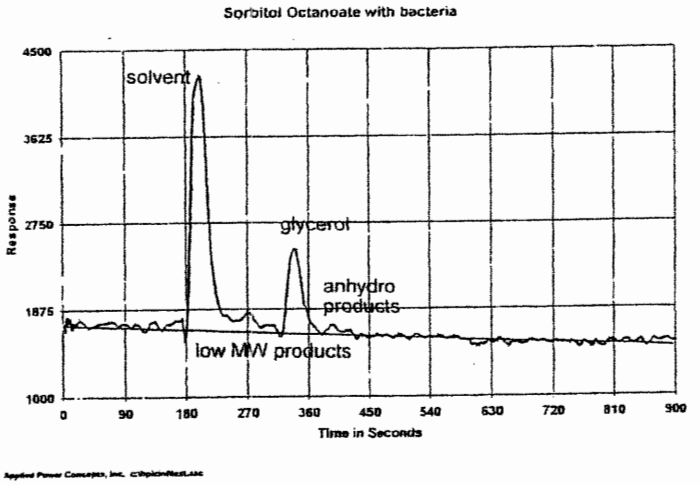


Figure 6. Typical analysis of water phase during bacterial degradation of sorbitol octanoate.

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

BIODEGRADATION OF SYNTHETIC DETERGENTS

COOPER H. WAYMAN, Ph.D.

Department of Chemistry, Colorado School of Mines, Golden, Colorado

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

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:

$$\text{substrate} + O_2 + \text{bacteria} \rightarrow CO_2 + H_2O + \text{nutrients} + \text{cellular mass} \qquad (1)$$

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:

$$RNHCH_2COOH \xrightarrow{HCl} (RNH_2CH_2COOH)^+Cl^-$$

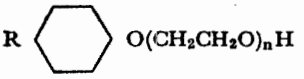
where R is the long-chain alkyl group and

$$RNHCH_2COOH \xrightarrow{NaOH} (RNHCH_2COO)^-Na^+$$

3.2. 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

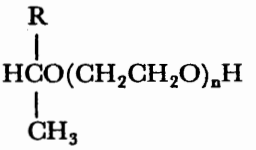


is prepared by the reaction of ethylene oxide (CH_2CH_2O) with an alkylated phenol. Usage of these surfactants is dependent upon the ethylene oxide content.

(b) Polyoxyethylene alcohols

(1) Primary alcohol ethoxylate $RO(CH_2CH_2O)_nH$

(2) Secondary alcohol ethoxylate

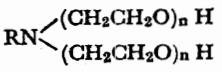


prepared by reacting an alcohol, usually a straight chain type with ethylene oxide. These types of surfactants behave as excellent emulsifiers as a result of their stability in hard water and at varying pH.

(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.

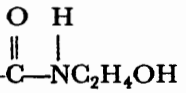
(d) Polyoxyethylene alkylamines



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



(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 rd.

(h) Polyol surfactants

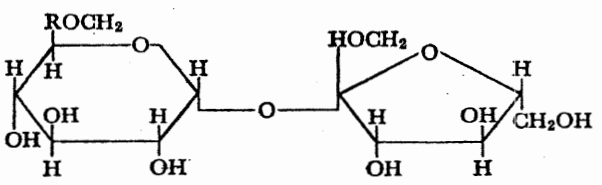
(1) Monoester of ethylene glycol $C_{15}H_{31}COO(CH_2CH_2O)OCC_{15}H_{31}$

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]



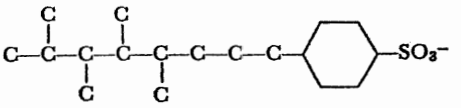
prepared from an alcoholysis reaction of a methyl ester of fatty acid with sucrose employing potassium carbonate as a catalyst. These surfactants could be a good 'bet' as our replacement if third-generation surfactants come into

3.3. Cationic Surfactants

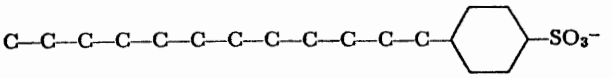
These types ionize in solution with a positive charge. The two major groups are the amines and quaternary ammonium compounds. Because of their germicidal nature in aqueous systems they are not generally biodegradable.

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



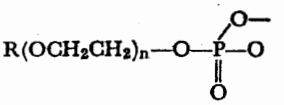
which is believed to be less resistive to biodegradation. Typical compositions of the commercially available packaged variety are:

	%
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)

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:

$$C_6H_{12}O_6 + 1{\cdot}26\ O_2 + 0{\cdot}96\ NH_3 \rightarrow 0{\cdot}96\ C_5H_{11}O_4N + 1{\cdot}20\ CO_2 + 2{\cdot}16\ H_2O \qquad (4)$$

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

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]

$$-k_1 A \Delta F_r = \Delta F_s + B \Delta F_m \tag{13}$$

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

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 osphate.[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

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:

$$X = \frac{Y_s}{1 + a/D} = \frac{X_{max}}{1 + a/D} \tag{21}$$

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 $= \dfrac{\text{cellular mass}}{\text{unit time}}$

$D = f/v = 1/t_r$; $1/t_r$ = mean retention time

e same writer has shown a very interesting relationship between k_1 and the piration 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 ployed to compute growth rates from respiration rates if the endogenous ate and slope are known.

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

$$\ln(X/X_0) = k_1 t \tag{30}$$

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:

$$-1/K\left\{\left(\frac{-K_s}{M_0 + aC_0} - \frac{1}{a}\right)\log e(M_0 + aC_0 - aC) + \left(\frac{K_s}{M_0 + aC_0}\right)\log e\left(\frac{CM_0}{C_0}\right) + \left(\frac{1}{a}\right)\log e M_0\right\} = t \tag{35}$$

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

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 f sucrose appears as second order

$$C_{12}H_{22}O_{11} + H_2O \rightarrow \text{fructose} + \text{glucose} \tag{46}$$

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.

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 wo, 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]

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

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 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/l) 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.

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 mber with overflow into a 2-l sedimentation chamber. Three hours resi- ıce 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 easures only the amount of total biodegradation but not the more desirable haracteristic, the rate. Table 3 lists results of both anionics and nonionics in a cooperative test program.[65]

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.

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

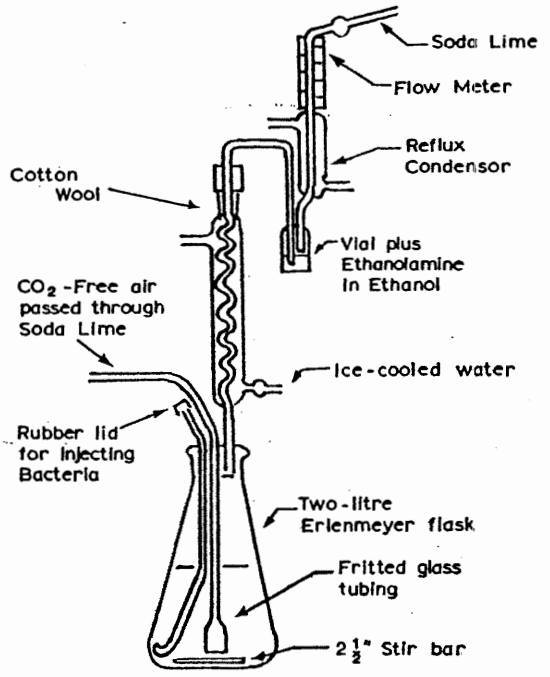


Figure 1

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 ompound is completely biodegradable to the innocuous substances, CO_2 and H_2O. Other methods for measurement of biodegradation merely measure the

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

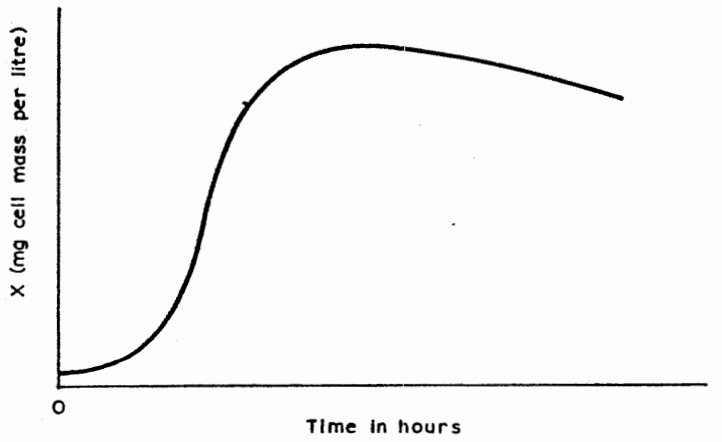


Figure 2

substrate is expressible in terms of an exponential of the form $X_0 e^{BT}$ 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

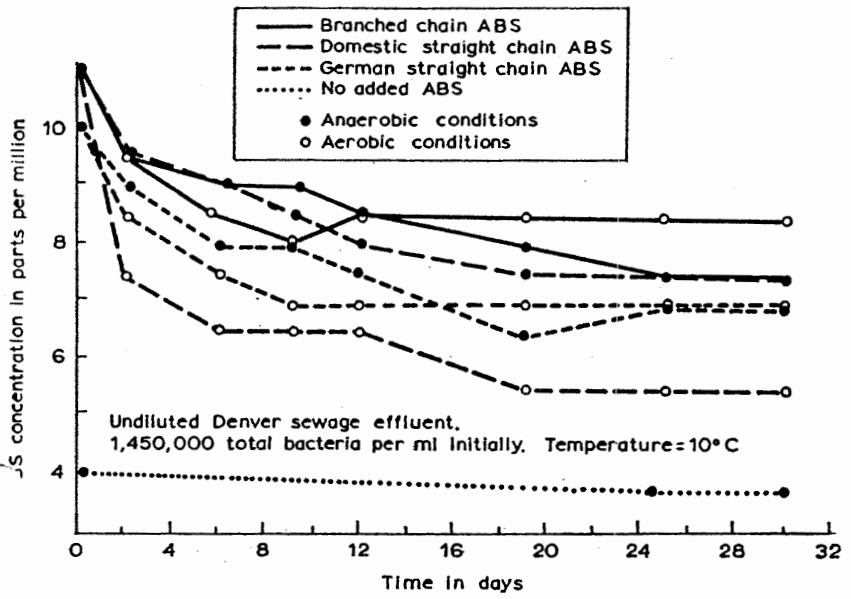


Figure 3

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.

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

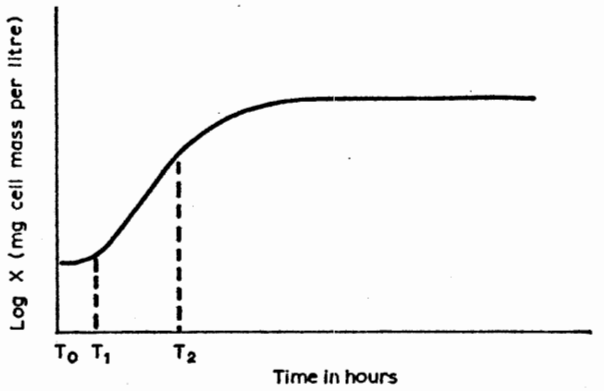


Figure 4

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-

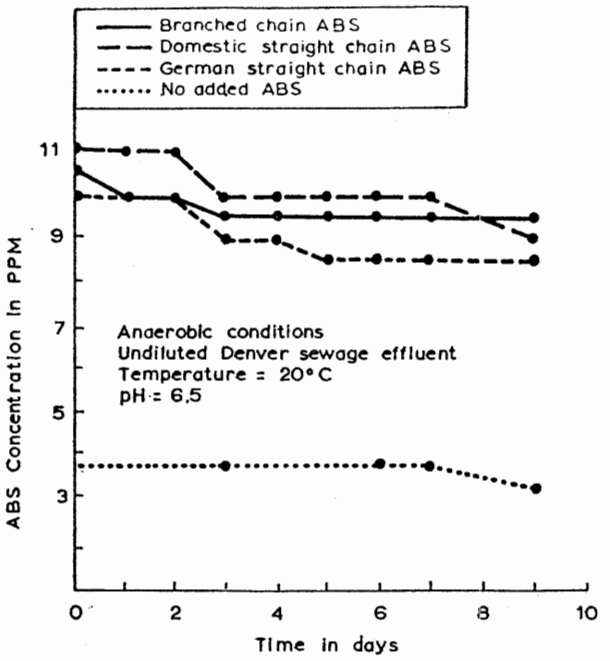


Figure 5

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

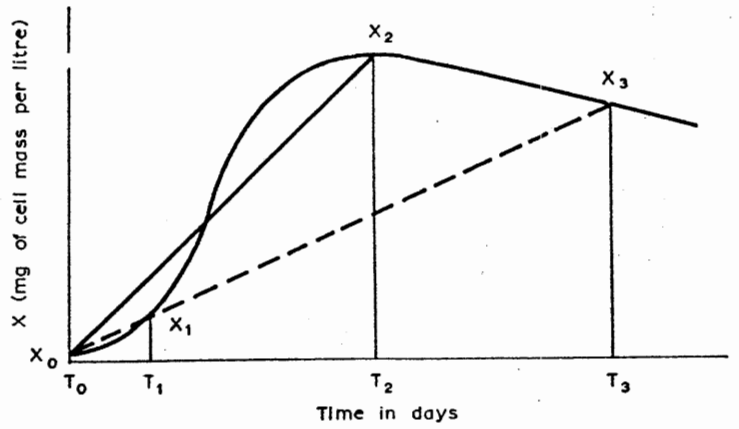


Figure 6

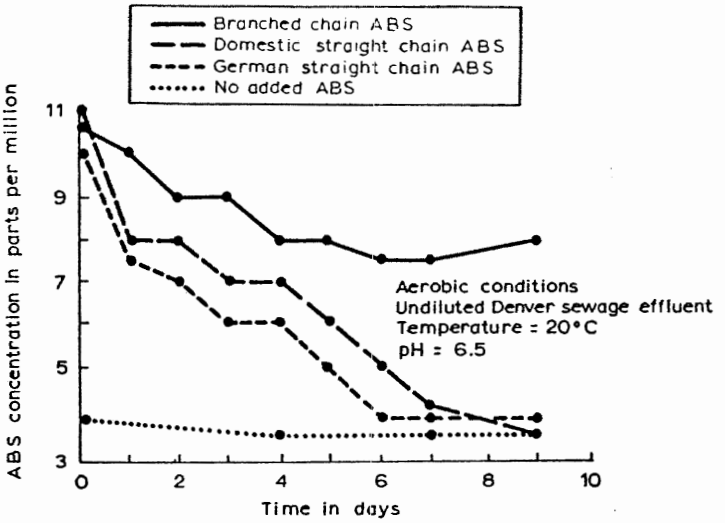


Figure 7

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

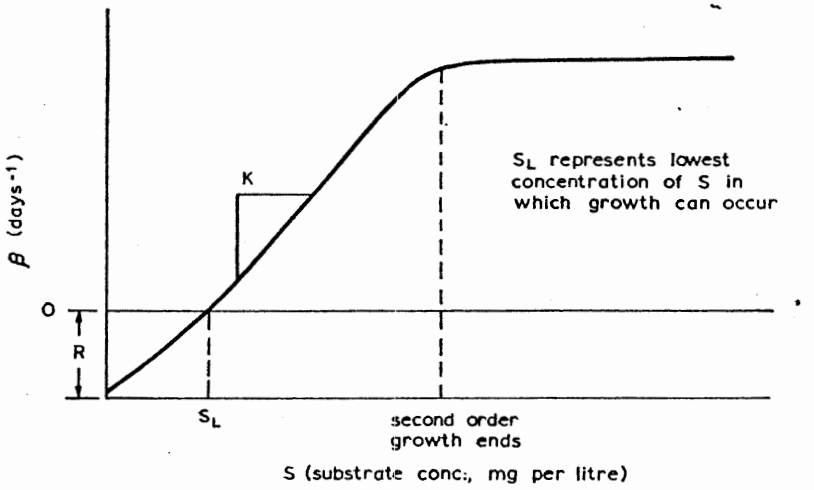


Figure 8

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.

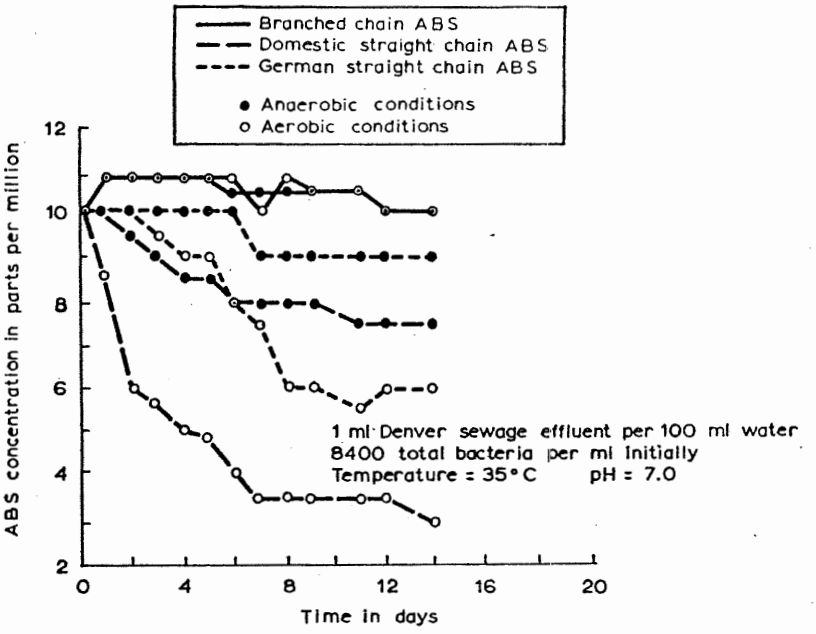


Figure 9

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

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

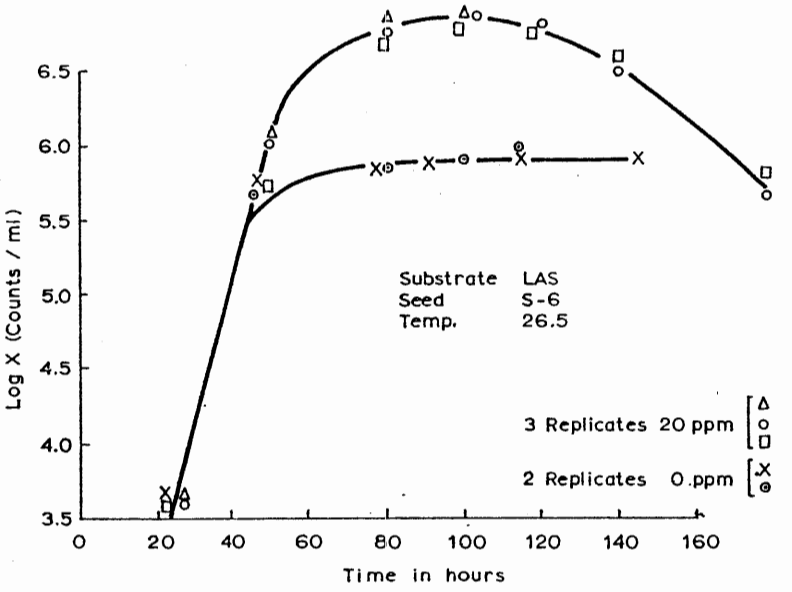


Figure 10

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]

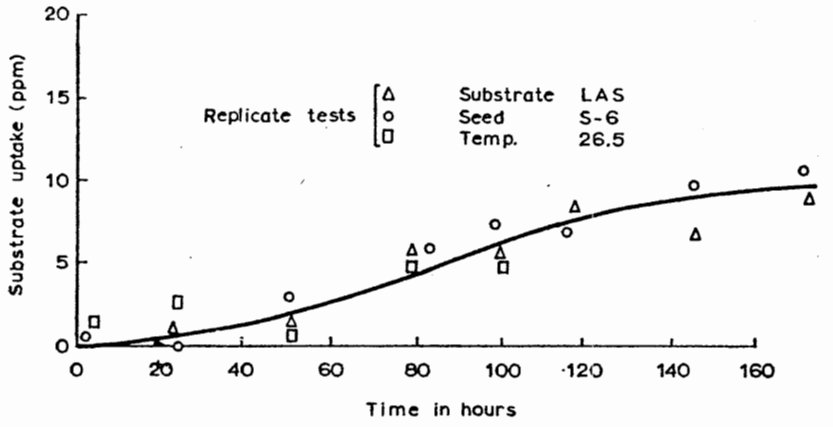


Figure 11

Though most procedures to date have employed river water-, soil-, or wage 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,

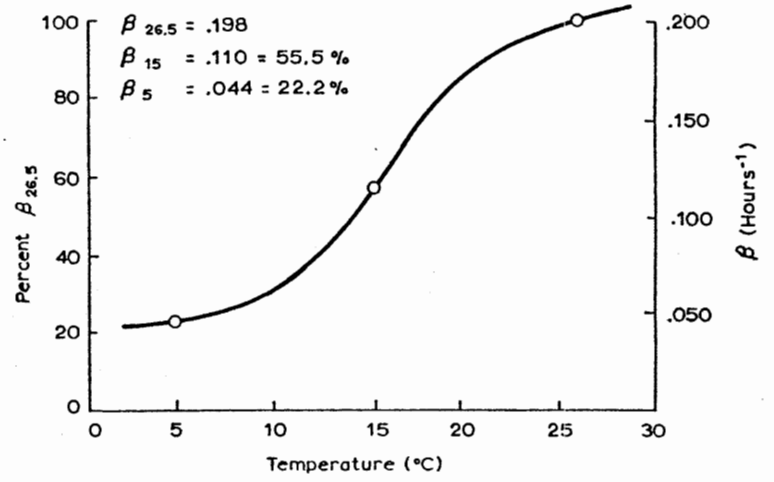


Figure 12

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

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 y acid, it seems clear that subsequent degradation by β-oxidation would substantially occur. If oxidation is the initiating force, then the terminal

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.

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

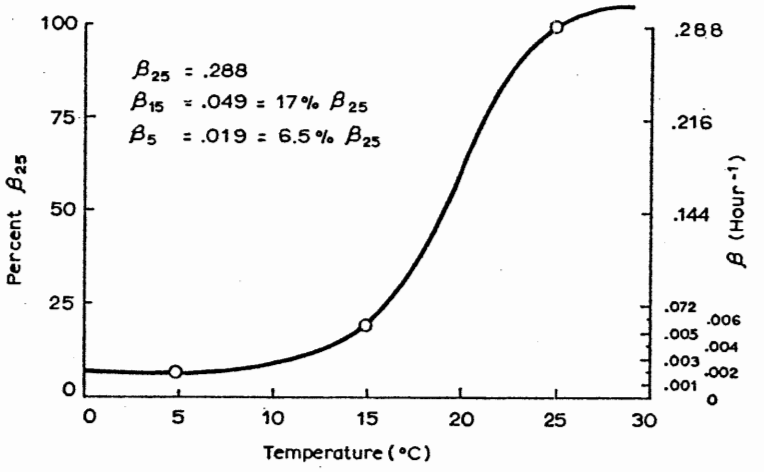


Figure 13

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

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

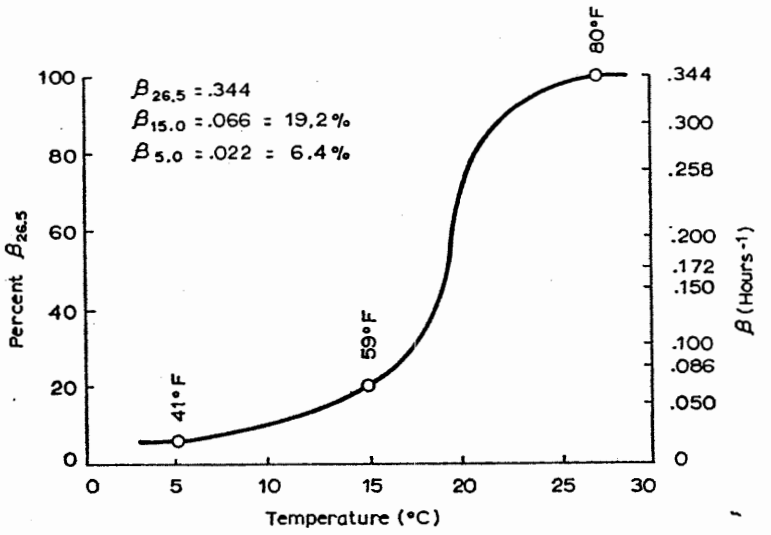


Figure 14

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]

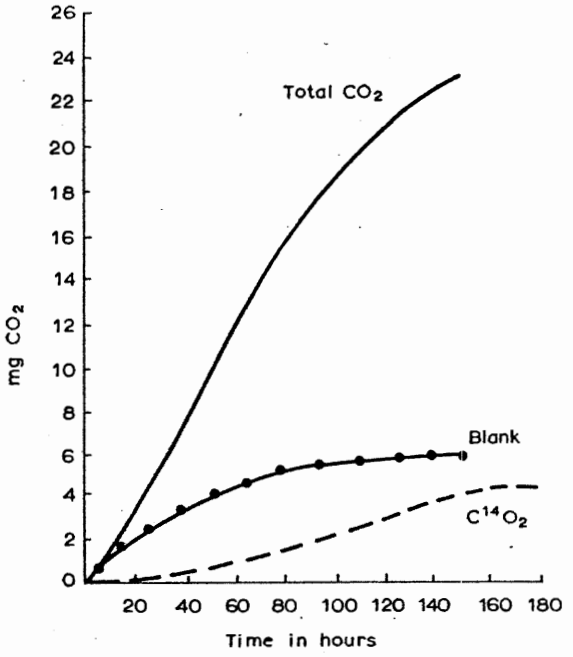


Figure 15

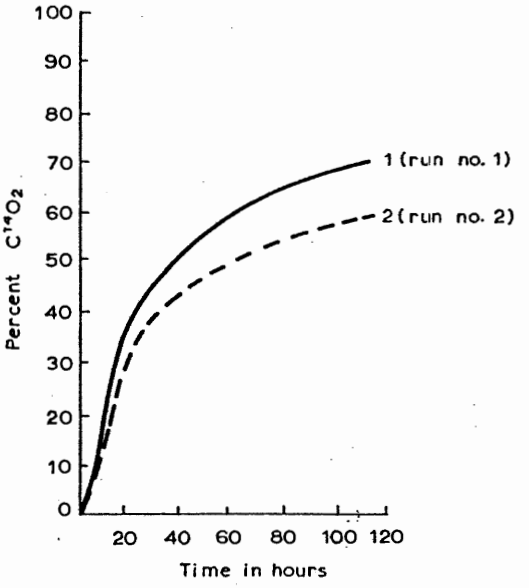


Figure 16

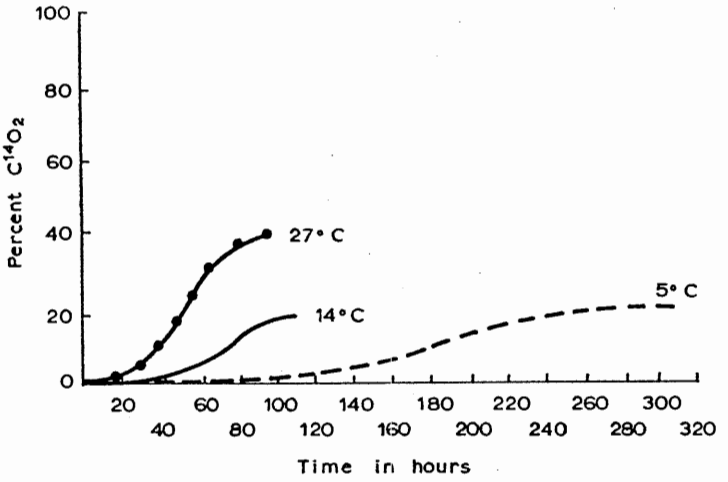


Figure 17

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-

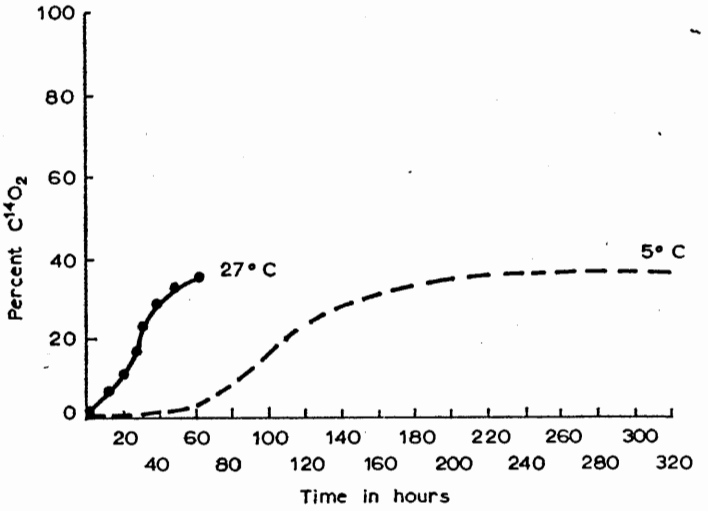


Figure 18

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

$$\lim_{t \to \infty} X_0 e^{Kt} \to \infty$$

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.

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

$$\int_0^T X\,dt.$$

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

$$Z = X^{1-n} = 1/X$$

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

$$X = \frac{2Y\beta^2 X_0}{2X_0\beta R + KRX_0\beta T - KRX_0 + e^{-\beta T}(2Y\beta^2 + X_0 RK - 2X_0\beta K)} \tag{21}$$

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).

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,

$$\frac{2Y\beta}{KRTX_0\,\mathrm{e}^{\beta T} + 2Y\beta} \overset{0}{=} 1$$

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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[Page 86-87]

TITLE 21--FOOD AND DRUGS

CHAPTER I--FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH AND HUMAN
SERVICES (CONTINUED)

PART 172--FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTION--Table of Contents

Subpart I--Multipurpose Additives

Sec. 172.842 Sorbitan monostearate.

The food additive sorbitan monostearate, which is a mixture of partial stearic and palmitic acid esters of sorbitol anhydrides, may be safely used in or on food in accordance with the following prescribed conditions:

(a) The food additive is manufactured by reacting stearic acid (usually containing associated fatty acids, chiefly palmitic) with sorbitol to yield essentially a mixture of esters.

(b) The food additive meets the following specifications:

Saponification number, 147-157
Acid number, 5-10
Hydroxyl number, 235-260

(c) It is used or intended for use, alone or in combination with polysorbate 60 as follows:

(1) As an emulsifier in whipped edible oil topping with or without one or a combination of the following:

(i) Polysorbate 60;

(ii) Polysorbate 65;
(iii) Polysorbate 80;

whereby the maximum amount of the additive or additives used does not exceed 0.4 percent of the weight of the finished whipped edible oil topping; except that a combination of the additive with polysorbate 60 may be used in excess of 0.4 percent: Provided, That the amount of the additive does not exceed 0.27 percent and the amount of polysorbate 60 does not exceed 0.77 percent of the weight of the finished whipped edible oil topping.

[[Page 87]]

(2) As an emulsifier in cakes and cake mixes, with or without one or a combination of the following:
(i) Polysorbate 65.
(ii) Polysorbate 60.

When used alone, the maximum amount of sorbitan monostearate shall not exceed 0.61 percent of the cake or cake mix, on a dry-weight basis. When used with polysorbate 65 and/or polysorbate 60, it shall not exceed 0.61 percent, nor shall the polysorbate 65 exceed 0.32 percent or the polysorbate 60 exceed 0.46 percent, and no combination of the emulsifiers shall exceed 0.66 percent of the weight of the cake or cake mix, calculated on a dry-weight basis.

(3) As an emulsifier, alone or in combination with polysorbate 60 in nonstandardized confectionery coatings and standardized cacao products specified in Secs. 163.123, 163.130, 163.135, 163.140, 163.145, and 163.150 of this chapter, as follows:

(i) It is used alone in an amount not to exceed 1 percent of the weight of the finished nonstandardized confectionery coating or standardized cacao product.

(ii) It is used with polysorbate 60 in any combination of up to 1 percent sorbitan monostearate and up to 0.5 percent polysorbate 60 provided that the total combination does not exceed 1 percent of the weight of the finished nonstandardized confectionery coating or standardized cacao product.

(4) As an emulsifier in cake icings and cake fillings, with or without one or a combination of the following:
(i) Polysorbate 65.
(ii) Polysorbate 60.

When used alone, the maximum amount of sorbitan monostearate shall not

exceed 0.7 percent of the weight of the cake icing or cake filling. When used with polysorbate 65 and/or polysorbate 60, it shall not exceed 0.7 percent, nor shall the polysorbate 65 exceed 0.32 percent or the polysorbate 60 exceed 0.46 percent, and no combination of these emulsifiers shall exceed 1 percent of the weight of the cake icing or cake filling.

(5) As an emulsifier in solid-state, edible vegetable fat-water emulsions intended for use as substitutes for milk or cream in beverage coffee, with or without one or a combination of the following:

(i) Polysorbate 60.

(ii) Polysorbate 65.

The maximum amount of the additive or additives shall not exceed 0.4 percent by weight of the finished edible vegetable fat-water emulsion.

(6) It is used alone as a rehydration aid in the production of active dry yeast in an amount not to exceed 1 percent by weight of the dry yeast.

(7) As an emulsifier, alone or in combination with polysorbate 60, in the minimum quantity required to accomplish the intended effect, in formulations of white mineral oil conforming with Sec. 172.878 and/or petroleum wax conforming with Sec. 172.886 for use as protective coatings on raw fruits and vegetables.

(d) To assure safe use of the additive, in addition to the other information required by the Act:

(1) The label of the additive and any intermediate premixes shall bear:

(i) The name of the additive.

(ii) A statement of the concentration or strength of the additive in any intermediate premixes.

(2) The label or labeling shall bear adequate directions to provide a final product that complies with the limitations prescribed in paragraph (c) of this section.

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[Page 81-83]

TITLE 21--FOOD AND DRUGS

CHAPTER I--FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH AND HUMAN
SERVICES (CONTINUED)

PART 172--FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTION--Table of Contents

Subpart I--Multipurpose Additives

Sec. 172.836 Polysorbate 60.

The food additive polysorbate 60 (polyoxyethylene (20) sorbitan monostearate) which is a mixture of polyoxyethylene ethers of mixed partial stearic and palmitic acid esters of sorbitol anhydrides and related compounds, may be safely used in food in accordance with the following prescribed conditions:

(a) The food additive is manufactured by reacting stearic acid (usually containing associated fatty acids, chiefly palmitic) with sorbitol to yield a product with a maximum acid number of 10 and a maximum water content of 0.2 percent, which is then reacted with ethylene oxide.

(b) The food additive meets the following specifications:

Saponification number 45-55.
Acid number 0-2.
Hydroxyl number 81-96.
Oxyethylene content 65 percent-69.5 percent.

(c) It is used or intended for use as follows:

(1) As an emulsifier in whipped edible oil topping with or without one or a combination of the following:
(i) Sorbitan monostearate;
(ii) Polysorbate 65;
(iii) Polysorbate 80;

[[Page 82]]

whereby the maximum amount of the additive or additives used does not exceed 0.4 percent of the weight of the finished whipped edible oil topping; except that a combination of the additive with sorbitan monostearate may be used in excess of 0.4 percent, provided that the amount of the additive does not exceed 0.77 percent and the amount of sorbitan monostearate does not exceed 0.27 percent of the weight of the finished whipped edible oil topping.

(2) As an emulsifier in cakes and cake mixes, with or without one or a combination of the following:
(i) Polysorbate 65.
(ii) Sorbitan monostearate.

When used alone, the maximum amount of polysorbate 60 shall not exceed
0.46 percent of the cake or cake mix, on a dry-weight basis. When used with polysorbate 65 and/or sorbitan monostearate, it shall not exceed 0.46 percent, nor shall the polysorbate 65 exceed 0.32 percent or the sorbitan monostearate exceed 0.61 percent, and no combination of these

emulsifiers shall exceed 0.66 percent of the cake or cake mix, all calculated on a dry-weight basis.

(3) As an emulsifier, alone or in combination with sorbitan monostearate, in nonstandardized confectionery coatings and standardized
cacao products specified in Secs. 163.123, 163.130, 163.135, 163.140, 163.145, and 163.150 of this chapter, as follows:

(i) It is used alone in an amount not to exceed 0.5 percent of the weight of the finished nonstandardized confectionery coating or standardized cacao product.

(ii) It is used with sorbitan monostearate in any combination of up to 0.5 percent of polysorbate 60 and up to 1 percent of sorbitan monostearate: Provided, That the total combination does not exceed 1 percent of the weight of the finished nonstandardized confectionery coating or standardized cacao product.

(4) [Reserved]

(5) As an emulsifier in cake icings and cake fillings, with or without one or a combination of the following:
(i) Polysorbate 65.

(ii) Sorbitan monostearate.

When used alone, the maximum amount of polysorbate 60 shall not exceed
0.46 percent of the weight of the cake icings and cake fillings. When used with polysorbate 65 and/or sorbitan monostearate, it shall not exceed 0.46 percent, nor shall the polysorbate 65 exceed 0.32 percent or

the sorbitan monostearate exceed 0.7 percent, and no combination of these emulsifiers shall exceed 1 percent of the weight of the cake icing or cake filling.

(6) To impart greater opacity to sugar-type confection coatings whereby the maximum amount of the additive does not exceed 0.2 percent
of the weight of the finished sugar coating.

(7) As an emulsifier in nonstandardized dressings whereby the maximum amount of the additive does not exceed 0.3 percent of the weight
of the finished dressings.

(8) As an emulsifier, alone or in combination with polysorbate 80, in shortenings and edible oils intended for use in foods as follows, when standards of identity established under section 401 of the act do not preclude such use:

(i) It is used alone in an amount not to exceed 1 percent of the weight of the finished shortening or oil.

(ii) It is used with polysorbate 80 in any combination providing no more than 1 percent of polysorbate 60 and no more than 1 percent of polysorbate 80, provided that the total combination does not exceed 1 percent of the finished shortening or oil.

(iii) The 1-percent limitation specified in paragraph (c)(8) (i) and (ii) of this section may be exceeded in premix concentrates of shortening or edible oil if the labeling complies with the requirements of paragraph (d) of this section.

(9) As an emulsifier in solid-state, edible vegetable fat-water emulsions intended for use as substitutes for milk or cream in beverage coffee, with or without one or a combination of the following:

(i) Polysorbate 65.

(ii) Sorbitan monostearate.

The maximum amount of the additive or additives shall not exceed 0.4 percent by weight of the finished edible vegetable fat-water emulsion.

[[Page 83]]

(10) As a foaming agent in nonalcoholic mixes, to be added to alcoholic beverages in the preparation of mixed alcoholic drinks, at a level not to exceed 4.5 percent by weight of the nonalcoholic mix.

(11) As a dough conditioner in yeast-leavened bakery products in an

amount not to exceed 0.5 percent by weight of the flour used.

(12) As an emulsifier, alone or in combination with sorbitan monostearate, in the minimum quantity required to accomplish the intended effect, in formulations of white mineral oil conforming with Sec. 172.878 and/or petroleum wax conforming with Sec. 172.886 for use
as protective coatings on raw fruits and vegetables.

(13) As a dispersing agent in artificially sweetened gelatin desserts and in artificially sweetened gelatin dessert mixes, whereby the amount of the additive does not exceed 0.5 percent on a dry-weight basis.

(14) As an emulsifier in chocolate flavored syrups, whereby the maximum amount of the additive does not exceed 0.05 percent in the finished product.

(15) As a surfactant and wetting agent for natural and artificial colors in food as follows:

(i) In powdered soft drink mixes in an amount not to exceed 4.5 percent by weight of the mix.

(ii) In sugar-based gelatin dessert mixes in an amount not to exceed 0.5 percent by weight of the mix.

(iii) In artificially sweetened gelatin dessert mixes in an amount not to exceed 3.6 percent by weight of the mix.

(iv) In sugar-based pudding mixes in an amount not to exceed 0.5 percent by weight of the mix.

(v) In artificially sweetened pudding mixes in an amount not to exceed 0.5 percent by weight of the mix.

(16) As an emulsifier in ice cream, frozen custard, fruit sherbet, and nonstandardized frozen desserts when used alone or in combination with polysorbate 65 and/or polysorbate 80, whereby the maximum amount of
the additives, alone or in combination, does not exceed 0.1 percent of the finished frozen dessert.

(d) To assure safe use of the additive, in addition to the other information required by the Act:

(1) The label of the additive and any intermediate premixes shall bear:

(i) The name of the additive.

(ii) A statement of the concentration or strength of the additive in any intermediate premixes.

(2) The label or labeling shall bear adequate directions to provide a final product that complies with the limitations prescribed in paragraph (c) of this section.

[42 FR 14491, Mar. 15, 1977, as amended at 43 FR 2871, Jan. 25, 1978; 45
FR 58836, Sept. 5, 1980; 46 FR 8466, Jan. 27, 1981; 64 FR 57976, Oct. 28, 1999]

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[Page 83-84]

TITLE 21--FOOD AND DRUGS

CHAPTER I--FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH AND HUMAN
SERVICES (CONTINUED)

PART 172--FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTION--Table of Contents

Subpart I--Multipurpose Additives

Sec. 172.838 Polysorbate 65.

The food additive polysorbate 65 (polyoxyethylene (20) sorbitan tristearate), which is a mixture of polyoxyethylene ethers of mixed stearic acid esters of sorbitol anhydrides and related compounds, may be

safely used in food in accordance with the following prescribed conditions:

(a) The food additive is manufactured by reacting stearic acid (usually containing associated fatty acids, chiefly palmitic) with sorbitol to yield a product with a maximum acid number of 15 and a maximum water content of 0.2 percent, which is then reacted with ethylene oxide.

(b) The food additive meets the following specifications:

Saponification number 88-98.
Acid number 0-2.
Hydroxyl number 44-60.
Oxyethylene content 46 percent-50 percent.

(c) The additive is used, or intended for use, as follows:

(1) As an emulsifier in ice cream, frozen custard, ice milk, fruit sherbet and nonstandardized frozen desserts when used alone or in combination with polysorbate 80, whereby the maximum amount of the additives, alone or in combination, does not exceed 0.1 percent of the finished frozen dessert.

(2) As an emulsifier in cakes and cake mixes, with or without one or a combination of the following:

(i) Sorbitan monostearate.

(ii) Polysorbate 60.

When used alone, the maximum amount of polysorbate 65 shall not exceed
0.32 percent of the cake or cake mix, on a dry-weight basis. When used

[[Page 84]]

with sorbitan monostearate and/or polysorbate 60, it shall not exceed 0.32 percent, nor shall the sorbitan monostearate exceed 0.61 percent or

the polysorbate 60 exceed 0.46 percent, and no combination of these emulsifiers shall exceed 0.66 percent of the cake or cake mix, all calculated on a dry-weight basis.

(3) As an emulsifier in whipped edible oil topping with or without one or a combination of the following:

(i) Sorbitan monostearate;

(ii) Polysorbate 60;

(iii) Polysorbate 80;

whereby the maximum amount of the additive or additives used does not exceed 0.4 percent of the weight of the finished whipped edible oil topping.

(4) As an emulsifier in solid-state, edible vegetable fat-water emulsions intended for use as substitutes for milk or cream in beverage coffee, with or without one or a combination of the following:

(i) Sorbitan monostearate.

(ii) Polysorbate 60.

The maximum amount of the additive or additives shall not exceed 0.4 percent by weight of the finished edible vegetable fat-water emulsion.

(5) As an emulsifier in cake icings and cake fillings, with or without one or a combination of the following:

(i) Sorbitan monostearate.

(ii) Polysorbate 60.

When used alone, the maximum amount of polysorbate 65 shall not

exceed
0.32 percent of the weight of the cake icing or cake filling. When used with sorbitan monostearate and/or polysorbate 60, it shall not exceed 0.32 percent, nor shall the sorbitan monostearate exceed 0.7 percent or the polysorbate 60 exceed 0.46 percent, and no combination of these emulsifiers shall exceed 1 percent of the weight of the cake icing or cake filling.

(d) To assure safe use of the additive, in addition to the other information required by the Act:

(1) The label of the additive and any intermediate premixes shall bear:

(i) The name of the additive.

(ii) A statement of the concentration or strength of the additive in any intermediate premixes.

(2) The label or labeling shall bear adequate directions to provide a final product that complies with the limitations prescribed in paragraph (c) of this section.

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[Page 84-85]

TITLE 21--FOOD AND DRUGS

CHAPTER I--FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH AND HUMAN
SERVICES (CONTINUED)

PART 172--FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTION--Table of Contents

Subpart I--Multipurpose Additives

Sec. 172.840 Polysorbate 80.

The food additive polysorbate 80 (polyoxyethylene (20) sorbitan monooleate), which is a mixture of polyoxyethylene ethers of mixed partial oleic acid esters of sorbitol anhydrides and related compounds, may be safely used in food in accordance with the following prescribed conditions:

(a) The food additive is manufactured by reacting oleic acid (usually containing associated fatty acids) with sorbitol to yield a product with a maximum acid number of 7.5 and a maximum water content of
0.5 percent, which is then reacted with ethylene oxide.

(b) The food additive meets the following specifications:

Saponification number 45-55.
Acid number 0-2.
Hydroxyl number 65-80.
Oxyethylene content 65 percent-69.5 percent.

(c) The additive is used or intended for use as follows:

(1) An emulsifier in ice cream, frozen custard, ice milk, fruit sherbet, and nonstandardized frozen desserts, when used alone or in combination with polysorbate 65 whereby the maximum amount of the additives, alone or in combination, does not exceed 0.1 percent of the finished frozen dessert.

(2) In yeast-defoamer formulations whereby the maximum amount of the additive does not exceed 4 percent of the finished yeast defoamer and the maximum amount of the additive in the yeast from such use does not exceed 4 parts per million.

(3) As a solubilizing and dispersing agent in pickles and pickle products, whereby the maximum amount of the additive does not exceed 500 parts per million.

(4) As a solubilizing and dispersing agent in:

(i) Vitamin-mineral preparations containing calcium caseinate in the absence of fat-soluble vitamins, whereby

[[Page 85]]

the maximum intake of polysorbate 80 shall not exceed 175 milligrams from the recommended daily dose of the preparations.

(ii) Fat-soluble vitamins in vitamin and vitamin-mineral preparations containing no calcium caseinate, whereby the maximum intake of polysorbate 80 shall not exceed 300 milligrams from the recommended daily dose of the preparations.

(iii) In vitamin-mineral preparations containing both calcium caseinate and fat-soluble vitamins, whereby the maximum intake of polysorbate 80 shall not exceed 475 milligrams from the recommended daily dose of the preparations.

(5) As a surfactant in the production of coarse crystal sodium chloride whereby the maximum amount of the additive in the finished sodium chloride does not exceed 10 parts per million.

(6) In special dietary foods, as an emulsifier for edible fats and oils, with directions for use which provide for the ingestion of not more than 360 milligrams of polysorbate 80 per day.

(7) As a solubilizing and dispersing agent for dill oil in canned spiced green beans, not to exceed 30 parts per million.

(8) As an emulsifier, alone or in combination with polysorbate 60, in shortenings and edible oils intended for use in foods as follows, when standards of identity established under section 401 of the act do not preclude such use:

(i) It is used alone in an amount not to exceed 1 percent of the weight of the finished shortening or oil.

(ii) It is used with polysorbate 60 in any combination providing no more than 1 percent of polysorbate 80 and no more than 1 percent of polysorbate 60, provided that the total combination does not exceed 1 percent of the finished shortening or oil.

(iii) The 1-percent limitation specified in paragraph (c)(8) (i) and (ii) of this section may be exceeded in premix concentrates of shortening or edible oil if the labeling complies with the requirements of paragraph (d) of this section.

(9) As an emulsifier in whipped edible oil topping with or without one or a combination of the following:

(i) Sorbitan monostearate;

(ii) Polysorbate 60;

(iii) Polysorbate 65;

whereby the maximum amount of the additive or additives used does not exceed 0.4 percent of the weight of the finished whipped edible oil topping.

(10) It is used as a wetting agent in scald water for poultry defeathering, followed by potable water rinse. The concentration of the additive in the scald water does not exceed 0.0175 percent.

(11) As a dispersing agent in gelatin desserts and in gelatin dessert mixes, whereby the amount of the additive does not exceed 0.082
percent on a dry-weight basis.

(12) As an adjuvant added to herbicide use and plant-growth regulator use dilutions by a grower or applicator prior to application of such dilutions to the growing crop. Residues resulting from such use are exempt from the requirement of a tolerance. When so used or intended
for use, the additive shall be exempt from the requirements of paragraph (d)(1) of this section.

(13) As a defoaming agent in the preparation of the creaming mixture for cottage cheese and lowfat cottage cheese, as identified in Secs. 133.128 and 133.131 of this chapter, respectively, whereby the amount of the additive does not exceed .008 percent by weight of the finished products.

(14) As a surfactant and wetting agent for natural and artificial colors for use in barbecue sauce where the level of the additive does not exceed 0.005 percent by weight of the barbecue sauce.

(d) To assure safe use of the additive, in addition to the other information required by the Act:

(1) The label of the additive and any intermediate premixes shall bear:

(i) The name of the additive.

(ii) A statement of the concentration or strength of the additive in any intermediate premixes.

(2) The label or labeling shall bear adequate directions to provide a final product that complies with the limitations prescribed in paragraph (c) of this section.

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SORBITAN MONOESTERS OF PALMITIC, STEARIC, OLEIC AND LAURIC ACIDS AND TRIESTERS OF STEARIC ACID

(FAS 17) (1982)

Explanation

Sorbitan monoesters of palmitic and stearic acids and triesters of stearic acid have been evaluated for acceptable daily intake by the Joint FAO/WHO Expert Committee on Food Additives in 1974 (see Annex I, Ref. 32) and a toxicological monograph was prepared (see Annex I, Ref. 33). Sorbitan monoesters of oleic and lauric acids have not previously been evaluated by JECFA. The previous published monograph has been expanded to include sorbitan esters of oleic and lauric acids.

BIOLOGICAL DATA

BIOCHEMICAL ASPECTS

The fatty acid moiety of sorbitan monostearate has a coefficient of digestibility of 53.3% (Oser & Oser, 1957b).

Experiments with ^{14}C-labelled sorbitan monostearate showed that at least 90% of the emulsifier, when fed to rats in oily solution, was hydrolysed to stearic acid and anhydrides of sorbitol. A significant fraction of the administered ^{14}C was expired as CO_2. When the labelled material was administered in water, 16-25% was recovered in the urine; when it was given in oil, 44-66% was recovered. Of the ingested radioactivity, 3-7% was recovered in the tissues 48 hours after feeding. Fractionation of the carcass fats showed that ^{14}C was incorporated into fatty acids, glycerol and a residue that did not sublime (Wick & Joseph, 1953).

Sorbitan trioleate prepared with a ^{14}C-label in the sorbitol or the oleate moieties was administered orally to rats. After administration of sorbitan ^{14}C-trioleate, the appearance of ^{14}C-CO_2 in the expired air reached a peak at about 6 hours and amounted to 30-35% of administered label. The faeces and gastrointestinal tract contained about 42% of the ^{14}C-label, indicating that it was incompletely absorbed, and 3% appeared in the urine; the liver contained about 3% and the carcass about 22%. After administration of the ^{14}C-sorbitan-labelled ester, less than 2% of the label was recovered as ^{14}C-CO_2; the proportions not absorbed were 24% from a water emulsion and 37% from a solution in oil; the proportions recovered of that absorbed were as follows: urine, 88%; expired air, 5%; liver, 1%; carcass, 6% (Treon et al., 1967).

TOXICOLOGICAL STUDIES

Acute toxicity

The physical properties and low toxicity of the partial esters of sorbitan are such that formal acute toxicity tests are for the most part impracticable.

Acute feeding tests on sorbitan monopalmitate have been conducted in the rat. No toxic symptoms were observed in 10 male rats, weighing 100-175 g, using the ester as their sole ad libitum diet (except for water) for 24 hours, during which they consumed an average of 1.5 g per animal or approximately 10 g/kg bw (Krantz, 1947c).

For 10 rats, sorbitan monostearate was mixed with a diet at a concentration of 50%. These animals weighed 175-250 g and, within 10 hours, had ingested about 3 g of the ester. They all appeared unharmed after 3 days.

No toxic symptoms were observed in any of 10 female rats, weighing 125-175 g, fed 10 ml/kg bw of sorbitan tristearate by stomach tube, in the form of a 50% aqueous emulsion, and observed over a 6-day period. Four of the 10 animals, all of which appeared normal, were sacrificed and gave normal histological findings in the livers and kidneys. In 10 female rats, weighing 125-175 g, intraperitoneal injection of sorbitan tristearate in doses of 10 ml/kg (50% aqueous emulsion form) produced 2 fatalities in 48 hours of observation; the other animals appeared normal (Krantz, 1947d).

Sorbitan monopalmitate ("Span 40"), sorbitan monostearate ("Span 60") and sorbitan tristearate ("Span 65"), in the maximal orally administerable doses (15.9 g/kg), produced no mortality in rats (Brandner, 1973).

The LD_{50} of orally administered sorbitan (monooleate) and sorbitan monolaurate in the rat was 39.8 and 37.5 g/kg, respectively (Quigley, 1966). Diarrhoea occurred in all rats given either sorbitan monooleate or laurate. Autopsy of the surviving animals 14 days after treatment showed a high incidence of hydronephrosis.

Short-term studies

Hamster

Hamsters averaging 46 g in weight were segregated by sex into groups varying in size from 14 to 22 and fed diets containing sorbitan monolaurate at levels of 0, 5 and 15% for 68 days. Treated animals developed mild diarrhoea and a depressed growth rate. The high-dose group suffered 22% mortality compared to 8% in the control group. Treatment-related effects were noted in the gastrointestinal tract,

including mucosal and intramural hyperaemia and oedema, with mild infiltration by inflammatory cells. In the kidney of treated animals, the cortical tubular epithelium exhibited fraying at the free edges of cells, eosinophilic granularity and nuclear pyknosis. The kidney alterations were regarded as reversible. Incomplete maturation in testes and ovaries was more frequent in treated animals (Harris, 1951b).

Rat

Groups of 10 young rats were fed for 6 weeks diets containing 1% or 4% of sorbitan monostearate. There was no effect on weight gain, nor were there any significant changes histopathologically in the liver, kidneys, intestine and bladder (Krantz, 1946).

Sodium monostearate was added to diets designed to induce hepatic necrosis in rats. Levels up to 10% tended to prolong the survival time

and had no significant effect upon the hepatic changes over periods up to 120 days (Gyorgy et al., 1958).

Rats in groups of 5 receiving sorbitan monostearate in their diet for 6 weeks at levels of 5% or 15% showed no change in bile duct size (Krantz, 1951).

Groups each of 30 Wistar rats (initial body weight 84-87 g) equally divided by sex were fed sorbitan monolaurate at dietary levels of 0, 2.5, 5.0 and 10%. Haematological and urinary analyses were carried out at weeks 2, 6 and 13 and serum clinical chemistry at weeks 6 and 13. At weeks 2 and 6, 5 male and 5 female animals from each group were sacrificed, and at the end of the thirteenth week all surviving animals were sacrificed. Animals fed 5% and 10% test diet showed statistically significant dose-related decreases in haemoglobin levels, packed cell volumes (2, 6, 13 weeks) and total leucocyte count (13 weeks, males only). No treatment-related effects were observed in clinical chemistry values or urinalyses. At autopsy, organ weights were determined, and a biological examination made of the brain, pituitary, thyroid, heart, liver, spleen, kidneys, adrenal glands, gonads, stomach, small intestine, caecum, lung, salivary gland, aortic arch, thymus, various lymph glands, urinary bladder, colon, rectum, pancreas, uterus and skeletal muscle. An increase in relative liver weight occurred in the high-dose animals, and increased relative kidney weight occurred in all test groups. These effects were first observed in animals maintained on test diets for 2 weeks. No adverse histological findings could be demonstrated in the kidney or other tissues, except in the livers of animals in the high-dose groups where there was an increased incidence of periportal vacuolation (Cater et al., 1978).

Groups of 10 male and 10 female Osborne-Mendel rats, initial weight 40-50 g, were fed diets containing 0, 15, 20 or 25% sorbitan monolaurate for 23 weeks. Treated animals exhibited diarrhoea, an unkempt appearance and severe growth retardation. At the 25% dose level, only one animal of each sex survived the study. Upon gross pathological examination, the livers of treated animals showed paleness and enlargement, with a marked enlargement of the common bile duct. Histological studies of the tissues showed marked liver damage at all dose levels which consisted of fatty changes and fibrosis. There was no bile duct proliferation but there was great common bile duct enlargement. Focal nephritis was observed in the kidney and there was a marked increase in the incidence of foamy alveolar macrophages in the lungs of treated animals. No other tissues exhibited treatment-related effects (Fitzhugh et al., 1960).

Weanling male Sprague-Dawley rats were distributed into groups of 14 and fed sorbitan monolaurate for 59 days at dietary levels of 0 and 25%. Treatment-related symptoms included reduced rate of growth, reduced food consumption, diarrhoea, nasal haemorrhage and gangrenous tails. Only 1 rat survived the treatment regimen, presenting a stunted appearance resembling starvation (Harris et al., 1951a). In another study, 14 male and 16 female rats were fed sorbitan monolaurate in doses increasing to 25% by day 10 of the study, and continuing at that level for 60 additional days. The toxic effects were similar to those reported in the previous study. Results from pair-fed controls indicate that the reduction in growth was not caused primarily by reduced food consumption. Haematology at termination of the study showed a treatment-related decrease in haemoglobin value. At autopsy, increased relative organ-to-body weight was observed for brain,

kidney, heart, spleen, lung and liver. Histological studies of tissues showed mild degenerative lesions in the kidney, necrosis of the liver, and incomplete maturation of testes. No other effects were reported (Harris et al., 1951a).

Groups of 6 female Holtzman rats, 21-24 days old, were fed diets containing 0, 15 and 20% of sorbitan monolaurate for 21 days. All animals on test diets suffered diarrhoea, alopecia, unthrifty appearance, reduced rate of growth and increased mortality. No pathological evaluation was made (Ershoff, 1960).

White male rats of unspecified strain, initial weight 60 g, were distributed into groups of 4 or 5 and fed diets containing 0, 1 and 4% sorbitan monolaurate for 6 weeks. A significant reduction in growth rate occurred in the high-dose group. No significant histopathological changes were reported in liver, intestine or bladder, but coagulated fluid was present in the renal tubules (Krantz, 1946).

Wistar rats, initial weight 89-94 g, were distributed into groups of 15 males and 15 females and fed sorbitan monooleate at dietary levels of 0, 2.5, 5.0 and 10% for 16 weeks. Animals on test diets showed a decreased weight gain that was related to a reduction in food intake. At weeks 2, 6 and 16, haematology, serum clinical chemistry and urinalysis were carried out. Haematology studies showed lower values for haemoglobin and packed cell volume in female rats fed 10% sorbitan monooleate, with a significantly lower mean erythrocyte count. Variations in clinical chemistry values did not appear to be compound related. No compound-related effects were observed in the urinalysis.

Autopsy of the test animals at the termination of the study showed kidney enlargement in females in the 5% and 10% test groups. Histological studies showed renal damage in these groups, as well as periportal fatty changes in the liver of female rats in the 10% group. No other compound-related histopathology was reported (Ingram et al., 1978).

Groups of 10 male white rats of unspecified strain, initial weight 100 g, were fed sorbitan monooleate at dietary levels of 0 and 10% for 6 weeks. Reductions in food consumption and in rate of growth, and mild diarrhoea occurred in treated animals. Histological sections from liver and kidney showed no differences between control and treated animals. Haematological and serum clinical chemistry were carried out at weeks 3 and 6 of the study. There were no significant differences between test and control animals. At autopsy (3 weeks and 6 weeks), gross pathological and histological studies of selected organs (liver and kidney) from 3 rats showed no compound-related effects (Krantz, 1953).

Dog

Dogs were fed sorbitan monostearate in a semi-synthetic diet at a level of 5% for 19-20 months. There was no appreciable difference in food intake, weight, maintenance and longevity between controls and those fed sorbitan monostearate. The microscopic examination of the tissues showed no changes attributable to the feeding of sorbitan monostearate, with the exception of some haemosiderosis of the liver (Fitzhugh et al., 1959).

Monkey

Two rhesus monkeys, fed daily with 0.7-0.8 g/kg bw of sorbitan monostearate for 5 weeks, appeared unharmed throughout. No damage to the liver and kidneys was seen post mortem (Krantz, 1946).

Two Macacus rhesus monkeys of unspecified sex (initial weights 2.25 and 2.85 kg) were fed 2 cc of sorbitan monolaurate per day in their diet for 6 weeks. Because no control animals were used in the study, the haematology and growth results are difficult to interpret. Histological sections from liver and spleen were reported to be normal in appearance. However, some kidney damage was evident, including

shrinking and slight serous exudation into some of the glomerular spaces, swelling of the lining epithelium of the convoluted tubules, and fragmentation and debris in the lumina of some tubules. Because of the absence of tissue sections from control animals, the apparent kidney damage cannot be unequivocally ascribed to treatment (Krantz, 1946).

Long-term studies

Rat

A life-span (2-year) study in 30 male rats given 5% sorbitan monopalmitate in their daily diet showed no alteration in the growth pattern or survival of the test rats, as compared with controls, and no abnormalities that could be attributed to the experimental diet. Blood studies and histopathological studies of the principal viscera were made during and at the end of the experiment. Examination of the brain, spleen, pancreas, adrenal gland, bladder, bone marrow, heart, lymph nodes, lung, testicle and muscle, revealed nothing of a pathological nature attributable to the experimental diet (Krantz, 1947a).

Thirty rats were fed on a diet containing 5% sorbitan monostearate for up to 2 years. Growth rate and survival were similar to those of the controls. There were no histological abnormalities post mortem that could be attributed to the diet (Krantz, 1947a).

Experiments were conducted over a period of 2 years on 4 generations of rats (30 rats in each group). At levels of 5% and 10% in the diet, no effects were observed on growth, food efficiency, reproduction, lactation, metabolism, behaviour, mortality, or the growth and histopathological appearance of the tissues. At a level of 20%, retardation of growth and impairment in lactation were noted, but mortality was not increased. The weight of the liver and kidneys was increased, but both appeared to be histologically normal (Oser & Oser, 1956a,b, 1957a,b).

Sorbitan monostearate was fed to groups of 24 rats at levels of 2, 5, 10 and 25% in the diet for 2 years. Levels of 2% and 5% did not produce evidence of toxicity. The substance caused a significant increase in mortality at the 10% and 25% levels, with growth depression and enlargement of the liver and kidneys (Fitzhugh et al., 1959).

A life-span (2-year) chronic feeding study was conducted on 30 male rats, using 5% sorbitan tristearate in their diet. From this experiment it was concluded that there was no alteration in the growth

pattern or survival of the test rats as compared to the controls, nor were there any abnormalities that could be attributed to the experimental diet (Krantz, 1947e).

A life-span (2-year) study was conducted with 30 white male rats (strain unspecified, initial weights 54-63 g) that were given 5% sorbitan monolaurate in their daily diet. No effect on growth or mortality of the test rats, as compared with controls, could be attributed to the test compound. Blood chemistry, haematology, histopathological examinations and gross pathological examinations of liver, kidney, spleen, brain, adrenals, urinary bladder, gastrointestinal tract, pancreas, thyroid, heart, lung, testicle, salivary gland, prostate, parathyroid, pituitary, striated muscle and bone marrow were carried out both during the study, following interim sacrifice at 6, 12 and 17 months, and at termination of the study. No treatment-related changes were reported (Krantz, 1950a).

Groups of 10 male and 10 female weanling Sprague-Dawley rats were fed diets containing 0, 5 and 10% sorbitan monolaurate for 2 years. Animals consuming a 10% sorbitan monolaurate diet suffered diarrhoea and a statistically significant reduction in growth, but animals fed the 5% diet showed no adverse treatment-related effect. Because food consumption data were inadequate, it could not be ascertained whether the reduced growth was caused by reduced food consumption or by a direct toxic effect. Liver, kidney, heart, aorta, spleen, pancreas and body fat exhibited no treatment-related abnormalities when examined grossly and histologically.

A group of 30 white male rats (strain unspecified, initial weight 54-63 g) were maintained on a diet containing 5% sorbitan monooleate for a period of 2 years. There was a minor retardation of growth, and no effect on mortality. Haematology and serum clinical chemistry tests were carried out at months 6, 12 and 17 of the study. No compound-related effects were reported. At months 6, 12 and 17, 1 control and 1 test animal were sacrificed for histopathological examination of liver, kidney and bone marrow, and at week 104, all surviving animals were sacrificed. At autopsy, gross pathological and histopathological examination of brain, spleen, pancreas, thyroid, parathyroid, prostate, pituitary gland, salivary gland, adrenal, bladder, bone marrow, heart, lymph node, lung, testicle and muscle did not show any compound-related effects (Krantz, 1950b).

OBSERVATIONS IN MAN

Sorbitan monostearate given to 9 human subjects in doses of 6 g/day for 28 days had no significant effect on the gastric activity or on the activity of the gastrointestinal tract (Steigmann et al., 1953).

Other experiments on 16 human subjects With the same doses and for the same period showed no deleterious effect. Blood chemistry (including cholesterol), other haematological findings, urine analyses and liver function tests were normal (Waldstein et al., 1954).

In another study 4 g of sorbitan monostearate were fed daily to 2 children for 32-37 days without harmful effects (Preston et al., 1953).

Comments

Metabolic studies indicate that a significant portion of the sorbitan esters are hydrolysed to the fatty acid moiety and anhydrides of sorbitol.

The partial esters of sorbitan have been investigated in both short-term and long-term experiments. Some have also been studied in man.

Some changes in liver and kidney have been reported in recent short-term studies with sodium monolaurate and sodium monooleate at dietary levels of 10%. The most significant changes occurred in the liver of rats fed sodium monolaurate, the changes being primarily fatty changes, intralobular fibrosis, and enlargement of the common bile duct; no bile duct proliferation was reported. The esters also caused some kidney enlargement, but there were no histological changes. The changes reported appeared to be related to the fatty acid moiety of the ester.

Long-term feeding studies in the rat are available for all the esters considered in this monograph. Although these are older studies, the available information shows that no compound-related effects were observed when the esters were fed at 5% of the diet.

From the toxicological point of view, the evidence provides a basis for evaluating the sorbitan esters as a group.

EVALUATION

Level causing no toxicological effect

Rat: 50 000 ppm (5%) in the diet equivalent to 2500 mg/kg bw.

Estimate of acceptable daily intake for man

0-25 mg/kg bw.*

* Group ADI. As the sum of the sorbitan esters of lauric, oleic, palmitic and stearic acid.

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See Also:
Toxicological Abbreviations

Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers and thickening agents

WHO FOOD ADDITIVES SERIES NO. 5

The evaluations contained in this publication were prepared by the Joint FAO/WHO Expert Committee on Food Additives which met in Geneva, 25 June - 4 July 1973[1]

World Health Organization
Geneva
1974

[1] Seventeenth Report of the Joint FAO/WHO Expert Committee on Food Additives, Wld Hlth Org. techn. Rep. Ser., 1974, No. 539; FAO Nutrition Meetings Report Series, 1974, No. 53.

SORBITOL

Explanation

This substance has been evaluated for acceptable daily intake by the Joint FAO/WHO Expert Committee on Food Additives (see Annex 1, Refs Nos. 7 and 13) in 1963 and 1965.

Since the previous evaluation, additional data have become available and are summarized and discussed in the following monograph. The previously published monograph has been expanded and is reproduced in its entirety below.

BIOLOGICAL DATA

BIOCHEMICAL ASPECTS

The absorption of sorbitol is much slower than that of glucose or fructose. Both normal and diabetic human subjects excreted in the urine less than 3% of an oral dose of 35 g. No sorbitol was detected in the faeces. In experiments with uniformly labelled ^{14}C-sorbitol (sorbitol-U-^{14}C), at least 75% of the dose given orally was metabolized to CO_2. In normal subjects, there was no significant

increase in the blood sugar levels; in diabetic subjects the blood sugar increased slightly. The concentration of sorbitol in the blood was immeasurably small (Adcock & Gray, 1957).

In experiments on rats given sorbitol-U-^{14}C by intraperitoneal injection, 57.4% of the activity was excreted as CO_2, 17.3% in the urine, 4.2% was found as liver glycogen and 0.6% as liver fatty acids. In diabetic rats, a smaller proportion was oxidized and the major portion was excreted in the urine (Stetten & Stetten, 1951). Sorbitol has a strong glycogenic effect in the fasted diabetic rat (Stetten & Stetten, 1951; Todd et al., 1939). The polyol has a more efficient antiketogenic effect in liver slices of fasted rats than glucose or fructose (Blakley, 1951, 1952), and behaved similarly in the intact rat (Todd, 1954). Sorbitol is oxidized to fructose by a DPN-linked polyol dehydrogenase (Embden & Griesbach, 1914; McCorkindale & Edson, 1954).

The intravenous infusion of sorbitol in rabbits caused a prompt fructosaemia and a variable secondary glucosaemia occurred later (Seeberg et al., 1955). Experiments in rats (Wick et al., 1955) given sorbitol-U-^{14}C support the view that at least two pathways exist for the oxidation of sorbitol in the body: (a) oxidation after conversion to glucose, and (b) the direct oxidation of the primarily formed fructose (Hers, 1955). Sorbitol was not metabolized by hepatectomized animals (Wick & Drury, 1951).

Sorbitol has a sparing effect for some B-vitamins (thiamine, pyridoxine, biotin) (Morgan & Yudkin, 1957, 1961a).

It has been shown that the slowly absorbed polyol promotes the proliferation of intestinal bacteria which synthesize B-vitamins (Griem & Lang, 1960; Griffith et al., 1957). When administered in amounts of 20 to 40 g daily, sorbitol increased the excretion of thiamine, riboflavin and N^1-methylnicotinamide in man (Treon, 1963).

According to Heinrich & Staak (1960), sorbitol is an inhibitor of the intestinal absorption of vitamin B_{12} in man, and in the rat, guinea-pig and pig, as shown by administering physiological doses of the ^{60}Co-labelled vitamin. On the other hand, Wolff & Herbeuval (1962), after Chow et al. (1956) and Greenberg et al. (1957), have shown that sorbitol exerts an important stimulating action on digestive absorption of vitamin B_{12} in man. The increase of vitaminaemia, that of the faecal contents, that of urinary elimination, and of its storage in the kidney in rats have been studied in succession in order to show up this action, the mechanism which is unknown.

In rats, sorbitol enhances the absorption of iron (Herndon et al., 1958) and also that of strontium.

Rats fed sorbitol, at a level of 16% in their diet for three months showed persistently high calcium absorption and retention with heavy citric acid excretion. Thickening of the skeleton was produced (Founier et al., 1967). These effects are similar to those produced by lactose in the same conditions. In feeding experiments on rats, sorbitol showed the same caloric value as glucose (Morgan & Yudkin, 1961b).

It has been shown that fructose present in the seminal vesicles is formed from glucose via sorbitol (Hers, 1960). The same pathway is present in the lens (Van Heyningen, 1959; Kuck, 1961; Kinoshita et al., 1963). The finding of relatively high levels of free fructose in mammalian nerve led to the identification of sorbitol in nervous tissue (Sherman & Stewart, 1966).

TOXICOLOGICAL STUDIES

Acute toxicity

Animal	Route	LD_{50} (mg/kg bw)	Reference
Mouse - male	oral	23 200	Treon, 1963
Mouse - female	oral	25 700	
Rat - male	oral	17 500	
Rat - female	oral	15 900	
Rat - male	i.v.	7 100	
Rat - female	i.v.	7 300	

Short-term studies

Rat

Five groups of 10 female Charles River rats, weighing 150 to 210 g, were administered, by stomach tube, three times daily for three days sorbitol at respective doses of 0.675. 1.35. 2.70, 3 (conc. 45%) 3 (conc. 25%) g/kg bw in aqueous solution at the concentration w/vol of 90% (undiluted sorbitol solution USP) for the three lower doses and of 45 and 25% for the highest one. Parallel experiments were made with glycerol. The animals were sacrificed about one hour after the second dose on the third day. The stomach and attached portion of the duodenum of each animal were removed and the mucosal surface was examined grossly and microscopically to estimate the degree of irritation. The observations made clearly indicate that, at equivalent undiluted oral doses, glycerol produced gastrointestinal irritation to a much greater degree than did sorbitol. The degree of severity of the irritant effect of each compound was dose dependent and was reduced by dilution of the dose (Staples et al., 1967).

Rabbit

The intravenous infusion of sorbitol together with amino acids for 10 days to three rabbits between 2.9 and 3 kg of weight was associated with a positive nitrogen balance. The histopathological examination of the organs showed no abnormalities Griem & Lang, 1960).

Dog

Sorbitol was excreted by glomerular filtration; the renal clearance in the dog was found to be 74-77 ml/min (Smith et al.,

1940).

Injection of 2.5 ml/kg bw of a 50% solution in different conditions (six dogs weighing from 6.4 to 18.9 kg) had a marked diuretic effect for about one hour (Leimdorfor, 1954).

Two adult mongrel dogs of either sex, weighing 8.9 to 16 kg, were given by stomach tube, three times daily for three days, sorbitol in 90% w/vol aqueous solution, at respective doses of 0.675 and 1.35 g/kg bw. Only at the highest dose, stomach appears hyperaemic. As in rats, glycerol, administered in the same conditions, produced a much more severe irritation (Staples et al., 1967).

Long-term studies

Rat

Fifteen weanling male rats Wistar given sorbitol at levels of 10% or 15% in the diet for 17 months showed no evidence of deleterious effect on weight gain, reproduction, lactation or histopathological appearances of the main organs. The only difference with the controls was slight diarrhoea and, consequently, a retardation in growth, with rapid return to the normal. In supplement, a reproduction study made on 30 rats (equally divided by sex) and extended over four generations did not reveal any abnormalities (Le Breton, 1956).

OBSERVATIONS IN MAN

In amounts of 40 g daily, spread throughout the day's intake of food, sorbitol was well tolerated for a long period by human subjects (Treon, 1963). A total of 25 g daily in two doses caused no laxative effect in 86 subjects. In about 5% of these subjects a somewhat increased amount of gas appeared in the bowel (Peters & Lock, 1958). Quantities greater than 50 g daily were laxative. This effect was presumably due to the relatively slow rate at which sorbitol was absorbed from the small bowel (Tacquet, 1957).

Sorbitol has been used for many years in the diet, especially of diabetics. There have been no indications of significant harmful effects.

Comments:

Considering the biochemical and toxicological data obtained on animals and man and the known facts about the nutritional properties of sorbitol, there appears to be no need for the limitation of sorbitol as a food additive on toxicological grounds.

EVALUATION

Estimate of acceptable daily intake for man

Not limited*

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See Also:
Toxicological Abbreviations
Sorbitol (FAO Nutrition Meetings Report Series 40abc)
Sorbitol (WHO Food Additives Series 13)
Sorbitol (JECFA Evaluation)

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SAFETY EVALUATION OF CERTAIN FOOD ADDITIVES AND CONTAMINANTS

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SATURATED ALIPHATIC ACYCLIC LINEAR PRIMARY ALCOHOLS, ALDEHYDES, AND ACIDS

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1. EVALUATION

1.1 Introduction

The Committee evaluated a group of 38 flavouring agents that includes selected saturated aliphatic acyclic linear primary alcohols, aldehydes and acids of chain length C_{1-18} using the Procedure for the Safety Evaluation of Flavouring Agents (the "Procedure") (see Figure 1 in the Introduction to the section on Substances Evaluated Using the Procedure for the Safety Evaluation of Flavouring Agents and Table 1 in this section).

Several substances in the group had been evaluated previously by the Committee. At the seventeenth meeting a group ADI "not limited" was allocated to acetic acid and its potassium and sodium salts, an ADI "not limited" was allocated to propionic acid, and a group ADI of 0-3 mg/kg bw was allocated to formic acid and ethyl formate (Annex 1, reference 32). A group ADI of 0-0.1 mg/kg bw was established for ocatanal and nonanal, singly or in combination, at the twenty-eighth meeting (Annex 1, reference 66). At the twenty-ninth meeting, ADIs "not specified" were allocated to the aluminium, ammonium, calcium, magnesium, potassium, and sodium salts of lauric, myristic, palmitic, and stearic acids (Annex 1, reference 70). At that meeting, the Committee did not establish ADIs for myristic, palmitic or stearic acids owing to lack of information on the manufacture or use of the food-grade material, but noted that these substances are normal constituents of coconut oil, butter and other edible oils. ADIs have not been allocated to butyl alcohol, decanal or propyl alcohol because the data were considered to be inadequate (Annex 1, references 38, 14, and 56, respectively).

One substance structurally related to the group, ethyl alcohol, was evaluated as a flavouring agent at the forty-sixth meeting of the Committee (reference Annex 122). The Committee determined that ethyl alcohol posed no safety concern at its current level of intake when used as a flavouring agent.

1.2 Estimated daily *per capita* intake

The total annual production volume of the 38 substances from their use as flavouring substances is approximately 2100 tonnes in the USA (NAS, 1987). In the USA, approximately 90% of the total volume (NAS, 1987) is accounted for by acetic acid, which includes uses (aciduvant or solvent) in food other than flavour use. Data are not available on the specific flavour use of acetic acid in Europe. Disregarding the annual volume of acetic acid, the total reported annual volume of the remaining 37 aliphatic substances is approximately 200 tonnes from use as flavouring substances in the USA (NAS, 1987) and 300 tonnes in Europe (IOFI, 1995). In the unlikely event that all of the substances in this group were simultaneously consumed on a daily basis, the estimated daily *per capita* intakes in Europe and the USA would be approximately 40 mg per day and 30 mg per day, respectively (excluding acetic acid and propionic acid which have ADIs "not limited"). According to the European and USA production statistics and derived intakes, acetaldehyde, butyl alcohol and butyric acid are the major flavouring substances in this group. Acetaldehyde and butyl alcohol constitute about 46% of the daily *per capita* intake of flavouring agents in this group in the USA and acetaldehyde and butyric acid constitute about 50% of the daily intake in Europe. Other flavouring agents in this group that are used at higher intake levels (i.e., >1800 µg per day) include butyric acid, propionic acid, propyl alcohol and stearic acid in the USA and octanoic acid, hexanoic acid, valeraldehyde, butyl alcohol and hexyl alcohol in Europe (Table 1).

Linear saturated aliphatic alcohols, aldehydes and acids are ubiquitous in nature. Low molecular weight alcohols and acids have been detected in almost every known fruit and vegetable (CIVO-TNO, 1996). However, there are relatively few reports for the natural occurrence of the corresponding aldehydes. In the USA, the available quantitative data indicate that the dietary consumption of saturated linear aliphatic alcohols, aldehydes and acids from naturally occurring sources exceeds the consumption from their use as flavouring substances (Stofberg & Kirschman, 1985; Stofberg & Grundschober, 1987).

1.3 Absorption, metabolism and elimination

Linear aliphatic acyclic alcohols (Lington & Bevan, 1994), aldehydes (Brabec, 1993) and carboxylic acids (von Oettingen, 1960; Dawson *et al.*, 1964; Katz & Guest, 1994) are absorbed through the gastrointestinal tract. Plasma half-lives are difficult to measure since many low molecular weight alcohols (e.g., ethanol), aldehydes and carboxylic acids (e.g., acetate and propionate) are endogenous in humans (Lington & Bevan, 1994). Acetaldehyde has been detected in whole blood (<0.2 mg/litre) and acetate is a blood buffer (Tietz, 1986).

The flavouring agents in this group of selected saturated aliphatic linear alcohols, aldehydes and acids are all metabolized via fatty acid and tricarboxylic acid pathways. Additional information can be found in introduction to this chapter on flavouring agents.

1.4 Application of the procedure for the safety evaluation of flavouring agents

Step 1. All of the flavouring agents in this group were classified in structural class I (Cramer *et al.*, 1978).

Step 2. All of the flavouring agents in this group are known or can be readily predicted to be efficiently metabolized to substances harmless to humans at the estimated intakes of the flavouring agents.

Step A3. Twenty-seven substances in this group fall below the human intake threshold for class I (i.e., 1800 µg per day) at their current levels of intake; therefore, these substances were determined to be of no safety concern on the basis of their structural class and low levels of estimated intake.

Step A4. Eleven substances in this group exceeded the human intake threshold for class I. In all cases, the substances can be predicted to undergo complete metabolism to endogenous products via the fatty acid and tricarboxylic acid pathways. In the opinion of the Committee the endogenous levels of metabolites from these substances would not give rise to perturbations outside the physiological range. Therefore, these 11 substances were also determined to be of no safety concern based on their structural class and known metabolism.

Table 1 summarizes the evaluation of the 38 saturated aliphatic, acyclic linear primary alcohols, aldehydes and acids using the Procedure.

Table 1. Summary of results of safety evaluations of saturated aliphatic acy

Step 1: All of the substances in the group are in structural class I, the human
Step 2: All of the substances in this group are metabolized to innocuous product

Substance	Step A3 Does intake exceed the human intake threshold?[1] Intake estimates (µg per person per day)	Step A4 Endogenous or metabolized to endogenous substances?	Commen
Formic acid	No USA: 160 Europe: 800	N/R	Formic is a n metabo
Acetaldehyde	Yes USA: 9 700 Europe: 11 000	Yes	Acetal is met acetal
Acetic acid	Yes USA: 360 000 Europe: N/D[2]	Yes	Acetic acetyl into pro
Propyl alcohol	Yes USA: 2700 Europe: 420	Yes	Propyl which metabo
Propionaldehyde	No USA: 140	N/R	See pr

	Europe: 33			
Propionic acid	Yes USA: 5200 Europe: 1100		Yes	See pr
Butyl alcohol	Yes USA: 8100 Europe: 1900		Yes	Butyl corres the ac tricar

Table 1. Continued...

Substance	Step A3 Does intake exceed the human intake threshold?[1] Intake estimates (µg per person per day)	Step A4 Endogenous or metabolized to endogenous substances?	Commen
Butyraldehyde	No USA: 17 Europe: 26	N/R	See bu
Butyric acid	Yes USA: 5 900 Europe: 10 000	Yes	See bu
Amyl alcohol	No USA: 44 Europe: 97	N/R	Amyl a aldehy acid; tricar
Valeraldehyde	Yes USA: 8.8 Europe: 3000	Yes	See am
Valeric acid	No USA: 850 Europe: 140	N/R	See am
Hexyl alcohol	Yes USA: 800 Europe: 1900	Yes	Hexyl corres oxidiz acid a
Hexanal	No USA: 260 Europe: 780	N/R	See he
Hexanoic acid	Yes USA: 1300 Europe: 3500	Yes	See he

Table 1. Continued...

Substance	Step A3 Does intake exceed the human intake threshold?[1] Intake estimates (µg per person per day)	Step A4 Endogenous or metabolized to endogenous substances?	Commen

Heptyl alcohol	No USA: 7 Europe: 12	N/R	Heptyl corres oxidiz acid a	
Heptanal	No USA: 3.2 Europe: 200	N/R	See he	
Heptanoic acid	No USA: 5.3 Europe: 170	N/R	See he	
1-Octanol	No USA: 32 Europe: 230	N/R	1-Octa aldehy acid; tricar	
Octanal	No USA: 90 Europe: 170	N/R	See 1-	
Octanoic acid	Yes USA: 650 Europe: 3800	Yes	See 1-	
Nonyl alcohol	No USA: 2.1 Europe: 8.1	N/R	Nonyl corres oxidiz acid a	

Table 1. Continued...

Substance	Step A3 Does intake exceed the human intake threshold?[1] Intake estimates (µg per person per day)	Step A4 Endogenous or metabolized to endogenous substances?	Commen
Nonanal	No USA: 17 Europe: 130	N/R	See no
Nonanoic acid	No USA: 63 Europe: 64	N/R	See no
1-Decanol	No USA: 7 Europe: 290	N/R	1-Deca corres oxidiz acid p pathwa
Decanal	No USA: 61 Europe: 288	N/R	See 1-

Decanoic acid	No USA: 980 Europe: 1400		Yes	See 1-decano
Undecyl alcohol	No USA: 11 Europe: 0.9		N/R	Undecy corres oxidiz acid a
Undecanal	No USA: 1.5 Europe: 480		N/R	See un

Table 1. Continued...

Substance	Step A3 Does intake exceed the human intake threshold?[1] Intake estimates (µg per person per day)	Step A4 Endogenous or metabolized to endogenous substances?	Commen
Undecanoic acid	No USA: 8.8 Europe: 4.6	N/R	See un
Lauryl alcohol	No USA: 80 Europe: 170	N/R	Lauryl corres oxidiz acid a
Lauric aldehyde	No USA: 21 Europe: 52	N/R	See la
Lauric acid	No USA: 1200 Europe: 590	N/R	See la
Myristaldehyde	No USA: 25 Europe: 9.4	N/R	Myrist corres acid a
Myristic acid	No USA: 72 Europe: 160	N/R	See my
1-Hexadecanol	No USA: 0.2 Europe: 3.6	Yes	1-Hexa corres oxidiz acid a

Table 1. Continued...

Substance	Step A3 Does intake exceed the human intake threshold?[1] Intake estimates (µg per person per day)	Step A4 Endogenous or metabolized to endogenous substances?	Commen

Palmitic acid		No	N/R	beta-O 2-carb acid c
	USA:	234		
	Europe:	89		
Stearic acid		Yes	Yes	beta-O 2-carb acid c
	USA:	1900		
	Europe:	58		

[1] N/R: Not required for evaluation because consumption of the substance was det
[2] N/D: No intake data reported.

1.5 Consideration of combined intakes from use as flavouring agents

In the unlikely event that all of the substances in this group of flavouring agents were simultaneously consumed on a daily basis, the estimated daily *per capita* intake in Europe and the USA would exceed the human intake threshold for substances in class I. All of the substances in this group and their metabolites are innocuous and endogenous, and their combined intake was judged by the Committee not to give rise to perturbations outside the physiological range.

1.6 Conclusions

The Committee concluded that the substances in this group would not present safety concerns at the current levels of intake.

No toxicity data were required for the application of the Procedure. The Committee noted that the available toxicity data were consistent with the results of the safety evaluation using the Procedure. In cases where ADIs were previously established, these ADIs were maintained at the present meeting.

2. RELEVANT BACKGROUND INFORMATION

2.1 Intake data

The most recent data on the annual production volumes of the flavouring agents in this group in the USA and in Europe are given in Table 2. The estimates of intake were calculated assuming under-reporting of the production data and consumption by 10% of the population, as indicated in the footnote to Table 2.

Table 2. Annual production and estimated per capita intake of saturated aliphatic acyclic linear primary alcohols, aldehydes and acids in the USA and Europe

Substance	Most recent annual production volume[1] tonnes	Daily Per Capita Intake[2] ("eaters only")	
		µg/day	µg/kg bw/day
1. Formic acid			
USA	0.84	160	2.7

Europe	5.6	800	13
2. Acetaldehyde			
USA	51	9700	160
Europe	78	11 000	180

Table 2. Continued...

Substance	Most recent annual production volume[1] tonnes	Daily Per Capita Intake[2] ("eaters only")	
		µg/day	µg/kg bw/day
3. Acetic acid			
USA[3]	1910	360 000	6000
Europe	0	0	0
4. Propyl alcohol			
USA	14	2700	45
Europe	2.9	420	6.9
5. Propionaldehyde			
USA	0.72	140	2.3
Europe	2.29	330	5.5
6. Propionic acid			
USA	27	5200	86
Europe	8.0	1100	19
7. Butyl alcohol			
USA	43	8100	140
Europe	13	1900	32
8. Butyraldehyde			
USA	0.09	17	0.29
Europe	0.19	26	0.44
9. Butyric acid			
USA	31	5900	98
Europe	73	10 000	170
10. Amyl alcohol			
USA	0.23	43	0.73
Europe	0.68	96	1.6
11. Valeraldhyde			
USA	0.046	8.7	0.15
Europe	21	3000	50
12. Valeric acid			
USA	4.4	850	14
Europe	0.97	140	2.3
13. Hexyl alcohol			
USA	4.3	810	14
Europe	13	1900	31

Table 2. Continued...

Substance	Most recent annual production volume[1] tonnes	Daily Per Capita Intake[2] ("eaters only")	
		µg/day	µg/kg bw/day
14. Hexanal			
USA	1.4	260	4.3
Europe	5.4	780	13
15. Hexanoic acid			
USA	6.8	1300	22
Europe	25	3500	59
16. Heptyl alcohol			
USA	0.037	7.0	0.12
Europe	0.081	11	0.19
17. Heptanal			
USA	0.017	3.2	0.05
Europe	1.5	210	3.5
18. Heptanoic acid			
USA	0.028	5.3	0.09
Europe	1.2	170	2.9
19. 1-Octanol			
USA	0.17	32	0.54
Europe	1.6	230	3.9
20. Octanal			
USA	0.47	90	1.5
Europe	1.2	170	2.8
21. Octanoic acid			
USA	3.43	650	11
Europe	27	3800	63
22. Nonyl alcohol			
USA	0.011	2.1	0.03
Europe	0.057	8.1	0.14
23. Nonanal			
USA	0.09	17	0.29
Europe	0.91	130	2.2
24. Nonanoic acid			
USA	0.33	63	1.0
Europe	0.45	64	1.1

Table 2. Continued...

Substance	Most recent annual production volume[1] tonnes	Daily Per Capita Intake[2] ("eaters only")	
		µg/day	µg/kg bw/day

25. 1-Decanol			
USA	0.037	7.0	0.12
Europe	0.2	28	0.48
26. Decanal			
USA	0.32	61	1.0
Europe	2.0	290	4.9
27. Decanoic acid			
USA	5.1	980	16
Europe	9.9	1400	24
28. Undecyl alcohol			
USA	0.06	11	0.19
Europe	0.006	0.86	0.01
29. Undecanal			
USA	0.008	1.5	0.03
Europe	3.4	480	8.0
30. Undecanoic acid			
USA	0.046	8.7	0.15
Europe	0.032	4.6	0.08
31. Lauryl alcohol			
USA	0.42	80	1.3
Europe	1.2	170	2.8
32. Lauric aldehyde			
USA	0.011	21	0.35
Europe	0.36	52	0.86
33. Lauric acid			
USA	6.5	1200	21
Europe	4.2	590	9.9
34. Myristaldehyde			
USA	0.13	25	0.41
Europe	0.066	9.4	0.16
35. Myristic acid			
USA	0.38	72	1.2
Europe	1.1	150	2.6

Table 2. Continued...

Substance	Most recent annual production volume[1] tonnes	Daily Per Capita Intake[2] ("eaters only")	
		µg/day	µg/kg bw/day
36. 1-Hexadecanol			
USA	0.0009	0.17	0.003
Europe	0.025	3.6	0.06
37. Palmitic acid			

USA	1.2	230	3.9
Europe	0.63	89	1.5
38. Stearic acid			
USA	9.9	1900	31
Europe	0.41	58	0.97
Totals			
USA	2110	400 000	6700
Europe	300	43 000	720
Total excluding acetic acid			
USA	200	38 000	640

[1] USA: National Academy of Science (NAS, 1987) Evaluating the safety of food chemicals. Washington, DC. Europe: International Organization of the Flavour Industry (IOFI, 1995) European inquiry on volume of use. Private communication to FEMA.

[2] Intake calculated as follows: [[(annual volume, kg) x (1 x 109 µg/kg)]/ [population x 0.6 x 365 days]], where population (10%, "eaters only") = 24 x 106 for the USA and 32 x 106 for Europe; 0.6 represents the assumption that only 60% of the flavour volume was reported in the survey [NAS, 1987; IOFI, 1995]. Intake (µg/kg bw/day) calculated as follows: [µg/day/body weight], where body weight = 60 kg. Slight variations may occur from rounding off.

[3] The USA production volume reported for acetic acid includes use of acetic acid as a solvent by the flavour and food industries.

2.2 Toxicological studies

2.2.1 Acute toxicity

Linear aliphatic alcohols, aldehydes and carboxylic acids exhibit low acute toxicity. For this group of saturated, aliphatic, acyclic, linear primary alcohols, aldehydes and acids used as flavouring agents, studies in rodents indicate LD_{50} values typically > 1 g/kg bw for 36 of the 38 substances. Generally, LD_{50} values of aldehydes and carboxylic acids having a carbon chain length greater than 3 are >2500 mg/kg bw. LD_{50} values were not available for undecanoic acid and palmitic acid. The acute toxicity studies that were available are summarized in Table 3.

Table 3. Acute toxicity studies for Saturated Aliphatic Acyclic Linear Prim

Substance	Species	Sex[1]	Route	LD_{50} (mg/kg bw)	Re
Formic acid	mouse	NR	oral	1100	M
Acetic acid	mouse	NR	gavage	4960	W
	rat	NR	gavage	3310	W
	rat	NR	oral	3530	S

Propionic acid	rat	male	gavage	4290	S
Butyric acid	rat	male & female	oral	8790	S
	rat	NR	oral	2940	S
Valeric acid	rat	NR	oral	1844	S
Hexanoic acid	rat	male	gavage	6440	S
	rat	male	gavage	3000	L
Heptanoic acid	rat	NR	oral	7000	G
Octanoic acid	rat	male	gavage	1283	S
	rat	male & female	gavage	10 080	J
Nonanoic acid	rat	NR	oral	3200	F
Decanoic acid	rat	male	gavage	3301	S
Lauric acid	mouse	NR	oral	1238	S
Myristic acid	rat	NR	oral	>5000	M
Stearic acid	rat	NR	oral	>5000	M
Acetaldehyde	rat	NR	oral	1930	S
Propionaldehyde	rat	NR	oral	1110	S
Butyraldehyde	rat	NR	oral	5890	S
Valeraldehyde	rat	male	gavage	3000-6400	S
Hexanal	rat	male	gavage	7740	S
	rat	male & female	oral	4890	S
Heptanal	rat	NR	oral	>5000	M
Octanal	rat	male	gavage	4600	S
Nonanal	rat	male & female	gavage	>5000	S
Decanal	mouse	NR	gavage	>4175	J
	rat	male & female	gavage	>3332	J
Undecanal	rat	male & female	gavage	>5000	S
Lauric aldehyde	rat	male & female	gavage	>23 100	C

Table 3. Continued...

Substance	Species	Sex[1]	Route	LD_{50} (mg/kg bw)	Re
Myristaldehyde	rat	male & female	gavage	>4000	L
	rat	NR	oral	4500	S
Propyl alcohol	rat	male & female	gavage	6500	J
	rat	male & female	gavage	6500	T
	rat	NR	oral	5000	L
	rat	male & female	oral	1870	S
	rat	NR	oral	5400	R
Butyl alcohol	rat	male & female	gavage	2510	J
	rat	male & female	gavage	790 (female); 2020 (male)	P
	rat	NR	oral	4360	S
Amyl alcohol	rat	male & female	gavage	3030	J
	rat	NR	oral	5730	C
Hexyl alcohol	rat	male & female	gavage	720 (female); 1800 (male)	P
	rat	NR	oral	4590	S
Heptyl	mouse	NR	oral	4300	Y
1-Octanol	rat	NR	oral	4135	L
Nonyl alcohol	mouse	NR	oral	19 000	Y
1-Decanol	rat	NR	oral	9800	S
Undecyl alcohol	rat	male	gavage	3000	S
Lauryl alcohol	rat	male & female	oral	1280	L
1-Hexadecanol	rat	NR	oral	8400	C

[1] NR = not reported.

2.2.2 Short-term and long-term toxicity and carcinogenicity

Although toxicity studies were not required to apply the Procedure to this group of flavouring agents, multiple dose toxicity studies lasting more than 21 days in were available for approximately half of the 38 substances in the group (see Table 4). The lowest NOELs derived from these studies were 50-60 mg/kg bw per day, reported for heptyl alcohol and propyl alcohol. Few multiple dose studies are available for aldehydes due to their volatility and reactivity. Not all of these studies were designed to provide comprehensive toxicological assessments of the substances tested; however, consideration of these studies did not raise concerns regarding the safe use of the substances in this group as flavouring agents.

Several studies were conducted to evaluate the irritant effects of alcohols and acids on the forestomach of the rat. There are several substances in this group for which data indicate that high doses given to rats cause lesions of the forestomach. These effects are not considered to be relevant to the human ingestion of these substances as flavouring agents in foods.

A brief summary of the available data on substances not previously evaluated by the Committee is given below.

2.2.2.1 Acetaldehyde

A NOEL of 125 mg/kg bw per day was reported for acetaldehyde added to the drinking-water of male and female rats for 4 weeks at level of 0, 25, 125 or 625 mg/kg bw per day (Til *et al.*, 1988); the only treatment-related effect was hyperkeratosis of the forestomach at 625 mg/kg bw per day. No adverse effects were seen when acetaldehyde in drinking-water at a daily intake level of 0.5 mg/kg bw was given to rats (Amirkanova & Latypova, 1967).

2.2.2.2 Propyl alcohol

No adverse effects on the liver were observed when male rats were give 1 or 2 M solutions of propyl alcohol (approximately 60 or 120 mg/kg bw per day) as a drinking-water substitute for 6 or 2 months, respectively. Mallory bodies were reported in some animals (Hillbom *et al.*, 1974a). In groups of rats given a 1 M solution of propyl alcohol as their sole source of drinking-water for 4 months, a lower ratio of weight gain to caloric intake compared to controls was observed, but there were no effects on the liver. A NOEL of 60 mg/kg bw per day was determined in this study (Hillbom *et al.*, 1974b).

In a study of the factors affecting the distribution of propionic acid in the forestomach of rats, no adverse effects on the forestomach mucosa were reported when male rats were fed a pellet diet containing 0 or 2-3% propionic acid (about 3800-5800 mg/kg bw per day) for 12 weeks (Bueld & Netter, 1993).

Table 4. Short-term and long-term toxicity studies for saturated aliphatic

Substance	Species, sex	Route	Time
Formic acid	rat, male & female	oral	2 years
Acetic acid	rat, male	oral	63 days
Propionic acid	rat, male	oral	24 weeks
Butyric acid	rat	oral	up to 500 day
Hexanoic acid	rat, male	diet	3 weeks
Decanoic acid	rat	diet	150 days
Lauric acid	rat, male	diet	18 weeks
10-Undecenoic acid[2]	rat	gavage	6-9 months
Palmitic acid	rats	diet	150 days
Stearic acid	mice	oral	3 weeks
Acetaldehyde	rats, male & female	oral	4 weeks
Hexanal	rat, male & female	oral	28 days
Myristaldehyde	mice	diet	130 days
Propyl alcohol	rat, male	oral	4 months
Butyl alcohol	rat, male	oral	28 days
Amyl alcohol	rat, male & female	oral	13 weeks
Hexyl alcohol	dog, male & female	oral	13 weeks
Heptyl alcohol	rabbit	gavage	6 months
1-Octanol	mice	gavage	one month
Nonyl alcohol	rabbit	diet	67 days
1-Hexadecanol	rat, male & female	diet	13 weeks

[1] A NOEL (no-observed-effect level) reported in this table as "greater than" (>) highest dose level in the study, and therefore an actual NOEL was not obtained.
[2] A structurally related substance.

2.2.2.3 Butyl alcohol

No adverse effects were observed when 6.9% butyl alcohol and 25% sucrose (about 5.6 mg/kg bw per day butyl alcohol) were added to the drinking-water of male rats for 13 weeks (Wakabayashi *et al.*, 1984).

In rats given control diets or diets with 0.69, 1.38, 2.75 or 5.5% butyl alcohol (equivalent to 690-5500 mg/kg bw), a statistically significant increase in the ratio of liver-to-body weight was reported in males at all but the lowest dose tested and in females only at the highest dose (PPG, 1991a).

In a 28-day study on male rats fed diets containing 0, 1000, 3500 or 10 000 mg butyl alcohol kg feed (about 90-940 mg/kg bw per day) in 2% corn oil, no deaths, gross lesions at necropsy or differences in liver and kidney weights were reported; there was a statistically significant increase in the ratio of adrenals-to-body weight at all doses compared to controls (Bio-Fax, 1969).

2.2.2.4 Butyric acid

In a study of the development of gastric lesions with diets containing fatty acids, rats fed a rice diet with 1% butyric acid (equivalent to 500 mg/kg bw per day) that was gradually increase to 10% (equivalent to 5000 mg/kg bw per day) over a period of 500 days

had forestomach lesions with prominent keratin cysts after being fed the diet for more than 50 days. No lesions were observed in the glandular stomach (Mori, 1953).

2.2.2.5 Amyl alcohol

Amyl alcohol given to rats by gavage for 13 weeks at a dose level of 1000 mg/kg bw per day produced no effects on body weight gain, food or water consumption, haematological values, serum and urine analyses, renal function, organ weight or histopathology (Butterworth *et al.*, 1978).

2.2.2.6 Valeric acid

Rats fed 5% valeric acid (about 2500 mg/kg bw per day) in a rice diet for 115-150 days had papillomatous growths in the forestomach (Mori, 1953).

2.2.2.7 Hexyl alcohol

Two groups of male and female rats were fed hexyl alcohol at dietary levels of 0.25 and 0.50% for 13 weeks; a third group was fed 1% (reported to be equivalent to 577 mg/kg bw per day) for weeks 1-10, then 2, 4 and 6% for weeks 11, 12 and 13, respectively. Food consumption was decreased in the high-dose females, but no significant haematological changes, differences in urine analyses or histopathological effects were observed (Eibert, 1992).

In a 13-week study, hexyl alcohol at levels of 0.5 and 1% in the diet, or at a dose level of 1000 mg/kg bw per day in gelatin capsules, was given to dogs. At a dose of 1000 mg/kg bw per day, 4 out of 5 dogs died. Haematology, serum chemistry and urine analyses revealed no differences in treated dogs relative to controls. There was gastrointestinal inflammation in the mid- and high-dose groups. Congestion of the viscera and testicular atrophy were observed at the high dose. A NOAEL of 1%, which corresponds to a daily intake of 230-695 mg/kg bw, was determined from this study (Eibert, 1992).

2.2.2.8 Hexanal

No adverse effects were reported when hexanal was given to rats in drinking-water at concentrations of 1, 10, 100 and 1000 mg/litre (calculated to provide doses of about 0.1, 1.2, 12.6 and 124.7 mg/kg bw per day) for 4 weeks (Komsta *et al.*, 1988).

2.2.2.9 Hexanoic acid

No effects on hepatic peroxisomes or peroxisomal enzymes were induced in male rats fed hexanoic acid in the diet at a level of 2% for 3 weeks (Moody & Reddy, 1978).

In rats fed 10% (about 5000 mg/kg bw per day) hexanoic acid for 150 days, no changes in the glandular stomach or forestomach were observed (Mori, 1953).

2.2.2.10 Heptyl alcohol

Heptyl alcohol, administered intragastrically to mice, in the form of a solution or suspension over a one-month period, showed no cumulative effects at a dose of 150 mg/kg bw per day (Voskoboinikova,

1966). The NOEL in rabbits given 0, 1.4, 14 or 50 mg/kg bw per day heptyl alcohol by gavage in sunflower oil for 6 months was 50 mg/kg bw per day (Voskoboinikova, 1966).

2.2.2.11 1-Octanol

No cumulative effects were observed in a study in which 1-octanol was administered intragastrically to mice in the form of a solution or suspension over a one-month period at a dose of 180 mg/kg bw per day (Voskoboinikova, 1966).

2.2.2.12 Octanoic acid

Rats gavaged on gestation days 6-15 with octanoic acid in corn oil at dose levels of 0, 1125 or 1500 mg/kg bw per day exhibited maternal toxicity and maternal mortality. There was an decrease in the number of live pups on post-gestational day 6, but no developmental toxicity was reported (Narotsky *et al.*, 1994).

2.2.2.13 Nonyl alcohol

No adverse effects were reported when an isomeric mixture of nonyl alcohol, 2-methyl-1-octanol and 3-methyl-1-octanol, calculated to provide a daily intake level of 148 mg/kg bw, was added to the diet of rabbits for 67 days of an 83-day period (Treon, 1963).

2.2.2.14 Decanoic acid

In a study of gastric lesions, 10% decanoic acid (about 5000 mg/kg bw per day) in the diet of rats for 150 days resulted in no observable changes in the forestomach or glandular stomach (Mori, 1953).

2.2.2.15 Undecanoic acid

In a study with undecanoic acid, there was a marked inhibitory effect on growth in rats give 2.5% (about 1250 mg/kg bw per day) for 8 weeks (Newell *et al.*, 1949).

2.2.2.16 Myristaldehyde

No adverse effects on mortality and body and organ weights were reported when myristaldehyde was fed to mice at a level of 166 mg/kg bw per day for 130 days (Galea *et al.*, 1965).

2.2.2.17 1-Hexadecanol

Two groups of male and female rats were fed 1-hexadecanol for 13 weeks at dietary levels of 1 or 2.5%; a third group was fed 5% for weeks 1-10, 7.5% for week 11, and 10% for weeks 12 and 13. Decreased food consumption (in females at the intermediate and high dose) and/or body weights (in males and females at the high dose and in females only at the intermediate dose) were observed at various times in rats in the intermediate and high-dose groups. No significant haematological findings, changes in urinalyses or pathological effects were reported between control and treated animals. A NOAEL of 1% (equal to 577 mg/kg bw per day) was determined from this study (Eibert, 1992).

In a 13-week study in dogs, at levels of 0, 0.5, 1 or 3%, no effects on body weight, organ weight or food consumption were

reported. No significant haematological findings, changes in urinalyses or gross pathological effects were reported between control and treated animals; however, serum glutamate oxaloacetate transaminase levels were elevated at all three doses. A NOAEL of 3% (equal to 807 mg/kg bw per day) was determined from this study (Eibert, 1992).

In addition to the multiple dose studies described above, the Committee was aware of the results of a long-term inhalation study in which hamsters that were administered acetaldehyde developed an excess of upper respiratory tract tumours (Kruysse et al., 1975). Respiratory lesions were also observed in 2-week and 13-week whole body

inhalation studies on formic acid (National Toxicology Program, 1992). No systemic effects resulted, but the NTP recommended caution in extrapolating the results of these studies to man because humans do not metabolize formate to CO_2 as rapidly as rodents. The Committee considered that, under conditions of use of acetaldehyde and formic acid as flavouring agents, these observations were not predictive of a response in humans because these substances are endogenous and oral ingestion from their use as flavouring agents is low.

2.2.3 Genotoxicity

In vitro and *in vivo* genotoxicity studies for the flavouring agents in this group are listed in Tables 5 and 6. Saturated aliphatic acyclic linear primary alcohols, aldehydes, and carboxylic acids generally exhibited consistent negative results in the Ames assay, the unscheduled DNA synthesis test, and the *in vitro* or *in vivo* mouse micronucleus test. However, genotoxic activity has been reported for some low molecular weight alcohols, carboxylic acids and aldehydes in varied assays, including the sister chromatid exchange (SCE) assay, the chromosomal aberration test and the forward mutation assays with mouse lymphoma and Chinese hamster lung cells.

The positive results in *in vitro* genotoxicity assays for aliphatic aldehydes is not surprising in light of the recognized reactivity of the aldehyde functional group. Acetaldehyde induced an increase in SCE in adult human lymphocytes (He & Lambert, 1985) and human peripheral lymphocytes (Helander & Lindahl-Kiessling, 1991). Acetaldehyde and propionaldehyde induced an increase in SCE in Chinese hamster embryonic diploid cells (Furnus *et al.*, 1990). However, aldehydes exhibit a short plasma half-life and are efficiently oxidized to the corresponding acids, which are metabolized in the fatty acid or citric acid pathways. These are important *in vivo* conditions that are difficult to establish in the above-mentioned *in vitro* assays. In one *in vivo* test, there was no evidence of an increase in micronucleated polychromatic erythrocytes in the bone marrow cells of $B6C3F_1$ mice given a single intraperitoneal injection of 95 or 100 mg acetaldehyde/kg bw. Dose levels of 190 mg/kg bw (approximately 50% of the subcutaneous LD_{50} value) or more did increase the number of mouse micronuclei (Ozawa *et al.*, 1994). Administration via intraperitoneal injection, however, bypasses the liver, where 80% of the acetaldehyde from the portal circulation is converted to acetate.

Table 5. In vitro mutagenicity/genotoxicity studies for saturated aliphatic

Substance name	Test system	Test cells
Formic acid	modified Ames test (preincubation method) Chromosomal aberration test	S. typhmiurium TA97, TA98, TA10 TA1535, Chinese hamster ovary c (CHO) K1
	Chromosomal aberration test	Chinese hamster ovary cells (CH
Acetic acid	modified Ames test (preincubation method) Chromosomal aberration test	S. typhmiurium strains TA97, TA T100, TA1535, Chinese hamster
	Chromosomal aberration test	Chinese hamster ovary K1 cells
	Sister chromatid exchange	Adult human lymphocytes
Propionic acid	Modified Ames test (preincubation method)	S. typhmiurium TA97, TA98, TA15 and TA1537
	DNA repair test (spot test)	E. coli strains WP2, WP67, polA uvrA-, CM871
	SOS chromotest	E. coli PQ37
	Ames test	S. typhmiurium TA98, TA100, TA1 TA1537
	Sister chromatid exchange	Adult human lymphocyte cells
	Sister chromatid exchange	Chinese hamster V79 cells
Butyric acid	Chromosomal aberration test	Chinese hamster fibroblast cell

Table 5. Continued...

Substance name	Test system	Test cells
	Ames test	S. typhmiurium TA92, TA1535, TA TA1537, TA94, TA98
Hexanoic acid	Mouse lymphoma assay	mouse lymphoma L5178Y TK+/-
	Unscheduled DNA synthesis	Rat hepatocytes
	Ames test (plate incorporation assay)	S. typhmiurium TA98, TA100, TA1 TA1535 and TA1537
Heptanoic acid	Mouse lymphoma assay	Mouse lymphoma L5178Y TK +/-
	Unscheduled DNA synthesis assay	Rat hepatocytes

	Ames test (plate incorporation assay)	S. typhmiurium TA98, TA100, TA1538, TA1535 and TA1537
	Modified Ames test (preincubation method)	S. typhmiurium TA97, TA98, TA10 TA104, TA1535 and TA1537
Octanoic acid	Plate and suspension assays	S. typhmiurium TA1535, TA1537 and TA1538
	Nonactivation suspension test	Saccharomyces cerevisiae D4
	Unscheduled DNA synthesis	Rat hepatocytes
	Ames test (plate incorporation assay)	S. typhmiurium TA98, TA100, TA1 TA1535 and TA1537

Table 5. Continued...

Substance name	Test system	Test cells
Decanoic acid	Rec assay	B. subtilis strains H17 and M45
	Modified Ames test (preincubation method)	S. typhimurium TA98, TA100, TA1 TA97 and TA1537
Lauric acid	Modified Ames test (preincubation method)	S. typhimurium TA98, TA100, TA1 TA97 and TA1537
Myristic acid	Cell mutagenesis assay	Mouse lymphoma L5178Y TK+/-
	Ames test (plate incorporation assay)	S. typhimurium TA98, TA100, TA1 TA1537 and TA1538
	Modified Ames test (preincubation method)	S. typhmiurium TA97, TA98, TA10 TA1535 and TA1537
Stearic acid	Modified Ames test (preincubation method)	S. typhmiurium TA98, TA100, TA1 TA1537 and TA1538
	Ames test	S. typhmiurium TA98, TA100, TA1 TA1537 and TA1538
Acetaldehyde	Sister chromatid exchange	Adult human lymphocytes
	Forward mutation assay	L5178y mouse lymphoma TK+/-
	Ames test	S. typhmiurium TA100, TA102 and
	Chromosomal aberration test	Chinese hamster embryonic diploid cells
	Sister chromatid exchange	Adult human peripheral lymphocy

Table 5. Continued...

Substance name	Test system	Test cells
Propionaldehyde	Ames test	S. typhmiurium TA98, TA100 and
	Forward mutation assay	V79 Chinese hamster lung cells
	Ames test	S. typhmiurium TA100, TA102 and
	Unscheduled DNA synthesis assay	Adult human hepatocytes
	Chromosomal aberration test	Chinese hamster embryonic diplo cells
Butyraldehyde	Ames test	S. typhmiurium TA100, TA102 and
	Unscheduled DNA synthesis assay	Adult human hepatocytes
	Forward mutation assay	V79 Chinese hamster lung cells
	Sister chromatid exchange	Chinese hamster ovary cells
	Chromosome aberration test	Chinese hamster ovary cells
	Sister chromatid exchange	Adult human lymphocytes
Valeraldehyde	Forward mutation assay	V79 Chinese hamster lung cells
	Unscheduled DNA synthesis assay	Adult human and rat hepatocytes
	Rec assay	B. subtilis strains H17 and M45

Table 5. Continued...

Substance name	Test system	Test cells
Hexanal	Forward mutation assay	V79 Chinese hamster lung cells
	Unscheduled DNA synthesis assay	Adult human and rat hepatocytes
	Ames test	S. typhmiurium TA102 and TA104
	Ames test (spot test)	S. typhmiurium TA98, TA100, TA1 and TA1537
Heptanal	Ames test (spot test)	S. typhmiurium TA98, TA100, TA1 and TA1537

	Ames test	S. typhmiurium TA97, TA98, TA10 TA1535 and TA1537
Octanal	Ames test (spot test)	S. typhmiurium TA98, TA100, TA1 and TA1537
Nonanal	Sister chromatid exchange	Rat (female Fischer 344 animals hepatocytes
	Unscheduled DNA synthesis	Adult human and rat hepatocytes assay
	Forward mutation assay	V79 Chinese hamster lung cells
	Modified Ames test (preincubation method)	S. typhmiurium TA98, TA100 and
	Ames test	S. typhmiurium TA102 and TA104
	Chromosomal aberration test	Rat hepatocytes

Table 5. Continued...

Substance name	Test system	Test cells
Decanal	Rec assay	B. subtilis strains H17 and M45
	Rec assay	E. coli WP2, uvrA
	Chromosomal aberration test	Chinese hamster fibroblast cell
	Ames test	S. typhmiurium TA92, TA1535, TA TA1537, TA94 and TA98
Undecanal	Ames test (spot test)	S. typhmiurium TA98, TA100, TA1 and 1537
Propyl alcohol Hine,	Ames test	S. typhmiurium TA100
	Sister chromatid exchange	V79 Chinese hamster lung fibrob
	Sister chromatid exchange	Chinese hamster ovary cells
	Micronucleus test	Chinese hamster lung fibroblast
Butyl alcohol	Ames test	S. typhimurium TA102
	Sister chromatid exchange	Chinese hamster ovary cells
	Forward mutation assay	Chinese hamster ovary cells
1-Octanol	Cell mutagenesis assay	Mouse lymphoma L5178Y TK+/-
	Ames test	S. typhmiurium TA1535, TA1537, TA98 and TA100

1-Decanol	Rec assay	B. subtilis strains H17 and M45

Table 5. Continued...

Substance name	Test system	Test cells
Undecyl alcohol	Rec assay	B. subtilis strains H17 and M45
	Rec assay	E. coli WP2 uvrA
Lauryl alcohol	Modified Ames test (preincubation method)	S. typhmiurium TA98, TA100, TA1 TA1537 and TA1538
1-Hexadecanol	Ames test	S. typhmiurium TA98, TA100, TA1 TA1537 and TA1538

[1] Both with and without metabolic activation.
[2] Without metabolic activation
[3] With metabolic activation
[4] Positive only at middle dose (2.5 mM), negative at lower (1.25 mM) and higher

Table 6. Mutagenicity/genotoxicity studies for saturated aliphatic acyclic line

Substance name	Test system	Test organism	Concent
Propionic acid	Micronucleus test, intraperitoneal injection	Chinese hamster cells	5 ml/kg
Acetaldehyde	Mouse bone marrow micronucleus test, intraperitoneal injection	Mouse	95-400

In mutation assays with mammalian cell lines, hexanoic acid and heptanoic acid exhibited an increase in the frequency of mutations in mouse lymphoma L5178Y cells with S9 metabolic activation at concentrations greater than 600 µg/ml. The authors noted that culture conditions of low pH and high osmolality, which may occur upon incubation with acidic substances, have been shown to produce false-positive results in this and other assays (Heck *et al.*, 1989). Therefore, these results must be cautiously interpreted. Formic acid and acetic acid, which initially gave an increase in SCE in Chinese hamster ovary cells, were later shown to be negative when tested at physiological pH (Morita *et al.*, 1990). In a forward mutation assay, butyl alcohol tested at concentrations of 0.2 to 1.6 µl/ml was mutagenic when incubated with Chinese hamster ovary cells (PPG, 1991b). This result is also probably due to perturbations in the pH of the test medium.

2.2.4 Reproductive and developmental toxicity

Reproductive and developmental toxicity studies on low molecular weight aliphatic alcohols (propyl alcohol and butyl alcohol) via inhalation at high concentrations have been associated with developmental effects in the presence of maternal toxicity (Nelson *et al.*, 1990). When butyric acid, valeric acid and octanoic acid were given daily by tracheal intubation on days 6 to 15 of gestation, only fetotoxicity was reported at the highest dose level (1500 mg/kg bw per day) with octanoic acid; no other evidence of fetotoxicity, developmental toxicity or teratogenicity associated with these three carboxylic acids was observed (Narotsky *et al.*, 1994). There is no evidence to conclude that, when ingested as flavouring substances, intake of any of the substances in the group of linear saturated aliphatic substances would be associated with reproductive or developmental toxicity.

2.2.4.1 Propionic Acid

Fetal abnormalities or effects on survival were not observed when the calcium salt of propionic acid was fed to pregnant rodents (up to 300 mg/kg bw per day for 10 days, hamsters (up to 400 mg/kg bw per day for 5 days) and rabbits (up to 400 mg/kg bw per day for 13 days) (FDRL, 1972).

2.2.4.2 Butyric Acid

Maternal weight loss and respiratory effects were observed in female rats given 100 or 133 mg/kg bw per day of butyric acid by tracheal intubation on days 6 to 15 of gestation (Narotsky *et al.*, 1994). In dams with peripartum respiratory symptoms, reduced pup weight and decreased progeny viability were reported, but no signs of significant development toxicity were reported at either dose. Gastric irritation was noted at necropsy.

2.2.4.3 Valeric Acid

Female rats given 0, 75 or 100 mg/kg bw per day valeric acid by gavage on days 6 to 15 of gestation exhibited signs of maternal toxicity including respiratory effects and decreased body weight, but no significant developmental toxicity at either dose. In Segment II of this study, valeric acid was associated with maternal toxicity and reduced fetal weights at dose levels from 50 to 200 mg/kg bw per day. No fetal skeletal malformations were reported, except for sternebrae variations. Gastric irritation was noted at necropsy (Narotsky *et al.*, 1994).

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See Also:
Toxicological Abbreviations

Final Report on the Safety Assessment of Sorbitan Caprylate, Sorbitan Cocoate, Sorbitan Diisostearate, Sorbitan Dioleate, Sorbitan Distearate, Sorbitan Isostearate, Sorbitan Olivate, Sorbitan Sesquiisostearate, Sorbitan Sesquistearate, and Sorbitan Triisostearate[1]

Sorbitan fatty acid esters are mono-, di-, and triesters of fatty acids and sorbitol-derived hexitol anhydrides. They function as surfactants in cosmetic formulations. Previously, the Cosmetic Ingredient Review (CIR) Expert Panel had reviewed the safety of several of these sorbitan fatty acid esters (Sorbitan Laurate, Sorbitan Oleate, Sorbitan Palmitate, Sorbitan Sesquioleate, Sorbitan Stearate, Sorbitan Trioleate, and Sorbitan Tristearate). This safety assessment is an addendum to that report that includes Sorbitan Caprylate, Sorbitan Cocoate, Sorbitan Diisostearate, Sorbitan Dioleate, Sorbitan Distearate, Sorbitan Isostearate, Sorbitan Olivate, Sorbitan Sesquiisostearate, Sorbitan Sesquistearate, and Sorbitan Triisostearate. Although concentrations of these ingredients up to 25% have been reported to be used, most commonly they are used at less than 10%. These esters may be hydrolyzed to one fatty acid and anhydrides of Sorbitol. Fatty Acids are absorbed and metabolized. Sorbitan fatty acid esters were relatively nontoxic via ingestion in acute and long-term studies. They were generally minimal to mild skin irritants in animal studies, except that Sorbitan Isostearate applied to the skin was a moderate irritant in one rabbit study and when injected intradermally caused mild to severe irritation in guinea pigs. Sorbitan fatty acid esters did not sensitize guinea pigs. The fatty acid component, tested alone, typically caused only slight irritation and sensitization, and was not photosensitizing. Sorbitan fatty acid esters were not ocular irritants. Fatty acids are normal components of diet for which no data were available concerning reproductive or developmental toxicity, but Sorbitol had no adverse effects on the reproduction of CD rats during a multigeneration feeding study and was not a reproductive toxin at doses of 3000 to 7000 mg/kg/day for 2 years. Overall these esters and their corresponding fatty acids were not mutagenic, but Sorbitan Oleate was reported to reduce DNA repair following ultraviolet radiation exposure in human lymphocytes in culture. Sorbitan Laurate and Sorbitan Trioleate were cocarcinogens in one mouse study, but Sorbitan Trioleate and Sorbitan Oleate were not tumor promoters in another study. In clinical tests, Sorbitan fatty acid esters were generally minimal to mild skin irritants and were nonsensitizing, but Sorbitan Sesquioleate did produce an allergic reaction in fewer than 1% of patients with suspected contact dermatitis and addition of Sorbitan Sesquioleate to the components of a fragrance mix used in patch testing increased both irritant and allergic reactions to the fragrance mix. Careful consideration was made of the data on the cocarcinogenesis of Sorbitan Laurate and Sorbitan Trioleate, but the high exposure levels, high frequency of exposure, and absence of a dose-response led to the conclusion that there was not a cocarcinogenesis risk with the use of these ingredients in cosmetic formulations. Accordingly, these ingredients were considered safe for use in cosmetic formulations under the present practices of use.

INTRODUCTION

Sorbitan Caprylate, Sorbitan Cocoate, Sorbitan Diisostearate, Sorbitan Dioleate, Sorbitan Distearate, Sorbitan Isostearate, Sorbitan Olivate, Sorbitan Sesquiisostearate, Sorbitan Sesquistearate, and Sorbitan Triisostearate are mono-, di-, and triesters of fatty acids and sorbitol-derived hexitol anhydrides.

The Cosmetic Ingredient Review (CIR) Expert Panel previously completed a safety assessment on other sorbitan fatty acid esters including Sorbitan Laurate, Sorbitan Oleate, Sorbitan Palmitate, Sorbitan Sesquioleate, Sorbitan Stearate, Sorbitan Trioleate, and Sorbitan Tristearate, concluding that these ingredients are safe as used in cosmetic formulations (Elder 1985). Summaries of selected data presented in that report, as well as new data on the ingredients previously reviewed, are included in this report.

This safety assessment completes the Panel's review of this family of sorbitan fatty acid esters.

As part of this safety assessment, the CIR Expert Panel considered its previous assessments of a number of related ingredients with findings as described below.

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Coconut Oil, Coconut Acid, Hydrogenated Coconut Oil, and Hydrogenated Coconut Acid are safe for use as cosmetic ingredients (Elder 1986).

Oleic, Lauric, Palmitic, Myristic, and Stearic Acids are safe in the present practices of use and concentration in cosmetics (Elder 1987).

Isostearic Acid is safe as a cosmetic ingredient in the present practices of use (Elder 1983).

Summaries of data from those reports and other published sources are included as a further basis for assessing the safety in cosmetics of the Sorbitan Fatty Acid Esters.

CHEMISTRY

Definition and Structure

The Sorbitan Fatty Acid Esters are mono- and diesters of fatty acids and hexitol anhydrides derived from sorbitol. They conform to the formulas given in Figure 1, but can be depicted using a six-membered ring—shown as the tetrahydropyran form. Formulas were not available for Sorbitans Sesquiisostearate and Sesquistearate, which are mixtures of mono- and diesters of isostearic and stearic acids. These ingredients have no CAS numbers and are also known as Sorbitan, Monohexadecanoate and Anhydrosorbitol Sesquistearate, respectively (Wenninger and McEwen 1997).

The ingredients of this safety assessment are esters of caprylic, coconut, oleic, isostearic, and stearic acids, as well as fatty acids derived from refined olive oil, with hexitol anhydrides derived from sorbitol. The ingredients of the previous safety assessment (Elder 1985) are mono- and triesters of lauric, stearic, oleic, and palmitic acids, or mixtures of oleic acid esters, with sorbitol anhydrides (Wenninger and McEwen 1997). Table 1 provides a complete list of ingredients previously reviewed and ingredients addressed in this addendum.

"Sorbitan" is a generic name for anhydrides (cyclic ether tetrahydric alcohols) derived from sorbitol by removal of one molecule of water. Sorbitol is a crystalline hexahydric alcohol that occurs naturally in berries, plums, cherries, pears, apples, seaweed, and algae. In mammals, it is formed from glucose and then converted to fructose. Sorbitol is also found in deposits of the lens of patients with diabetes mellitus (Taylor 1988; Lewis 1993).

Chemical and Physical Properties

Sorbitan Caprylate has an acid value <6.00 mg KOH/g, a saponification value of 250 to 280 mg KOH/g, and an iodine value of <5 g I_2/100 g (Gattefossé S.A. 1998).

Sorbitan Olivate is an ivory-colored, waxy solid at 20°C with a slight, characteristic odor. It consists of 99.0% (minimum) of the active substance, and contains a maximum of 1.0% moisture. The melting point is 52°C to 55°C. Sorbitan Olivate has acid, iodine, and saponification values of 10 to 12, 3.0 (maximum), and 155 to 165, respectively. It is soluble in ethanol, almost soluble in vegetable oils, and dispersible in warm water (B&T Srl 1998).

Sorbitan fatty acid esters are waxy solids or viscous liquids that are soluble in organic solvents. For Sorbitans Stearate, Laurate, Sesquioleate, Oleate, Tristearate, Palmitate, and Trioleate, the maximum moisture contents were 1% to 2%, and the specific gravities (at 25°C) were generally 0.95 to 1.05. The acid values were 5 to 15, the saponification values were 135 to 190, and the hydroxyl values ranged from 55 to 80 for Sorbitans Trioleate and Tristearate to 182 to 360 for Sorbitans Stearate, Laurate, Sesquioleate, Oleate, and Palmitate.

Reactivity

Undiluted Sorbitan Fatty Acid Esters, as well as neutral, mildly alkaline, or mildly acidic solutions of these esters are stable at room temperature and within a pH range of 2 to 12. Hydrolysis occurs in the presence of water at high or low pH conditions (Elder 1985).

Analytic Methods

Commercially available (food-grade) Sorbitan Fatty Acid Esters have been analyzed using high-performance liquid chromatography (Garti et al. 1983). Sorbitan Palmitate consisted of 52% monoesters, 39% diesters, and 9% triesters. Sorbitan Stearate consisted of 45% to 56% monoesters, 33% to 40% diesters, and 9% to 17% triesters. Sorbitan Tristearate consisted of 38% monoesters, 31% diesters, and 31% triesters. Sorbitan Oleate consisted of 44% to 52% monoesters, 34% to 38% diesters, and 14% to 18% triesters. Sorbitan Trioleate consisted of 31% to 35% monoesters, 32% to 33% diesters, and 32% to 37% triesters. Sorbitan Sesquioleate was comprised of 36% monoesters, 38% diesters, and 26% triesters. Sorbitan Isostearate consisted of 44% monoesters, 33% diesters, and 23% triesters.

Sorbitan Stearate was also analyzed using gas chromatography (Tsuda et al. 1984; Brüschweiler and Hautfenne 1990). Confectionery products contained 0.1% to 0.63% Sorbitan Stearate, and average recoveries from samples spiked with 1.0% of the ester were 91% to 96% for isosorbide, 83% to 99% for 1,4-sorbitan, and 92% to 98% for D-sorbitol. Sorbitan Stearate content was calculated using the formula:

$$C = (W1 + W2 + W3)/(10{,}000 \times W \times f)$$

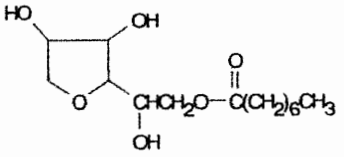
where C is the Sorbitan Stearate content (%); $W1$, $W2$, and $W3$ are isosorbide, 1,4-sorbitan, and D-sorbitol contents (μg), respectively; W is the sample weight (g); and f is a conversion factor of 0.27 (Tsuda et al. 1984).

Method of Manufacture

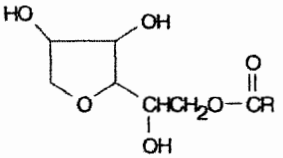
In general, Sorbitan Fatty Acid Esters are prepared by the dehydration of sorbitol (Figure 2) to form a hexitan, which is then esterified with the desired fatty acid (Gennaro 1990; Canterbery 1997).

Sorbitan Caprylate is produced by the esterification of sorbitol with caprylic acid (Gattefossé S.A. 1998) and Sorbitan

SORBITAN CAPRYLATE
No CAS No. or Synonyms

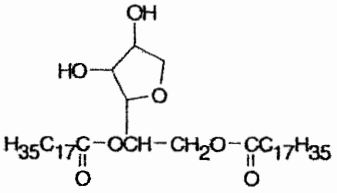


SORBITAN COCOATE
CAS No. 68154-36-9
Synonyms: Anhydrosorbitol Monococoate
Fatty Acids, Coco, Monoesters with Sorbitan
Sorbitan Monococoate

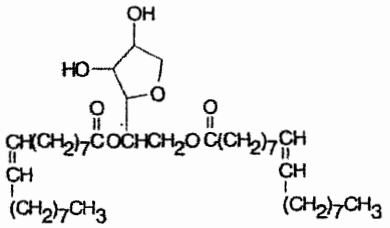


where RCO- represents the fatty acids derived from coconut oil

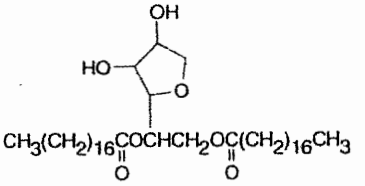
SORBITAN DIISOSTEARATE
CAS No. 68238-87-9
Synonym: Anhydrohexitol Diisostearate



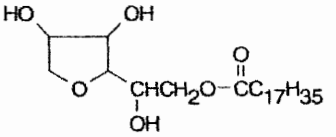
SORBITAN DIOLEATE
CAS No. 29116-98-1
Synonyms: Anhydrosorbitol Dioleate
Sorbide Dioleate
Sorbitan, Di-9-Octadecanoate



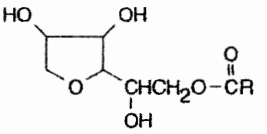
SORBITAN DISTEARATE
CAS No. 36521-89-8
Synonyms: Anhdrosorbitol Distearate
Sorbitan Dioctadecanoate



SORBITAN ISOSTEARATE
CAS No. 54392-26-6
Synonyms: 1,4-Anhydro-D-Glucitol, 6-Isooctadecanoate
Anhydrosorbitol Monoisostearate
D-Glucitol, 1,4-Anhydro-, 6-Isooctadecanoate
Sorbitan, Monoisooctadecanoate
Sorbitan Monoisostearate

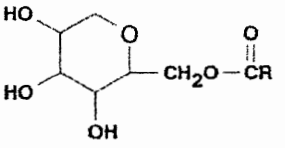


SORBITAN OLIVATE
No CAS No.
Synonyms: Anhydrosorbitol Monoolivate
Fatty Acids, Olive, Monoesters with Sorbitan
Sorbitan Monoolivate



where RCO- represents the fatty acids derived from olive oil

GENERIC STRUCTURE - TETRAHYDROPYRAN FORM



where RCO⁻ equals the fatty acid moiety

FIGURE 1
Formulas for specific sorbitan esters, including a generic structure for the tetrahydropyran form.

TABLE 1
Ingredients previously reviewed by CIR and ingredients addressed in this report

New ingredients reviewed (this report)	Ingredients previously reviewed	Reference
Sorbitan Caprylate	Sorbitan Laurate	Elder 1985
Sorbitan Cocoate	Sorbitan Oleate	
Sorbitan Diisostearate	Sorbitan Palmitate	
Sorbitan Dioleate	Sorbitan Sesquioleate	
Sorbitan Distearate	Sorbitan Stearate	
Sorbitan Isostearate	Sorbitan Trioleate	
Sorbitan Olivate	Sorbitan Tristearate	
Sorbitan Sesquiisostearate		
Sorbitan Sesquistearate	Coconut acid	Elder 1986
Sorbitan Triisostearate	Hydrogenated Coconut acid	
	Isostearic acid	Elder 1983
	Oleic acid	Elder 1987
	Lauric acid	
	Palmitic acid	
	Myristic acid	
	Stearic acid	

Olivate is formed by the esterification of sorbitan with the wax obtained by partial hydrogenation of olive oil (B&T Srl 1998).

Impurities

Impurities such as free acid and alcohol, arsenic (<3 ppm), lead (<10 ppm), and water may be found in the Sorbitan Fatty Acid Esters (Elder 1985).

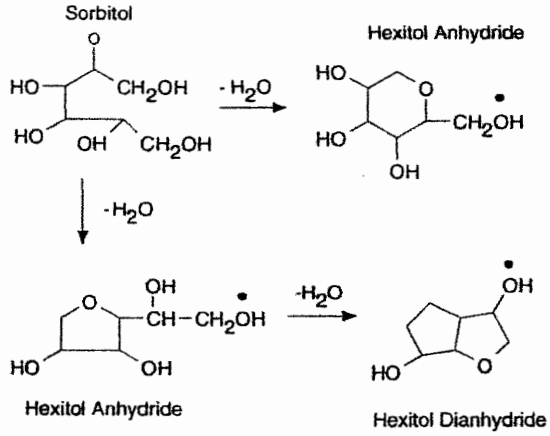


FIGURE 2
Mechanisms of Hexitol Anhydride Derivation (Canterbery 1997).

Polycyclic aromatic hydrocarbons and aflatoxins have been found as contaminants of copra and crude Coconut Oil; these impurities are removed by conventional refining processes (Elder 1986).

Cosmetic grade fatty acids occur as mixtures of several fatty acids, the content varying with method of manufacture and source. Fatty acid preparations can include up to 1.5% unsaponifiable matter, glyceryl monoesters of fatty acids, and butylated hydroxytoluene (Elder 1987).

Ultraviolet Absorption

Sorbitan Laurate at a concentration of 26,244 mg/l (in absolute ethanol) had maximum absorbance (2.0) at 230 nm; the absorbance was 0.1/2.0 at a wavelength of 350 nm. Sorbitan Sesquioleate (8,397 mg/l) had an absorbance of 1.98/2.0 at 245 nm and 0.1/2.0 at 320 nm. Sorbitan Palmitate (27,982 mg/l) had maximum absorbance at 220 nm and an absorbance of 0.1 at 350 nm. Sorbitan Trioleate (8,093 mg/l) had maximum absorbance at 250 nm and an absorbance of 0.1 at 320 nm (Elder 1985).

USE

Cosmetic

The Sorbitan Fatty Acid Esters function as surfactants—emulsifying agents in cosmetics (Wenninger and McEwen 1997). It was also reported that Sorbitan Isostearate functions as a pigment dispersant in creams (Unichema International 1996). In 1998, Sorbitan Isostearate, Sorbitan Laurate, Sorbitan Oleate, Sorbitan Palmitate, Sorbitan Sesquiisostearate, Sorbitan Sesquioleate, Sorbitan Stearate, Sorbitan Trioleate, and Sorbitan Tristearate were reported to the Food and Drug Administration (FDA) as used in 37, 93, 68, 39, 16, 170, 308, 20, and 8 product formulations, respectively (Table 2). Sorbitans Caprylate, Cocoate, Dioleate, Diisostearate, Distearate, Olivate, Sesquistearate, and Triisostearate were not reported used in cosmetics (FDA 1998).

In 1984, Sorbitan Isostearate was used at concentrations of 1% to 5%; Sorbitan Laurate was used at concentrations of 5% to 10%, but was mostly used at 1% to 5%; Sorbitan Oleate was used at concentrations of 10% to 25%, but was mostly used at 0.1% to 1%; Sorbitan Palmitate was used at concentrations of 0.1% to 5%; Sorbitan Peroleate was used at concentrations of 0.1% to 1%; Sorbitan Sesquiisostearate was used at concentrations of up to 5%; Sorbitan Sesquioleate was used at concentrations up to 5% to 10%, but was mostly used at concentrations of 0.1% to 1%; Sorbitan Stearate was used at concentrations up to 10% to 25%, but was mostly used at concentrations of 1% to 5%; Sorbitan Trioleate was used at concenrations up to 1% to 5%; and Sorbitan Tristearate was used at concentrations up to 5% to 10%, but was mostly used at 0.1% to 1% (FDA 1984).

Data submitted by industry indicated that Sorbitan Isostearate was used in concealers at concentrations up to 2.5% and in eye creams at concentrations of 4% (Cosmetic, Toiletry, and

TABLE 2
Product formulation data (FDA 1998)

Product category	Total no. of formulations in category	Total no. of formulations containing ingredient
Sorbitan Isostearate		
Baby lotions, oils, powders, and creams	53	3
Eyebrow pencil	91	1
Eyeliner	514	1
Eye shadow	506	12
Other eye makeup preparations	120	2
Tonic, dressings, and other hair-grooming aids	549	1
Blushers (all types)	238	7
Foundations	287	2
Makeup bases	132	5
Other personal cleanliness products	291	1
Body and hand preparations (excluding shaving)	796	1
Other skin care preparations	692	1
1998 Sorbitan Isostearate total		**37**
Sorbitan Laurate		
Eyeliner	514	2
Eye lotion	18	2
Mascara	167	3
Other eye makeup preparations	120	2
Other fragrance preparations	148	5
Shampoos (noncoloring)	860	1
Other hair preparations	276	1
Foundations	287	14
Lipstick	790	15
Makeup bases	132	5
Makeup fixatives	11	2
Other makeup preparations	135	3
Aftershave lotion	216	2
Other shaving preparation products	60	2
Cleansing preparations	653	5
Body and hand preparations (excluding shaving)	796	7
Moisturizing preparations	769	10
Paste masks (mud packs)	255	4
Other skin care preparations	692	5
Suntan gels, creams, and liquids	136	2
Indoor tanning preparations	62	1
1998 Sorbitan Laurate total		**93**
Sorbitan Palmitate		
Bath oils, tablets, and salts	124	1
Eyebrow pencil	91	5
Eyeliner	514	3
Other eye makeup preparations	120	2
Other fragrance preparations	148	1
Hair conditioners	636	1
Hair straighteners	63	1
Lipstick	790	3
Other makeup preparations	135	3
Aftershave lotion	216	1

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TABLE 2
Product formulation data (FDA 1998) *(Continued)*

Product category	Total no. of formulations in category	Total no. of formulations containing ingredient
Cleansing preparations	653	7
Body and hand preparations (excluding shaving)	796	1
Moisturizing preparations	769	3
Night preparations	188	1
Paste masks (mud packs)	255	3
Other skin care preparations	692	1
Suntan gels, creams, and liquids	136	1
Indoor tanning preparations	62	1
1998 Sorbitan Palmitate total		**39**
Sorbitan Oleate		
Eyeliner	514	1
Eye shadow	506	3
Eye makeup remover	84	1
Other fragrance preparations	148	4
Hair conditioners	636	2
Permanent waves	192	1
Tonics, dressings, and other hair-grooming aids	549	1
Other hair preparations	276	1
Blushers (all types)	238	2
Foundations	287	8
Lipstick	790	1
Makeup bases	132	2
Makeup fixatives	11	1
Other makeup preparations	135	2
Nail creams and lotions	17	1
Other manicuring preparations	61	2
Cleansing preparations	653	3
Body and hand preparations (excluding shaving)	796	4
Moisturizing preparations	769	18
Night preparations	188	3
Paste masks (mud packs)	255	2
Skin fresheners	184	3
Other skin care preparations	692	1
Other suntan preparations	38	1
1998 Sorbitan Oleate total		**68**
Sorbitan Sesquiisostearate		
Eye shadow	506	5
Other eye makeup preparations	120	1
Face powders	250	3
Foundations	287	6
Other makeup preparations	135	1
1998 Sorbitan Sesquiisostearate total		**16**
Sorbitan Sesquioleate		
Baby lotions, oils, powders, and creams	53	2
Other bath preparations	159	1
Eyebrow pencil	91	1
Eyeliner	514	3
Eye shadow	506	11

(Continued on next page)

TABLE 2
Product formulation data (FDA 1998) *(Continued)*

Product category	Total no. of formulations in category	Total no. of formulations containing ingredient
Eye lotion	18	1
Eye makeup remover	84	1
Mascara	167	20
Other eye makeup preparations	120	5
Tonics, dressings, and other hair-grooming aids	549	1
Other hair preparations	276	1
Blushers (all types)	238	4
Face powders	250	10
Foundations	287	19
Lipstick	790	16
Makeup bases	132	1
Rouges	12	1
Other makeup preparations	135	5
Nail creams and lotions	17	1
Other manicuring preparations	61	1
Aftershave lotion	216	1
Cleansing preparations	653	11
Face and neck preparations (excluding shaving)	263	3
Body and hand preparations (excluding shaving)	796	6
Moisturizing preparations	769	12
Night preparations	188	10
Other skin care preparations	692	12
Suntan gels, creams, and liquids	136	8
Other suntan preparations	38	1
1998 Sorbitan Sesquioleate total		**170**
Sorbitan Stearate		
Baby lotions, oils, powders, and creams	53	4
Other baby products	29	1
Eyebrow pencil	91	15
Eyeliner	514	5
Eye shadow	506	3
Eye lotion	18	2
Eye makeup remover	84	1
Mascara	167	12
Other eye makeup preparations	120	3
Other fragrance preparations	148	9
Hair conditioners	636	4
Tonics, dressings, and other hair-grooming aids	549	4
Other hair preparations	276	1
Foundations	287	8
Makeup bases	132	2
Other makeup preparations	135	5
Cuticle softeners	19	3
Deodorants (underarm)	250	5
Other personal cleanliness products	291	1
Aftershave lotion	216	2
Shaving cream	139	1
Cleansing preparations	653	24

(Continued on next page)

TABLE 2
Product formulation data (FDA 1998) *(Continued)*

Product category	Total no. of formulations in category	Total no. of formulations containing ingredient
Face and neck preparations (excluding shaving)	263	19
Body and hand preparations (excluding shaving)	796	57
Foot powders and sprays	35	2
Moisturizing preparations	769	56
Night preparations	188	11
Paste masks (mud packs)	255	11
Other skin care preparations	692	29
Suntan gels, creams, and liquids	136	3
Indoor tanning preparations	62	4
1998 Sorbitan Stearate total		**308**
Sorbitan Trioleate		
Eye shadow	506	1
Tonics, dressings, and other hair-grooming aids	549	1
Blushers (all types)	238	5
Face powders	250	1
Foundations	287	2
Makeup bases	132	2
Other makeup preparations	135	2
Cleansing preparations	653	2
Body and hand preparations (excluding shaving)	796	1
Moisturizing preparations	769	1
Night preparations	188	1
Other skin care preparations	692	1
1998 Sorbitan Trioleate total		**20**
Sorbitan Tristearate		
Makeup bases	132	1
Face and neck preparations (excluding shaving)	263	1
Moisturizing preparations	769	2
Paste masks (mud packs)	255	2
Other skin care preparations	692	1
Other suntan preparations	38	1
1998 Sorbitan Tristearate total		**8**

Fragrance Association [CTFA] 1998a). Sorbitan Caprylate functioned as an antistatic agent and was used at concentrations of 1% to 5% (Gattefossé S.A. 1998) and 2.5% to 7.5% Sorbitan Olivate served as an emulsifier (B&T Srl 1998).

Further data submitted by industry reported that Sorbitan Isostearate was used at a maximum concentration of 1% in eyebrow pencils, eyeliner, eye shadow, and all types of blushers, of 0.5% in other makeup preparations, 0.8% in moisturizing creams, lotions, powders, and sprays and at a maximum concentration of 0.2% in suntan gels, creams and liquids. Reported uses of Sorbitan Sesquiisostearate indicate maximum concentrations of 1% in eye shadow and all types of blushers and 3% in foundations, depilatories, and face powders (CTFA 1998d, 1999a).

The Sorbitan Esters of Fatty Acids and the Sorbitans Distearate, Isostearate, Cocoate, Isostearate, Laurate, Oleate, Palmitate, Stearate, Sesquiisostearate, Sesquioleate, Sesquistearate, Trioleate, and Tristearate are listed in the Japanese *Comprehensive Licensing Standards of Cosmetics by Category* (CLS) (Rempe and Santucci 1997).

Sorbitans Isostearate, Laurate, Oleate, Palmitate, Stearate, Sesquiisostearate, Sesquioleate, Sesquistearate, Trioleate, and Tristearate, which conform to the specifications of the *Japanese Standards of Cosmetic Ingredients* (JSCI) and *Japanese Cosmetic Ingredient Codex* (JCIC), have precedent for use without restriction in all CLS categories. Sorbitan Distearate and Sorbitan Trioleate, which conform to the specifications of the JCIC and JSCI, respectively, have precedent for use without restriction

all CLS categories except Eyeliner Preparations, for which there is no precedent for use. Sorbitan Cocoate, which conforms to the specifications of the JCIC has precedent for use without restriction in all CLS categories except Eyeliner Preparations, Lip Preparations, Oral Preparations, and Bath Preparations, for which there is no precedent for use.

Sorbitan Isostearate and Sorbitan Sesquiisostearate are used in Japan at concentrations less than 5% (CTFA 1998b).

Noncosmetic

Polyalcohol isostearate esters, including Sorbitan Stearate, are used as lubricants or ingredients in lubricants, but "are not allowed to be used in any application implying (possible) food contact." These ingredients are not listed in any pharmacopoeia or national formulary (Unichema International 1996).

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, and Excretion

Sorbitan fatty acids were reported to affect the metabolism and excretion of other materials.

The Sorbitans Laurate, Palmitate, Stearate, Oleate, Sesquioleate, and Trioleate at concentrations of 50% and 100% increased the cumulative urinary excretion of pirenzepine dihydrochloride after oral administration to rats (dose = 2 mg/kg) within 24 hours of treatment. In this study, rats of the control group (12 rats) had cumulative urinary excretion of 2.7% pirenzepine dihydrochloride, whereas rats given Sorbitan Fatty Acid Esters (3–6/group) excreted 3.8% to 15.7% of the drug (Nakagawa et al. 1988).

When applied daily for 81 days to the skin of rabbits at test concentrations of 1% to 60%, Sorbitans Laurate, Stearate, Oleate, and Trioleate caused two- to threefold increases in oxygen consumption of the skin and increased numbers of inflammatory cells were observed in the dermis. In another study, treatment for 4 days with 10% Sorbitan Trioleate resulted in a 27% to 58% increase in phosphorus content using DNA content as a reference standard. After 10 days of treatment, phosphorus content increased 18% to 35%, suggesting that damage to the biological membranes had occurred. During a third study, 10% Sorbitan Trioleate increased the rate of water loss from rabbit skin, compared to control water loss time, but no significant difference in water content (Elder 1985).

Coconut Oil was used as a saturated fat control for metabolism studies and caused slight increases in serum cholesterol concentrations. The longevity of experimental animals in metabolism studies was not affected by diets containing Coconut Oil (Elder 1986).

Although data were unavailable on the absorption, distribution, and excretion of the Sorbitans Caprylate, Cocoate, Dioleate, Diisostearate, Distearate, Isostearate, Olivate, Sesquiisostearate, Sesquistearate, and Triisostearate, information from earlier safety assessments is provided below.

Sorbitan Stearate was hydrolyzed to stearic acid and anhydrides of sorbitol when ingested. Approximately 90% of the Sorbitan Stearate was absorbed and hydrolyzed when fed to rats in oil solution, and 50% was absorbed and hydrolyzed when fed as a water emulsion. Sorbitan Stearate did not accumulate (<0.5%) in the fat stores of the rat (Elder 1985).

Results of dietary studies suggest that 95% to 98% of ingested Coconut Oil is absorbed. No specific data were available indicating the extent of percutaneous absorption of Coconut Oil (Elder 1986).

Fatty acids are absorbed, digested, and transported in animals and humans. Radioactivity from labeled fatty acids administered orally, intravenously, intraperitoneally, and intraduodenally has been found in various tissues and in blood and lymph. β-Oxidation of the fatty acids involves serial oxidation and reduction reactions yielding acetyl coenzyme A (CoA). Although placental transfer of fatty acids has been documented in several species and fetal lipid metabolism has been studied, no studies on the teratogenicity of Oleic, Lauric, Palmitic, Myristic, or Stearic Acids were found. High intake of dietary saturated fatty acids has been associated with the incidence of atherosclerosis and thrombosis (Elder 1987).

Results of studies with rat liver homogenate have suggested that Isostearic Acid is readily metabolized following ingestion (Elder 1983).

Cytotoxicity

The cytotoxicity of Sorbitan Oleate was investigated using in vitro skin recombinants and primary cultures of human keratinocytes (Roguet, Dossoe, and Rougier 1992). These recombinants were comprised of human epidermal cells cultured at the air-medium interface on dead de-epidermized dermis. After a 24-hour exposure, 10% aqueous Sorbitan Oleate induced mild to no change in morphology of the skin recombinant. The ester (at concentrations up to 200 mg/ml) had only a small effect on membrane integrity of the keratinocytes, as measured by the amount of lactic dehydrogenase leakage to the media.

In addition, Sorbitan Oleate had no effect on mitochondrial activity, which was assessed by measuring the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a MTT-formazan precipitate. The IC_{50} in the MTT assay was 2 mg/ml for the monolayer keratinocytes, and >200 mg/ml for the skin recombinants. In contrast, the IC_{50} values for 6% aqueous sodium dodecyl sulfate (SDS) were 1 and 0.07 mg/ml, respectively, and SDS induced a complete separation of the epidermis from the dermis.

ANIMAL TOXICOLOGY

The no-effect dose of Sorbitan Stearate was 7.5 g/kg/day after rats were treated with the ester for up to 2 years. Rats were fed Sorbitan Stearate concentrations of up to 25.0 g/kg/day and dogs were fed 5.0 g/kg/day of the ester. No adverse effects were noted after 24 months of treatment, with the exception of

tarded growth in rats of the high-dose group (Fitzhugh et al. 59).

Five female ddY mice were treated with a single oral dose of Sorbitan Sesquiisostearate at a volume of 10 ml/kg body weight. The acute oral LD_{50} was 25 ml/kg, which was considered "practically nontoxic" under the conditions of the study (CTFA 1998c).

The results of oral toxicity studies of the Sorbitan Fatty Acid Esters indicated that these Sorbitans at low concentrations were relatively nontoxic via ingestion. The lowest LD_{50} for the rat in the 20 Sorbitan Ester studies was 31 g/kg for Sorbitan Stearate.

Prolonged feeding (8 weeks) of Sorbitan Stearate to rats did not affect growth, and other studies indicated that Sorbitan Stearate had nutritive value for rats and dogs. In subchronic feeding experiments of Sorbitan Laurate in a variety of species (chickens, rats, monkeys, and hamsters), no toxic effects were noticed when the ester concentration in the feed was less than 10%. When the feed concentration was $\geq$10%, growth depression, decreased organ weights, diarrhea, unkempt appearance, hepatic and renal abnormalities, and gastrointestinal tract irritation were generally observed. Subchronic feeding of Sorbitan Oleate to rats produced no abnormalities until the ester comprised at least 10% of the diet. At this concentration, the same types of abnormalities were observed that occurred in the Sorbitan Laurate-fed animals.

Chronic feeding studies have been conducted using Sorbitans Stearate, Laurate, and Oleate. At a 5% dietary concentration, Sorbitan Laurate and Sorbitan Oleate had no adverse effects n rats over a 2-year period. Dogs fed 5% Sorbitan Stearate for 20 months had no compound-related changes. A feed concentration of $\geq$10% Sorbitan Stearate was required to produce depressed growth and hepatic and renal abnormalities. Mice appeared more sensitive to toxic effects of Sorbitan Stearate than rats. In other studies, a 0.5% dietary concentration produced growth abnormalities in male rats, and a 4% dietary concentration produced renal abnormalities (Elder 1985).

Coconut Oil and Hydrogenated Coconut Oil are relatively nontoxic when ingested. Administered as a single 5-g/kg dose to rats, neither compound caused deaths over a 7-day observation period. In a 90-day subchronic feeding study, rats fed a diet containing 25% Coconut Oil had slight fatty change of the liver. The results of a chronic lifetime study in which mice were fed diets supplemented with 15% Hydrogenated Coconut Oil indicated no effect on life spans of the test animals (Elder 1986).

Little acute toxicity was observed when Oleic, Lauric, Palmitic, Myristic, or Stearic Acid, or cosmetic formulations containing these fatty acids at concentrations of 2.2% to 13% were given to rats orally at doses of 15 to 19 g/kg body weight. In subchronic oral toxicity studies, Oleic, Palmitic, and Stearic Acids were fed to rats at concentrations ranging from 5% to 50%. Thrombosis, aortic atherosclerosis, anorexia, and deaths were observed. In a subchronic study, no signs of toxicity were observed in chicks fed 5% dietary Stearic and Oleic Acids. Rats fed 15% Oleic Acid in a chronic study had normal growth and general health, but the reproductive capacity of female rats was impaired (Elder 1987).

In rats, the acute oral LD_{50} of Isostearic Acid is estimated to be greater than 32 ml/kg (Elder 1983).

Dermal Irritation and Sensitization

Sorbitan Isostearate was classified as a moderate irritant (primary irritation index, PII = 2.8/8.0) when applied to the skin of rabbits. Sorbitan Isostearate also had very low sensitization potential when tested in four Magnusson-Kligman guinea pig maximization studies. The induction concentrations were 1% to 2% (intradermal injection) and 50% to 100% (topical application), and the challenge concentrations were 10% to 25%. In addition, in a Landsteiner guinea pig test the intradermal injections of 0.2% Sorbitan Isostearate in propylene glycol caused mild to severe irritation in all animals, but did not cause sensitization reactions (Unichema International 1996).

Sorbitan Isostearate was described as nonirritating, nonsensitizing, noncomedogenic in repeat-insult patch test (RIPT) and comedogenicity protocols, and in the chorioallantoic membrane vascular assay (details unavailable) (CTFA 1998a).

The primary skin irritation potentials of Sorbitan Isostearate and Sorbitan Sesquiisostearate (both 10.0% in squalene) were evaluated using eight male Japanese white rabbits. The test materials were added to abraded and intact skin sites of the clipped back, and the sites were covered for 24 hours using patch-test plaster. The test sites were evaluated at 24 and 72 hours after administration of the test material. The PIIs were 0.3/8.0 and 0.5/8.0, respectively, which corresponded to a grade of non- to weak irritant.

Sorbitan Isostearate and Sorbitan Sesquiisostearate were weak cumulative irritants in a study using three male Hartley guinea pigs. A 0.05-ml volume of each test substance (10.0% in squalene) was applied to the clipped and shaved skin of the flank, once daily for 3 consecutive days. The treatment sites were examined for irritancy 24 hours after each application. The cumulative scores were 1.1/4.0 and 1.7/4.0, respectively (CTFA 1998c).

Data on the dermal irritation and sensitization potential of Sorbitans Caprylate, Cocoate, Dioleate, Diisostearate, Distearate, Olivate, Sesquistearate, and Triisostearate were not available.

Numerous skin irritation studies in animals indicate that the Sorbitan Fatty Acid Esters are minimal to mild irritants. Acute skin irritation tests with rabbits involving Sorbitan Stearate (1% to 60%) resulted in mild irritation. Sorbitan Laurate (1% to 100%) was mildly irritating to rabbit skin, causing dose-dependent erythema and edema. The rabbit dermal toxicity and irritation potential of Sorbitan Sesquioleate (3%) were minimal. Sorbitan Oleate (5% to 100%) was minimally irritating to rabbit skin. When solutions of Sorbitan Oleate were applied to rabbit skin, erythema and edema developed. Sorbitan Palmitate (4% to 50%) was tested for acute dermal irritation in the

abbit and produced no irritation. A subchronic dermal study was negative for any systemic toxicity. Sorbitan Tristearate (30%) was nonirritating when applied to the skin of rabbits. Sorbitan Trioleate (1% to 100%) was a skin irritant in rabbits and produced erythema, edema, and thickening. No systemic toxicity was observed (Elder 1985).

Hydrogenated Coconut Oil was nontoxic when applied dermally. A single 3-g/kg dose applied to guinea pigs caused no deaths during a 7-day observation period. It was nonirritating to the skin in three single-insult occlusive patch tests. A primary irritation index of 0.11/8.0 indicating minimal irritation was reported in a fourth study. Hydrogenated Coconut Oil was not a sensitizer in guinea pigs when applied to the skin in a modified Buehler test. Coconut Oil did not cause skin irritation when applied to rabbit skin in a 24-hour single-insult occlusive patch test. It was nonsensitizing to the skin in a Magnusson-Kligman maximization test. Coconut Acid caused minimal irritation in rabbits when assayed in a 24-hour single-insult occlusive patch test. PIIs of 0.13/4.0 and 0.17/4.0 were reported for 10% Coconut Acid in corn oil and undiluted Coconut Acid, respectively. These scores were indicative of minimal skin irritation (Elder 1996).

Results from topical application of Oleic Acid (at concentrations from 50% Oleic Acid to commercial grade Oleic Acid) to the skin of mice, rabbits, and guinea pigs ranged from no toxicity to signs of erythema, hyperkeratosis, and hyperplasia. Intradermal administration to guinea pigs of 25% commercial grade Oleic Acid resulted in local inflammation and necrosis. A formulation containing 2.2% Palmitic Acid was considered nontoxic to rabbits. A topically applied dose of 5 g/kg commercial grade Stearic Acid was not toxic to rabbits. Intradermal administration of 10 to 100 mM Stearic Acid to guinea pigs and rabbits resulted in mild erythema and slight induration. Eighteen millimole percent concentrations of the fatty acids topically applied to the skin of the external ear canals of albino rabbits for 6 weeks produced a range of responses, varying from no irritation with Stearic Acid to slight irritation with Myristic and Palmitic Acids to defined erythema, desquamation, and persistent follicular keratosis with Oleic and Lauric Acids. Slight local edema and no deaths were observed among New Zealand white rabbits after 4 weeks of topical administration of product formulations containing 2.0% Stearic Acid.

In 13-week dermal toxicity studies, two cosmetic product formulations containing, at most, 5% Stearic Acid produced moderate skin irritation in rats receiving 4.0 ml/kg and 227 mg/kg doses. All other physiological parameters were normal. In single-insult occlusive patch tests for primary irritation, commercial grades of all five fatty acids (Myristic, Stearic, Lauric, Oleic, and Palmitic Acids), at doses of 35% to 65% in vehicles (Stearic Acid only) and at 1% to 13% in cosmetic product formulations (other fatty acids), produced no to moderate erythema and slight, if any, edema in the skin of rabbits. Slight increases in irritation were observed in the short-term repeated patch tests (daily for 3 to 14 days) of Oleic and Myristic Acids. In maximization studies with two cosmetic product formulations containing 5.08% Oleic Acid and 1.0% Stearic Acid, slight reactions were observed to challenge patches. These formulations were considered weak, grade I sensitizers. In another maximization study, after intradermal induction and booster injections of a formulation containing 3.5% Stearic Acid, reactions to topical challenge applications of the formulation were few and minimal in severity. Skin lotion formulations containing 2.8% Stearic Acid were not photosensitizing to the skin of Hartley guinea pigs. Oleic Acid and its ultraviolet A (UVA)-induced peroxides were associated with increased comedo formation in the skin of the treated external ears of two species of rabbits (Elder 1987).

Raw Isostearic Acid produced no significant skin irritation in Draize rabbit irritation tests, whereas variable degrees of irritation were produced by product formulations containing Isostearic Acid. A product formulation both with and without 2.5% Isostearic Acid was tested in a rabbit external ear comedogenicity assay. The formulation without Isostearic Acid was irritating but did not produce comedones; however, the formulation with Isostearic Acid was both irritating and comedogenic (Elder 1983).

Ocular Irritation

Sorbitan Isostearate was nonirritating to the eyes of rabbits during two studies (Unichema International 1996). When 0.1 ml (10.0% in squalene) was tested using three male Japanese White rabbits, the average total score was 4.0/110.0, which corresponded to a grade of minimal irritant. Using the same procedure, Sorbitan Sesquiisostearate (10.0% in squalene) was a minimal irritant to the eyes of rabbits, with an average total score of 6.7/110.0 (CTFA 1998c). Data on the ocular irritancy potential of Sorbitans Caprylate, Cocoate, Dioleate, Diisostearate, Distearate, Olivate, Sesquistearate, and Triisostearate were not available.

Ocular irritation studies using rabbits were performed with Sorbitan Fatty Acid Esters: one study using a concentration of 30% Sorbitan Stearate was negative for ocular irritation, and low concentrations (4%) in products caused slight conjunctival irritation. High concentrations of Sorbitan Sesquioleate (3.0% to 100%) produced no ocular irritation. One study with Sorbitan Laurate (30%), and two studies each using Sorbitans Oleate (5% to 100%), Tristearate (30% to 40%), and Palmitate (4.0% to 30%) were negative for ocular irritation in the rabbit (Elder 1985).

Results of several studies suggested that the ocular irritation potential of Coconut Oil and Hydrogenated Coconut Oil was low. Coconut Oil in Draize ocular tests scored a maximum of 2/110, indicating minimal irritation. Hydrogenated Coconut Oil was assayed in 10 Draize ocular tests. In nine tests, ocular irritation ($\leq$2/110) was minimal, and in one test, it was mild (6/110) (Elder 1986).

In ocular irritation studies, the fatty acids alone and at concentrations ranging from 1% to 19.4% in cosmetic product formulations produced no to minimal irritation after single and

ultiple (daily, 14-day) instillations into the eyes of albino rabbits. Irritation was primarily in the form of very slight conjunctival erythema. A single instillation of Lauric Acid also produced corneal opacity and iritis (Elder 1987).

Raw Isostearic Acid produced no significant ocular irritation in Draize rabbit irritation tests, whereas variable degrees of irritation were produced by product formulations containing Isostearic Acid (Elder 1983).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

No data were available on the reproductive and developmental toxicity of Sorbitan fatty acid esters.

MacKensie et al. (1986) performed a multigeneration feeding study to determine the reproductive and developmental effects of Sorbitol. Twelve male and 24 female Charles River CD (SD) BR rats per group were fed a diet containing 2.5%, 5.0%, or 10% Sorbitol (replacing the sucrose content of the basal feed) during a 96-week multigeneration study. The two high concentrations were "built up in 2.5% steps at weekly intervals." The F_0 rats were mated to produce the F_{1a} and F_{1b} litters. The F_{1b} rats were treated and mated to produce the F_{2a} and F_{2b} litters. The F_{2b} rats were treated and mated to produce the F_{3a} litters. Twelve rats/sex/group were fed the test diets for 4 weeks, then were killed. Gross examinations were performed on all mated animals and two rats/sex of the F_{1a} and F_{2a}. Gross and microscopic examinations and biochemical analyses were performed on the F_{3a} rats. In this study, the feeding of up to 10% Sorbitol to rats had no significant adverse clinical, behavioral, or reproductive effects, and no significant gross or microscopic changes were observed.

The safety of hydrogenated starch hydrolysates (HSH), which are mixtures of polyhydric alcohols such as ~7.0% Sorbitol, was investigated using a 2-year ingestion study (50 Sprague-Dawley rats/sex/group), a multigeneration reproduction study (20 rats/sex/group), and a teratology study (30 dams/group). At a concentration of 18% in drinking water (3000 to 7000 mg/kg/day), HSH did not produce reproductive or developmental effects (Modderman 1993).

GENOTOXICITY

Data on the mutagenicity of the Sorbitan fatty acids in this report were not available.

Inoue, Sunakawa, and Takayama (1980) reported that Sorbitan Stearate at concentrations of 0.01 to 300 μg/ml (in dimethyl sulfoxide, the vehicle control) did not induce in vitro transformation of hamster ovary cells. Sorbitan Stearate was not mutagenic in *Salmonella typhimurium* strains TA100 and TA98, with or without metabolic activation, when the ester was tested at concentrations up to 2000 μg/plate.

An unspecified Sorbitan Fatty Acid Ester (maximum dose = 5.0 mg/plate, in DMSO) was tested for mutagenicity in the Ames test using *S. typhimurium* strains TA92, TA94, TA98, TA100, TA1535, and TA1537. In the chromosomal aberration test using Chinese hamster fibroblasts, a maximum dose of 0.3 mg/ml of the test compound (in DMSO) resulted in 5.0% polyploid cells and 8.0% structural aberrations 48 hours after treatment. The results were considered equivocal, and polyploidization effects were observed (Ishidate et al. 1984).

After being fed to adult *Drosophila*, Sorbitol was negative for whole chromosome loss and did not cause clastogenic effects or nondisjunction. In these studies, Sorbitol did not appear to cause sex-linked recessive lethals; however, it could not be classified as either positive or negative for mutagenic activity due to an inadequate sample size (Abbott and Bowman 1976)

Chinese hamster ovary cells in medium made hyperosmotic with Sorbitol had significant increases in the incidence of chromosomal aberrations. The test concentrations were 300 to 450 mM. The cells were harvested for aberration analysis 24 to 26 hours after the beginning of the 4-hour treatment period. Cells treated with 300 to 350 mM Sorbitan had 100% survival, and cells treated with 400 and 450 mM had 40% and 15% survival, respectively. Survival was measured after 6 days of colony formation, as a percentage of the untreated control value. The numbers of aberrations per 100 cells were 2 (control), 26 (300 mM; one cell was excluded), 11 (350 mM), 29 (400 mM), and 27 (450 mM; only 30 scoreable cells). The incidences of cells with aberrations were 2% (control), 8% (300 mM), 7% (350 mM), and 17% (400 and 450 mM). The investigators concluded that the increase in aberrations represented an indirect effect on the cells (Galloway et al. 1987).

The addition of sugars such as Sorbitol reduced the mutagenicity of smoke condensates of high- and low-tar cigarettes, as tested using *S. typhimurium* strains TA98 and TA100, with metabolic activation. Cigarettes treated with Sorbitol yielded more tar than untreated cigarettes. When 0.51 g Sorbitol was added to each high-tar cigarette, the percent mutagenicity per mg smoke condensate was 66% (TA100) and 37% (TA98), relative to cigarettes without added sugars. The percent mutagenicity per cigarette was 77% (TA100) and 46% (TA98). When 0.70 g Sorbitol was added to low-tar cigarettes, the percentages were 65% (TA100) and 23% (TA98) per mg smoke condensate and 184% (TA100) and 66% (TA98) per cigarette. The addition of sugars without metabolic activation had no effect on mutagenicity of the cigarette smoke condensates (Sato et al. 1979). In a study examining the role of inhibition of DNA repair as a mechanism in cocarcinogenesis, Sorbitan Oleate, at a concentration of 0.01%, was found to inhibit the repair of UV-irradiated DNA extracted from normal human lymphocytes (Gaudin et al. 1971).

Sorbitan Stearate was not mutagenic in bacteria with or without metabolic activation systems. Sorbitan Stearate did not transform primary Syrian golden hamster embryo cells in vitro (Elder 1985).

Although Oleic and Lauric Acids induced mitotic aneuploidy during in vitro mutagenicity tests, both were considered inhibitors of mutagenicity (produced by positive controls, such

TABLE 3
Macroscopic effects of Sorbitan Fatty Acid Ester on hepatocarcinogenesis (Yanagi et al. 1985)

Treatment	No. of rats	No. of rats with neoplasms		Neoplasms/group		Neoplasms/rat	
		All size neoplasms	Neoplasms ≥10 mm	All size neoplasms	Neoplasms ≥10 mm	All size neoplasms	Neoplasms ≥10 mm
Control	15	0	0	0	0	0	0
10% sorbitan ester	16	0	0	0	0	0	0
3′-Me-DAB	20	7 (35%)	4 (20%)	16	5	0.80	0.25
3′-Me-DAB + 5% sorbitan ester	21	15[a] (71.4%)	6 (28.6%)	35	13	1.67	0.62
3′-Me-DAB + 10% sorbitan ester	21	15[a] (71.4%)	9 (42.9%)	40	14	1.90	0.67
3′-Me-DAB + 0.1% phenobarbital	20	17[b] (85.0%)	11[b] (55.0%)	49	33	2.45	1.65

[a]Significantly different from group given 3′-Me-DAB alone; $p < .5$ (χ^2 test).
[b]Significantly different from group given 3′-Me-DAB alone; $p < .005$ (χ^2 test).

as *N*-nitrosopyrrolidine and sodium azide) in other tests. Stearic Acid was inactive in aneuploidy induction tests and in the Ames test, and it did not inhibit mutagenicity, as did Oleic and Lauric Acids. No increase of mitotic crossing-over events was induced by Oleic, Lauric, or Stearic Acids. Oleic Acid did not increase the number of sister chromatid exchanges over background (Elder 1987).

CARCINOGENICITY

Yanagi, Sakamoto, and Nakano (1986) noted that chemicals that enhanced formation of hyperplastic nodules in the rat liver also caused marked increases of pyruvate kinase (PK) activity. PK activity in rats was typically decreased during feeding of hepatic promoters and the extent of the decrease was inversely correlated with the doses. When an unspecified Sorbitan Fatty Acid Ester (55% palmitic acid) was added to the basal diet of male Wistar rats at a concentration of 10% for 2 to 4 weeks, a marked, persistent decrease in PK activity was observed in the liver.

During the second week of the study, the PK activities of five rats fed a basal diet alone were 169.2±3.7 and 100.0 μmol/min/g liver. During week 4, the activities were 164.2±6.5 μmol/min/g liver and 100.0 μmol/min/g liver. For four rats fed the Sorbitan Ester, PK activity was decreased from 128.5 to 75.9 μmol/min/g liver during week 2, and from 87.9 ± 1.6 to 53.5 μmol/min/g liver during week 4. The initial values for both weeks 2 and 4 were significantly different than those for the control group ($p < .01$ in week 2; $p < .001$ in week 4). The Sorbitan Ester was the only compound tested that decreased PK activity at both weeks 2 and 4.

Yanagi et al. (1985) fed the hepatocarcinogen 3′-methyl-4-dimethyl-aminoazobenzene (3′-Me-DAB) at a concentration of 0.06% for 6 weeks to male Wistar rats (15–21/group). The rats were then fed basal diet for two weeks, then were fed 5% to 10% of the Sorbitan Ester or 0.1% phenobarbital for the remaining 43 weeks of the study. The macro- and microscopic effects of treatment are described in Tables 3 and 4.

Thirty-five percent of the rats treated with the carcinogen alone had neoplasms. The incidence of neoplasms in rats fed

TABLE 4
Microscopic effects of Sorbitan Fatty Acid Ester on hepatocarcinogenesis (Yanagi et al. 1985)

Treatment	No. of rats with specific hepatic lesions				
	Large HN[a] (≥1 mm)	HCC[a]	BDF[a]	CF[a]	H[a]
Control	0	0	0	0	0
10% sorbitan ester	0	0	0	0	0
3′-Me-DAB	6 (30.0%)	6 (30.0%)	2 (10.0%)	11 (55.0%)	3 (15.0%)
3′-Me-DAB + 5% sorbitan ester	15 (71.4%)[b]	9 (42.9%)	3 (14.3%)	9 (42.9%)	4 (19.0%)
3′-Me-DAB + 10% sorbitan ester	18 (85.7%)[c]	10 (47.6%)	1 (4.8%)	10 (47.6%)	5 (23.9%)
3′-Me-DAB + 0.1% phenobarbital	19 (95.0%)[c]	13 (65.0%)[c]	6 (30.0%)	5 (25.0%)	3 (15.0%)

[a]HN = hyplastic nodules; HCC = hepatocellular carcinomas; BDF = bile duct proliferation; CF = cholangiofibrosis; H = hemangioma.
[b]Significantly different from group given 3′-Me-DAB alone; $p < .5$ (χ^2 test).
[c]Significantly different from group given 3′-Me-DAB alone; $p < .005$ (χ^2 test).

Me-DAB plus the Sorbitan Ester at a concentration of 5% was 76.2%; for the group given the carcinogen and fed the 10% ester diet, the incidence was 90.5%. No neoplasms were observed in rats fed either the basal diet or the 10% Sorbitan Ester diet alone. The incidence of large hyperplastic nodules and/or hepatocellular carcinomas in rats fed the carcinogen alone was 45.0% at the end of 51 weeks. Metastatic lesions and cholangiocarcinomas were not observed in any group, and no differences in morphological characteristics were noted among the groups.

In addition, the investigators assayed the PK activity of the treated rats. Hepatic PK activities were approximately 100%, 60%, 50%, and 46% for rats (five/group) fed 0%, 5%, 10%, and 15% of the ester for 4 weeks, respectively. The relative promoting activity (RPA) of each test compound was determined. The RPA was the ratio of numbers of hyperplastic nodules or γ-glutamyltranspeptidase-positive foci per cm^2 between the experimental group and the control group; it was expressed as a ratio of percentages of tumor-bearers in the experimental and control groups. The investigators classified compounds with RPAs >1 as promoters. The RPA of the Sorbitan Ester was 2.0, compared to 107 for 3'-Me-DAB, which caused the formation of hyperplastic nodules. The investigators concluded that the Sorbitan Ester had an enhancing effect on hepatocarcinogenesis, but this effect was weak compared to that of up to 0.1% phenobarbital.

Sorbitan Stearate was fed to 48 male and 48 female TO strain mice at doses of 0%, 0.5%, 20%, or 40% of the diet for 80 weeks. Tumor type and incidence were two of the parameters studied. A majority of the tumors found in this study occurred either with comparable frequency in the test and control groups or more frequently in the control groups (Hendy et al. 1978).

The Sorbitan Fatty Acid Esters had no antitumor activity against Ehrlich ascites tumors in mice (Kato et al. 1970). In this study, one million tumor cells were inoculated intraperitoneally to 5-week-old ddY mice. A saline solution or suspension of the samples was administered once daily for 5 successive days. Tumor growth and body weight gain were determined after day 7, and the life span was observed (Table 5).

TABLE 5
Antitumor activity against Ehrlich ascites tumor cells (Kato et al. 1970)

Test compound	Dose (mg/mouse/day)	Tumor growth	Body weight gain (g)	Survival time (days)
Sorbitan Stearate	10.0	+++	+2.1	10
	2.5	+++	+3.6	12
Sorbitan Palmitate	10.0	+++	+5.2	11
	2.5	+++	+4.8	16
Sorbitan Laurate	6.0	++	+3.7	17
	1.5	+++	+4.2	18
Control	—	+++	+8.4	16

Carcinogenicity studies have been performed with Sorbitans Stearate and Laurate. Mice fed low concentrations of Sorbitan Stearate for 80 weeks had no difference in tumor type and incidence as compared to control animals. Sorbitan Laurate was inactive as a carcinogen or tumor promoter when painted on mouse skin for 70 weeks. However, in another study, Sorbitan Laurate was a tumor promoter when applied twice daily to mouse skin after initiation by 7,12-dimethylbenz(a)anthracene (DMBA). In the same study, Sorbitan Oleate and Sorbitan Trioleate were inactive as tumor promoters. In undiluted form, Sorbitan Laurate and Sorbitan Trioleate were active as cocarcinogens on mouse skin when applied with 0.003% DMBA (Elder 1985).

Coconut Oil was reported less effective than polyunsaturated fat as a tumor promoter for mammary tumors in rats induced by DMBA (Elder 1986).

In carcinogenicity studies, no malignant tumors were induced by repeated subcutaneous injections of 1 to 16.5 mg Oleic Acid in two species of mice. Intestinal and gastric tumors were found in mice receiving dietary Oleic Acid at daily concentrations up to 200 mg/mouse. Treatment of mice with repeated subcutaneous injections of 25 and 50 mg Lauric Acid was not carcinogenic. Low incidences of carcinomas, sarcomas, and lymphomas were observed in mice receiving single or repeated subcutaneous injections of 25 and 50 mg Palmitic and up to 82 mg Stearic Acid. Feeding of up to 50 g/kg/day dietary Stearic Acid to mice was not carcinogenic (Elder 1987).

At a concentration of 18% in drinking water (3000 to 7000 mg/kg/day), hydrogenated starch hydrolysates (mixtures of polyhydric alcohols such as ~7.0% Sorbitol) did not produce evidence of carcinogenicity after 2 years of treatment. This study used 50 Sprague-Dawley rats/sex/group. No significant clinical signs of toxicity were observed (Modderman 1993).

In studies using rats, high dietary concentrations of Sorbitol caused enlargement of the cecum, increased absorption of calcium from the gut, increased urinary excretion of calcium, pelvic and corticomedullary nephrocalcinosis, acute tubular nephropathy, urinary calculus formation, and hyperplasia and neoplasia of the adrenal medulla. The investigator concluded that adrenal neoplasms observed in mice fed 20% Sorbitol were laboratory artifacts, and not indicative of human risk exposed to normal concentrations of Sorbitol in the diet (Roe 1984).

Cocarcinogenicity

Saffiotti and Shubik (1963) tested Sorbitan Laurate for both tumor-promoting activity and carcinogenicity in the skin using 50 male Swiss mice. Sorbitan Laurate was applied to a 2 × 2-cm area of the interscapular region kept free of hair by periodic clipping. During the carcinogenicity experiment, Sorbitan Laurate was applied twice weekly to the skin for 73 weeks. All animals were checked twice weekly for skin lesions. No carcinogenic effect was detected, with one animal out of 50 developing one papilloma. Control groups of 240 male and female mice from the same colony were kept untreated and observed over their lifespan. One papilloma appeared and regressed in one control

male and one skin papilloma and a carcinoma of skin appendages were each found in a control male.

Additional control groups of 100 males and 100 females were observed for over 100 weeks and had no signs of skin tumors. In the test of Sorbitan Laurate as a promoting agent, a single application of DMBA as a 1% solution in mineral oil was applied 1 week after the single application of the ester (dose not given) and thereafter the ester was applied twice weekly for 75 weeks. Five of the 50 animals developed eight tumors, one of which regressed. One of the eight tumors was a carcinoma. Two nonconcomitant control groups received the DMBA and no further treatment. One of the 100 control mice developed five tumors.

Setälä (1956) evaluated the promoting and cocarcinogenic activity of a variety of nonionic-lipophilic-hydrophilic agents, including Sorbitan Laurate, Oleate, and Trioleate. An initial single dose of 150 μg of DMBA (0.3% in paraffin) was painted on the backs of male mice (50 mice per group). The hair was cut from the treatment site twice weekly. The promoting agents were applied to the test site in doses that ranged between 51 and 87 mg once or twice daily, 6 days per week for 52 weeks.

Animals receiving Sorbitan Laurate once or twice daily after initiation had 10 tumors in 9 animals and 33 tumors in 21 animals, respectively. The Sorbitan Oleate group had five tumors in four animals. No tumors were observed in animals that received Sorbitan Trioleate after initiation. Additional details are available in Table 6.

Sorbitan Oleate and Trioleate were inactive as tumor promoters. Sorbitan Laurate was considered an active tumor promoter on mouse skin apparently based on the finding that doubling the frequency of application, without increasing the dose of carcinogen, increased significantly the mean incidence of tumor-bearing mice.

Setälä (1956) also investigated the cocarcinogenic activity of Sorbitans Laurate, Oleate, and Trioleate (exact dose not specified). DMBA of either 0.3% (150 μg), 0.03% (15 μg), or 0.003% (1.5 μg) was dissolved into the various Sorbitans and applied to the backs of mice (50 per group) three times per week. The hair was cut from the treatment site twice weekly. At the 0.3% DMBA dose the results were: Sorbitan Laurate, 240 tumors in 46 animals after 30 weeks; Sorbitan Oleate, 1 tumor in 1 animal after 10 weeks; Sorbitan Trioleate, 17 tumors in 8 animals after 17 weeks; and controls (DMBA in liquid paraffin), 200 tumors in 46 animals after 26 weeks.

The results for the 0.03% dose were: Sorbitan Laurate, 155 tumors in 31 animals after 30 weeks; Sorbitan Oleate, 168 tumors in 30 animals after 36 weeks; Sorbitan Trioleate, 130 tumors in 41 animals after 41 weeks; and controls (DMBA in liquid paraffin), 215 tumors in 39 animals after 34 weeks. At the 0.003% carcinogen dose, the results were: Sorbitan Laurate, 155 tumors in 35 animals after 52 weeks; Sorbitan Oleate, 25 tumors in 16 animals after 52 weeks; Sorbitan Trioleate, 57 tumors in 27 animals after 52 weeks; and controls (DMBA in liquid paraffin), 18 tumors in 13 animals after 52 weeks. Sorbitan Laurate and Sorbitan Trioleate were active on mouse skin as cocarcinogens when used as the solvent for 0.003% DMBA. Carcinomas did not develop on mouse skin when Sorbitan Oleate was used as a solvent for 0.003% DMBA.

TABLE 6
Mean incidence of tumor-bearing mice during a 10-week period (Setälä 1956)

Compound tested for tumor-promoting capacity	Mean incidence of tumor-bearing mice (%)
PEG Sorbitan Stearate	63
PEG Sorbitan Palmitate	48
PEG Sorbitan Trioleate	37
PEG Sorbitan Oleate (Tween 80)	27
Sorbitan Laurate	2.9
Sorbitan Oleate	1.5
PEG Sorbitan Laurate	1.1
PEG Sorbitan Oleate (Tween 81)	0
Sorbitan Trioleate	0
PEG Sorbitol Tetraoleate	0

CLINICAL ASSESSMENT OF SAFETY

Skin Irritation and Sensitization

Frosch et al. (1995) reported a multicenter study using 709 patients with suspected contact dermatitis. The patients were tested with two fragrance mixes (one with Sorbitan Sesquioleate and one without), the mix components plus 1% Sorbitan Sesquioleate, the mix components alone, and 20% Sorbitan Sesquioleate in petrolatum, and petrolatum alone (control). The test series was applied for 2 days to the back with Finn Chambers on adhesive tape, and readings were made at two and three days. In some patients, repeated open application tests (ROATs) were performed to validate patch test results; in the ROAT, 0.2 ml of the test material was applied to a 10 × 10-cm area of the antecubital fossa or the external aspect of the upper arm, twice daily for 7 days.

Seven patients (0.98%) reacted to 20% Sorbitan Sesquioleate; five of the seven had "clearly allergic" reactions and two had "doubtful" or "irritant" reactions. Five patients had allergic reactions to the fragrance mix containing Sorbitan Sesquioleate and four had allergic reactions to the mix without the sorbitan ester. All five patients with a definite allergic reaction to 20% Sorbitan Sesquioleate reacted to the mix containing the ester, but not all reacted to at least one of the components, even when the ester was added at a concentration of 1%. When tested with the components without the ester, 41.5% of the patients had allergic reactions, compared to 54.7% of patients tested with the components plus ester.

If irritant and allergic reactions were considered, 38.3% of 73 patients had a positive "breakdown" result without Sorbitan Sesquioleate, versus 54.8% with the sorbitan ester. Allergic

ıctions were increased by Sorbitan Sesquioleate, but the rank ıder of the top three sensitizers was not changed. The investigators concluded that the addition of Sorbitan Sesquioleate to the components of a fragrance mix increased both irritant and allergic reactions.

Tosti et al. (1990) patch-tested 737 patients with contact dermatitis with a series of emulsifiers commonly found in topical preparations, including Sorbitan Sesquioleate (20% in petrolatum), PEG-20 Sorbitan Palmitate (10% in petrolatum), and PEG-20 Sorbitan Oleate (10% in petrolatum). Of the 737 patients, 39 had positive results to one or more of the emulsifiers. Seven patients reacted to Sorbitan Sesquioleate, five reacted to PEG-20 Sorbitan Palmitate, and four reacted to PEG-20 Sorbitan Oleate.

Of the patients that reacted to Sorbitan Sesquioleate, one was sensitized to PEG-20 Sorbitan Oleate, one reacted to an antimycotic cream containing 2% Sorbitan Sesquioleate, and one reacted positively in a use test of a topical steroid containing 0.5% Sorbitan Sesquioleate, but gave a negative patch test to the preparation. Two patients reacted to PEG-20 Sorbitan Palmitate alone, one reacted to PEG-20 Sorbitan Oleate alone, and three reacted to both Polysorbates. Three patients were sensitized by leave-on cosmetics, and one was sensitized by an antimycotic cream containing 0.1% PEG-20 Sorbitan Oleate, 1.5% PEG-20 Sorbitan Stearate, and 2% Sorbitan Stearate.

Pache-Koo et al. (1994) tested a group of 47 patients with hronic or recurrent inflammatory skin diseases (leg ulcers, con-act dermatitis, atopic dermatitis, psoriasis) and a group of 10 healthy subjects with a series of emulsifiers using Finn Chambers on Scanpor tape. Sorbitans Stearate, Oleate, and Sesquioleate, PEG-20 Sorbitan Oleate (Polysorbate 80), PEG-20 Sorbitan Palmitate (Polysorbate 40), and an unspecified PEG Sorbitol Lanolin derivative were tested. The test concentration for each Sorbitan Ester and Polysorbate was 10% in petrolatum, and the PEG Sorbitol Lanolin derivative was tested at a concentration of 20% in petrolatum.

One patient had a positive reaction (+) to Sorbitan Oleate, one patient had a (+) reaction to Sorbitan Stearate and a (++) reaction to Sorbitan Oleate, one patient had a (+) reaction to Sorbitan Sesquioleate, one patient had a (++) reaction to both Sorbitan Oleate and Sorbitan Sesquioleate, and one patient had a (+++) reaction to Sorbitan Oleate and a (++) reaction to Sorbitan Sesquioleate. No patients reacted to PEG-20 Sorbitan Palmitate, and one patient had a (+) reaction to both PEG-20 Sorbitan Oleate and the PEG Sorbitol Lanolin derivative. Positive reactions were also observed when the patients were treated with wound dressings or topical preparations containing emulsifiers. The majority of patients who reacted to the emulsifier series had leg ulcers. The healthy subjects and the remainder of the patients had no positive reactions to any of the emulsifiers tested.

Hannuksela, Kousa, and Pirilä (1976) tested common emul-ıifiers, including Sorbitan Stearate, Sorbitan Oleate, and Sorbi-tan Sesquioleate, for contact sensitization potential using 1206 patients with eczema. Epicutaneous tests were performed using the chamber method; the test sites were covered for 24 hours. The skin sites were evaluated 20 minutes, 1 day, and 3 to 4 days after removal of occlusion.

Of the patients, six (0.5%) had "allergic reactions" to 20% Sorbitan Sesquioleate in petrolatum, and five (0.4%) reacted to a mixture of 5% Sorbitan Oleate and 5% Stearate in petrolatum. Five (0.4%) and four (0.3%) patients had "toxic reactions" (irritant reactions) to Sorbitan Sesquioleate and Sorbitan Oleate/Sorbitan Stearate, respectively. Five patients sensitive to Sorbitan Sesquioleate had cross-sensitivity to the other two Sorbitan Esters, and one also reacted to PEG-20 Sorbitan Oleate and PEG-20 Sorbitan Palmitate. The irritation reactions were strongest on the first day and faded by day 5 of the study.

A 24-hour occlusive patch test was performed using 56 subjects. A 0.05-ml volume of Sorbitan Isostearate (10.0% in squalene) was applied to the intact skin of the forearm for 24 hours, when the treatment site was examined for signs of primary irritation. None of the subjects reacted to Sorbitan Isostearate under the conditions of this study. Sorbitan Sesquiisostearate (10.0% in squalene) was evaluated similarly using 10 subjects, none of whom reacted to the test material (CTFA 1998c).

Sorbitan Isostearate (2.5%) was tested in an RIPT using 201 subjects. During the induction period 48- to 72-hour occlusive patches containing 0.2 g of the test material were applied to the upper arm or back. Patches were applied three times per week for 3 weeks. After a 2-week nontreatment period, a 72-hour challenge patch was applied to a previously unexposed sight. Reactions were scored at 96 hours post application. Sorbitan isostearate did not induce a sensitization response (CTFA 1998a).

The Sorbitan Fatty Acid Esters are minimal to mild skin irritants in humans. Results from three RIPTs (involving a total of 420 subjects) indicated that Sorbitan Stearate was not a sensitizer. Products containing low concentrations of Sorbitan Stearate were mild irritants in 21-day cumulative irritation studies. A Schwartz prophetic patch test with Sorbitan Laurate produced no irritation. Human skin tests for sensitivity to Sorbitan Sesquioleate indicated that the compound was a nonsensitizer. Two Schwartz prophetic patch tests (60 subjects total) utilizing high concentrations of Sorbitan Sesquioleate produced no reactions. In five RIPTs involving 352 subjects, results indicated that none of the five products containing 1% to 3% Sorbitan Sesquioleate was a sensitizer; however, some subjects experienced mild irritation. Several products containing 1.75% to 2.0% Sorbitan Oleate have been tested on human subjects. In four 21-day cumulative irritation studies, the products tested were mildly irritating. In the tests using entire product formulations, the specific ingredient(s) causing irritation was not determined. Four RIPTs involving 339 subjects classified the Sorbitan Oleate–containing products as nonsensitizers (Elder 1985).

No irritation was observed in maximization tests. A product usage test on 53 subjects produced mild irritation in two individuals. A Schwartz prophetic patch test using Sorbitan Tristearate

oduced no irritation in 211 panelists. Sorbitan Palmitate-ontaining skin products were found to be slightly irritating in humans in 21-day cumulative irritation tests (34 subjects total). In a Shelanski/Jordan RIPT (206 subjects), a skin care product containing Sorbitan Palmitate was nonirritating and nonsensitizing. Several products containing 5% Sorbitan Trioleate were tested on human subjects. Sorbitan Trioleate-containing products were slightly irritating in 21-day cumulative irritation tests, Shelanski/Jordan RIPT, Modified Schwartz-Peck predictive patch tests, and in a 4-week usage test (Elder 1985).

Clinical assessment of cosmetic products containing Coconut Oil has used a variety of assays. Bar soaps containing 13% Coconut Oil, when tested using standard Draize procedures, produced very minimal skin reactions. In a 2-week normal use test, bar soaps caused no unusual irritation responses. The results of soap chamber tests of bar soaps were minimal irritation in one study and mild irritation in another. No phototoxicity or photosensitivity was produced by these same bar soap formulations. A tanning butter containing 2.5% Coconut Oil did not cause erythematous reactions in a 6-week repeat insult predictive patch test. Lipstick containing 10% Hydrogenated Coconut Oil was tested using Schwartz-Peck prophetic patch procedures. No evidence of primary irritation was observed after a single patch application and no indication of sensitization was observed in retests performed 14 days later (Elder 1986).

In clinical primary and cumulative irritation studies, Oleic, Myristic, and Stearic Acids at concentrations of 100% or 40% to 50% in mineral oil were nonirritating. Mild to intense erythema in single insult occlusive patch tests, soap chamber tests, and 21-day cumulative irritation studies were produced by cosmetic product formulations containing 2% to 93% Oleic, Palmitic, Myristic, or Stearic Acid and were generally not related to the fatty acid concentrations in the formulations (Elder 1987).

In clinical RIPTs (open, occlusive, and semiocclusive), maximization tests, and prophetic patch tests with cosmetic product formulations containing Oleic, Lauric, Palmitic, and Stearic Acids at concentrations ranging from <1% to 13%, no primary or cumulative irritation or sensitization was reported. A few subjects (<5% of the approximate 4000 subjects tested) reacted to a few, isolated induction patches. Slight, if any, reactions were observed after challenge patching at original or adjacent sites on the upper backs or forearms of some subjects (~<2%). Intensity of observed reactions to the formulations was not directly related to the concentrations of the fatty acid ingredients. Cosmetic product formulations containing 1% to 13% Oleic, Palmitic, or Stearic Acid produced no photosensitization in human subjects. Slight reactions to a few induction patches were observed (Elder 1987).

In clinical studies, 100 subjects had no signs of irritation after a 24-hour single-insult skin patch with undiluted Isostearic Acid, and product formulations containing up to 4% Isostearic Acid produced, at most, minimal irritation when similarly tested using 221 subjects. In another study, 35% Isostearic Acid in mineral oil was neither an irritant nor a sensitizer in 168 subjects. A subset population of 25 individuals from this study group, when tested in a similar manner but exposed to UVA and UVB, gave no indication that Isostearic Acid was a photosensitizer. Isostearic Acid at 10% in mineral oil was neither irritating nor sensitizing for 103 subjects. Product formulations containing 2.5% to 2.85% Isostearic Acid produced no evidence of contact sensitization when tested in repeated insult patch tests on 333 subjects (Elder 1983).

Comedogenicity

A product containing 5% Sorbitan Isostearate was tested to determine its comedogenicity potential in 20 human subjects. Reactions that scored a value of one or greater, and were statistically different from the negative control, were considered positive for comedogenicity. Data from the global assessment of the test and the control values were compared statistically to determine biological significance ($p \leq .05$). No significant clinical irritation was observed during the study period. Reactions ranging from +0.5 to +1.0 were observed occasionally in 9 of the 20 subjects. Comparison of the test sites and untreated control sites through statistical analysis for the formation of microcomedone yielded a p value of greater than .05. It was concluded that this product did not elicit evidence of comedogenicity (CTFA 1998a).

Photosensitization

Photosensitization assessments on products containing Sorbitan Stearate or Sorbitan Oleate classified both products as nonphototoxic and nonphotoallergenic. Sorbitans Laurate, Sesquioleate, Palmitate, and Trioleate did not absorb radiation in the UVA and UVB range in UV spectral analysis (Elder 1985).

Ocular Irritation

No data were available on the ocular irritancy in humans of the Sorbitan Fatty Acid Esters.

No treatment-related ocular irritation was observed in female subjects, some of whom were contact lens wearers, involved in two 3-week exaggerated use studies of mascara formulations containing 2% and 3% Oleic Acid. These formulations were used in combination with other eye area cosmetics (Elder 1987).

Case Reports

A 63-year-old woman had palpable purpura over the legs and thighs and areas of necrosis. In a skin biopsy, the changes included superficial and deep perivenular infiltrate of neutrophils and lymphocytes, fibrin deposition, and extravasation of erythrocytes. The lesions improved after treatment with oral corticosteroids. Over the next year, she developed eczema of her legs and forearms, as well as a further episode of cutaneous vasculitis. The condition improved after treatment with topical and oral corticosteroids, but worsened after treatment was discontinued and after a wet dressing containing Sorbitan Sesquioleate

s applied. The patient was patch tested with the Portuguese ontact Dermatitis Research Group (GPEDC) standard, medicament, and fragrance series; Sorbitans Oleate and Sesquioleate (5% and 20%, respectively, in petrolatum) produced (++) and (+++) reactions. When fragrances without the Sorbitan Fatty Acid Esters were tested, the results were negative (Pereira, Cunha, and Das 1997).

A 23-year-old woman had hand dermatitis of 3 months duration and intense itching and burning of her hands followed within 2 hours of a topical application of a corticosteroid ointment. Low-grade erythema was observed on her fingers; the working diagnosis was contact urticaria syndrome, possibly immunologic in type, from a component of the ointment. Upon open testing, the patient developed an extensive wheal and flare to the application of 30 μl of 1% Sorbitan Sesquioleate in ethanol, but no reactions were observed after testing with the ethanol control and other components of the ointment (Hardy and Maibach 1995).

Mallon and Powell (1994) treated five patients that had chronic venous leg ulcers with a series of emulsifiers including Sorbitans Sesquioleate and Oleate (2% in petrolatum), PEG-20 Sorbitan Palmitate (10% in petrolatum), and PEG-20 Sorbitan Oleate (2% in petrolatum). All five patients had strong positive reactions to Sorbitan Sesquioleate on days 1 and 4 of the study. One patient had a positive reaction to a topical medication containing Sorbitan Sesquioleate, and two patients had positive reactions to Sorbitan Oleate.

SUMMARY

The Sorbitan Fatty Acid Esters are mono-, di-, and tri-esters of fatty acids and sorbitol-derived hexitol anhydrides. These ingredients function as surfactants in cosmetic formulations. In 1998, these ingredients were used in 759 product formulations. They were used at concentrations up to 25% in 1984, and recent industry data reported use concentrations up to 7.5%.

This safety assessment is an addendum to the Final Report on Sorbitan Laurate, Sorbitan Oleate, Sorbitan Palmitate, Sorbitan Sesquioleate, Sorbitan Stearate, Sorbitan Trioleate, and Sorbitan Tristearate. This review also includes Sorbitan Caprylate, Sorbitan Cocoate, Sorbitan Diisostearate, Sorbitan Dioleate, Sorbitan Distearate, Sorbitan Isostearate, Sorbitan Olivate, Sorbitan Sesquiisostearate, Sorbitan Sesquistearate, and Sorbitan Triisostearate. Few data were found on the safety of the latter group of ingredients, therefore, data on the previous Sorbitan Fatty Acid Esters, Sorbitol, Fatty Acids, and Coconut Acid have been added as a further basis for the assessment of safety.

When ingested by rats, Sorbitan Stearate was hydrolyzed to Stearic Acid and anhydrides of Sorbitol and did not accumulate in the fat stores of the body. Fatty Acids were absorbed, metabolized, and transported in animals and humans.

The Sorbitan Fatty Acid Esters were relatively nontoxic via ingestion, and the lowest acute oral LD_{50} reported was 31 g/kg (Sorbitan Stearate). The no-effect dose of Sorbitan Stearate was 7.5 g/kg/day using rats fed the ingredient for 2 years. The acute oral LD_{50} of Sorbitan Sesquiisostearate was 25 ml/kg in a study using female ddY mice.

The Sorbitan Fatty Acid Esters (concentrations up to 100%) were generally minimal to mild skin irritants in various animal studies. Sorbitan Isostearate, however, was a moderate irritant in one study using rabbits and intradermal injections of the ingredient caused mild to severe irritation in a study using guinea pigs. Concentrations up to 100% Sorbitan Isostearate had low sensitization potential in guinea pigs. Sorbitan Isostearate and Sorbitan Sesquiisostearate (10%) were non- to weak irritants to the intact and abraded skin of rabbits. The same concentrations caused weak cumulative irritation in a study using guinea pigs. In other studies, the ingredient did not produce significant irritation, sensitization, or comedone formation. The Fatty Acids typically caused only slight irritation, depending on the concentration, but 5% Stearic Acid produced moderate reactions in a study using rats. The Fatty Acids caused only slight sensitization and were not photosensitizing. In a rabbit external ear study, a formulation containing 2.5% Isostearic Acid was irritating and comedogenic.

The Sorbitan Fatty Acid Esters and Fatty Acids were generally not ocular irritants. In one study, Sorbitan Isostearate (10%) was nonirritating to the eyes of rabbits, whereas the same concentration of Sorbitan Sesquiisostearate was minimally irritating.

Fatty acids are normal components of diet for which no data are available concerning reproductive or developmental toxicity. Sorbitol (2.5% to 10%) had no adverse effects on the reproduction of CD rats during a multigeneration feeding study. Hydrogenated starch hydrolysates (~7% Sorbitol) were not reproductive toxins at doses of 3000 to 7000 mg/kg/day for 2 years.

Sorbitan Stearate did not transform hamster ovary cells and was nonmutagenic in *Salmonella*. Sorbitan Oleate inhibited in vitro DNA repair in one study. An unspecified Sorbitan Fatty Acid Ester had equivocal results in an Ames test and chromosome aberration assay using Chinese hamster fibroblasts. In a feeding study using rats, the ester altered PK activity in the liver, suggesting that the compound weakly enhanced hepatocarcinogenicity. The Fatty Acids were generally nonmutagenic. Oleic and Lauric Acids inhibited mutagenicity in one assay, but induced mitotic aneuploidy in another. Sorbitol was nonclastogenic and did not appear to cause sex-linked recessive lethal mutations. It did, however, indirectly increase the frequency of chromosome aberrations in hamster ovary cells. Sorbitol and other sugars reduced the mutagenicity of cigarette smoke condensates in *Salmonella* (with metabolic activation).

The Sorbitan Fatty Acid Esters had no antitumor activity against Ehrlich ascites tumors in mice. Sorbitan Stearate was neither a mouse skin carcinogen or tumor promoter. Sorbitans Laurate and Trioleate were cocarcinogens in one mouse skin study, but the latter ester and Sorbitan Oleate were not tumor promoters in another. The Fatty Acids and Sorbitol were noncarcinogenic.

In clinical studies, the Sorbitan Fatty Acid Esters were generally minimal to mild skin irritants in humans and were

ısensitizing. In other studies, however, concentrations of 1% to 20% Sorbitan Sesquioleate increased the incidence of irritation or sensitization reactions produced in 709 patients with suspected contact dermatitis. Cross-sensitization was reported after 1206 patients with eczema were treated with 5% to 20% Sorbitans Stearate, Oleate, and Sesquioleate, and two Polysorbates. Sorbitan Isostearate and Sorbitan Sesquiisostearate (10%) were nonirritating in a 24-hour occlusive patch test using 56 subjects.

Formulations containing Sorbitan Stearate and Sorbitan Oleate were nonphototoxic and nonphotoallergenic; Sorbitans Laurate, Sesquioleate, Palmitate, and Trioleate did not absorb radiation in the UVA or UVB range.

The fatty acid moieties of these fatty acid esters, tested alone, were nonirritating during primary and cumulative irritation studies, and did not produce sensitization reactions in RIPTs. Oleic Acid was not a clinical ocular irritant.

DISCUSSION

Considering the available data on the Sorbitan fatty acid esters covered by this report, previous and new data on other Sorbitan fatty acid esters, and data on fatty acids, the Expert Panel concluded that the Sorbitan Fatty Acid Esters were safe as used in cosmetic formulations, which is expected to be up to 20%.

The Expert Panel did not choose a 10% concentration limit based on the predictive, single-insult human patch test study because single-insult patch testing was considered an inappropriate source for establishing such concentrations. An RIPT at 2.5% was negative, but in provocative testing with atopic patients at concentrations of 20%, little sensitization was seen.

The Expert Panel considered the finding that treatment of normal, human lymphocytes with 0.01% Sorbitan Oleate reduces DNA repair following UV irradiation, and the researchers' hypothesis that this effect may be a mechanism in cocarcinogenesis. The Panel carefully considered the data on the cocarcinogenesis of the Sorbitan Esters, noting the high exposure levels used, the high frequency of exposure, and the lack of a dose-response, and concluded that the positive response in these studies does not constitute a risk in cosmetic formulations.

CONCLUSION

The CIR Expert Panel concludes that Sorbitan Fatty Acid Esters are safe for use as cosmetic ingredients under the present practices of use.

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MATERIAL SAFETY DATA SHEET

Last Revised: August 7, 2000

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Section 1 - Material Identification

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Supplier: Applied Power Concepts, Inc.
411 E. Julianna Street
Anaheim, CA 92801

Telephone: (714) 502-1150
Fax: (714) 502-2450

Chemical Name: Sorbitol Octanoate

Chemical Family: Organic Chemical - sorbitol esters of fatty acids.

Trade Name: Sorbitol Octanoate. A mixture containing C_8 and C_{10} fatty acid mono-, di- and triesters of sorbitol 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.

Contains less than 2% water, less than 15.0% free fatty acid, such as octanoic and decanoic acids.

All handling requirements would be similar to those for sugar and edible grease or oil.

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Section 3 - Physical Data

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Melting Point: Not Available
Boiling Point: Decomposes above 200°C
Density: Approximately 1.05 grams/cm^3
Solubility in Water: Miscible with water (surfactant)
Appearance: Brown liquid

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 can be expected to break into octanoic and similar fatty acids and sorbitol upon ingestion and no harmful effects are expected.

Eye or respiratory contact is expected to cause some irritation.

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 gives 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.

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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, and 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.

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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.