ALAMO ORCHARD
10280 Wahluke Rd SW
Mattawa, WA 99349
(509) 932-4718

October 4, 2002

Mr. Owusus Bandele
Crops Committee Chairman
National Organic Standards Board
USDA – NOP

Dear Mr. Bandele:

I have been growing apples and peaches organically in Washington State since the early years of our venerable state certification program about 1987. I am writing with great alarm and concern upon hearing the news that mating disruption will not be allowed in organic production because of the class IV inerts used in stabilizing the pheromone in the dispenser. Before we had mating disruption as a tool for controlling codling moth and oriental fruit moth, these pests had the potential of completely devastating a crop in spite of whatever biopesticides or cultural practices were employed. To return to those control measures would mean higher chemical and fuel costs, more soil compaction, and high concentrations of summer oil and chemical residue associated with multiple sprays. The use of mating disruption has allowed the farmer to focus, and invest time and money into soil building instead of running his tractor and sprayer up and down the rows 16-20 times per season.

I implore you to favorably consider the petition submitted by Pacific Biocontrol Corporation to allow the use of the three inert stabilizers used in their dispenser. It will be a win for farmers to be able to control these difficult pests effectively. It will be a win for consumers to get a product without high concentrations of allowed, but undesirable residues. It will be a win for the environment to reduce fuel consumption and soil compaction. This is a product that is allowed in virtually all organic programs worldwide and I can see no benefit and many costs associated with disallowing it here. Thanks for your consideration.

Orlin Knutson

PACIFIC BIOCONTROL CORPORATION

Kathy Bolan, Registration Agent
PO Box 1551
Healdsburg, CA 95448
Tel/Fax: 707-433-4397
E-mail: bolan@interx.net

Corporate Headquarters
14615 NE 13th Court, Suite A
Vancouver, WA 98685
Tel: 360-571-2447
Fax: 360-571-2448

May 22, 2002

National Organic Standards Board
c/o Toni Strother
USDA/AMS/TM/NOP
Room 4008-So., Ag Stop 0268
Washington, D.C. 20250-0200
Phone: 202-690-2624
E-mail: Toni.Strother2@usda.gov

Dear Ms. Strother,

I have enclosed the following petition for amending the National List of the USDA's National Organic Program:

Petition for Butylated Hydroxytoluene (BHT) as an Inert Ingredient in Mating Disruptant End-Use Products (Solid Polymeric Matrix Pheromone Dispensers) For Inclusion on the National List under Category 205.601: Synthetic Substances Allowed for Use in Organic Crop Production.

The Confidential Business Information has been included in the CBI-Copy. This includes manufacturing and formulation information including research and quality control tests and data. I have included copies of all of the references (by number) with this copy. The CBI-Deleted copy does not include these references except the product labels and MSDS for BHT. Please contact me at my phone number or e-mail if you have any questions or need additional data. We appreciate your attention to our petition. Thank you.

Sincerely,

Kathy A. Bolan

Kathy A. Bolan
Registration Agent
Pacific Biocontrol Corporation
PO Box 1551, Healdsburg, CA 95448 USA
Phone/Fax: 707-433-4397
E-mail: bolan@interx.net

PACIFIC BIOCONTROL CORPORATION

Kathy Bolan, Registration Agent
PO Box 1551
Healdsburg, CA 95448
Tel/Fax: 707-433-4397
E-mail: bolan@interx.net

Corporate Headquarters
14615 NE 13th Court, Suite A
Vancouver, WA 98685
Tel: 360-571-2447
Fax: 360-571-2448

May 1, 2002

National Organic Standards Board
c/o Toni Strother
USDA/AMS/TM/NOP
Room 4008-So., Ag Stop 0268
Washington, D.C. 20250-0200
Phone: 202-690-2624
E-mail: Toni.Strother2@usda.gov

PETITION FOR AMENDING THE NATIONAL LIST OF THE USDA'S NATIONAL ORGANIC PROGRAM:

Petition for Butylated Hydroxytoluene (BHT) as an Inert Ingredient in Mating Disruptant End-Use Products (Solid Polymeric Matrix, Pheromone Dispensers) For Inclusion on the National List under Category 205.601: Synthetic Substances Allowed for Use in Organic Crop Production

COMMON NAME:

Butylated Hydroxytoluene
BHT

MANUFACTURER'S NAME:

Sumilizer BHT-R

OTHER NAMES:

2,6-Di-tert-butyl-methylphenol
2-6-Di-tert-butyl-p-cresol,
2-6-Bis(1,1-dimethyl-ethyl)-4-methylphenol

LIST OF USES, RATES AND APPLICATIONS FOR CROPS AND LIVESTOCK USES, MODE OF ACTION FOR HANDLING USES:

Pacific Biocontrol's Mating Disruption Formulations and NOP Standards

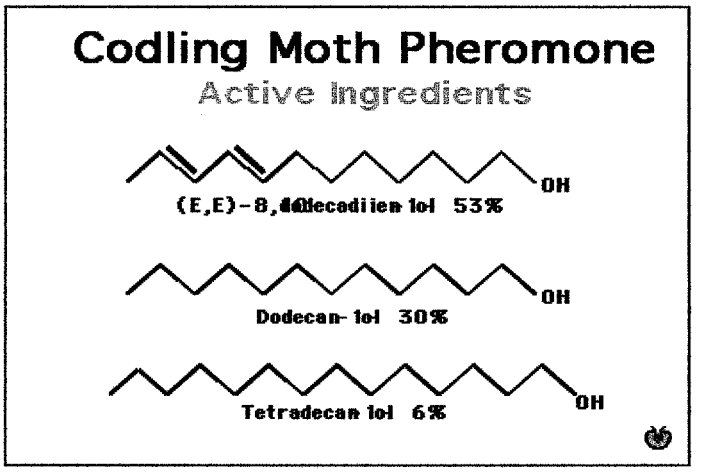
Mating-disruption has become an effective method for control of important insect pests. As of 1997 there were more than 30 mating-disruption products registered with the EPA (1). The number has increased over the past five years. Mating disruption is key component in many area-wide pest management programs often resulting in reduced use of toxic insecticides (2, 3, 4, 5). Furthermore, this technique has become an indispensable tool for organic growers.

The new National Organic Program (NOP) standards threaten the elimination of many mating disruption products, including Pacific Biocontrol's ISOMATE® and PB-ROPE L formulations, due to restrictions on EPA's List 3 inert ingredients. These inert ingredients are used in relatively small amounts and are contained within the plastic slow-release substrates. Contact with fruit or fiber is minimal. Nevertheless, these inerts are on List #3 and therefore not acceptable for organic production under the NOP standards. The elimination of mating disruption for organic growers will create an economic crisis due to much lower levels of control.

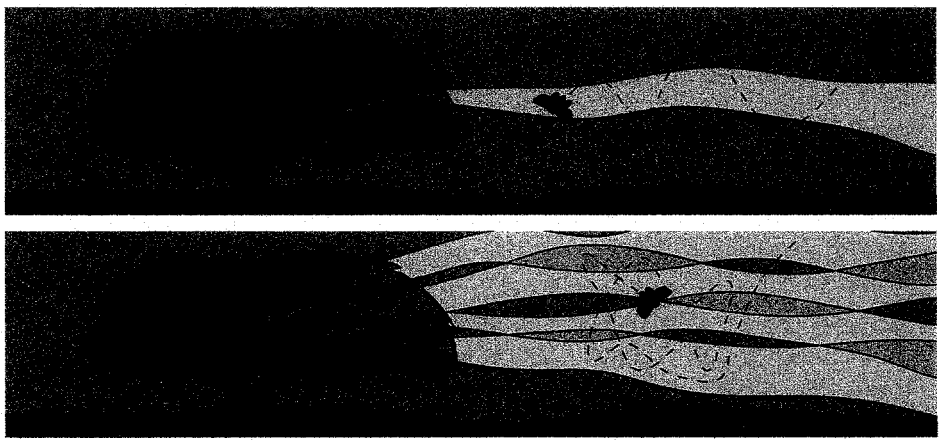
Pacific Biocontrol's Mating Disruption Technology

Pacific Biocontrol's mating disruption technology consists of the biochemical mixture being impregnated in a polyethylene tube, which is slowly released into the atmosphere and affects the mating pattern of the insect to be controlled. This material is very specific to those moths that use the biochemical mixture as part of the sex pheromone.

For example, Isomate®-C Plus is the discrete hand-applied synthetic formulation of the sex pheromone of the codling moth, *Cydia pomonella*, and is used to control the codling moth on apples and pears. Isomate®-C Plus consists of the three chemical blend of E,E–8,10-Dodecandien-1-ol, 1-Dodecanol and 1-Tetradecanol. Each of these three chemicals is important in affecting the full range of behavioral events, which are important in successful sexual communication between male and female codling moths.



The mode of action by the female moth is the volatile release of the pheromone into the atmosphere, the diffusion of the biochemicals in the atmosphere, and the antennal reception of the dispersed molecules by the male insect. Mating disruption functions solely by interfering with the insect's mating behavior. This results in diminished reproductive success.



Top picture: a male moth easily finds the female by following the pheromone plume she emits.

Bottom picture: shows how **Isomate®** disrupts normal communication between male and female codling moths.

Pheromone can be very unstable and easily broken down by UV light and oxidation. Therefore, in addition to the biochemical mixture consisting of the active ingredients, the formulated product also includes inert ingredients (stabilizers) that are added to assist in the protection of the pheromone active ingredients from these outside forces. BHT is an inert ingredient that is added to assist in the protection of the pheromone from oxidation during field use of the product.

As the mode of action of this technology is non-toxic, but behavioral, it is not expected that the formulation, consisting of the biochemical mixture and the inert ingredients, would pose any potential hazard to humans, environment, or non-target species.

What is an Antioxidant?

An antioxidant is a substance that prevents or slows the breakdown of another substance by oxygen. This type of ingredient is added to the pheromone mixture to prolong the field life of the product. These solid polymeric matrix, pheromone dispensers are designed to provide longevity, to eliminate the need for multiple applications and to reduce the probability of uncontrolled mating between applications. Without this type of inert ingredient, antioxidant, the field life of the dispenser would be diminished and economics of mating disruption would be in jeopardy.

Pacific Biocontrol's Use of Sumilizer BHT-R

At the present, Pacific Biocontrol has eight end-use products that are formulated with Sumilizer BHT-R as an inert ingredient. The amount of BHT in Pacific Biocontrol's products is as follows:

CBI-deleted

Codling Moth and Organic Production

Pacific Biocontrol's formulated product, Isomate®-C Plus, is used to control the codling moth on apples and pears. However, the added inert ingredient, BHT, is on EPA's List 3 and not acceptable for organic production under NOP standards. Therefore, organic growers will not be allowed to use mating disruption products with List 3 inerts, and lower levels of control could create an economic crisis. This is especially true for the organic apple and pear growers who have few options for control of the codling moth.

Codling moth is a key pest of pome fruits throughout world and is primarily controlled by one or more applications of broad-spectrum insecticides. Much effort has been put into the development of alternative control methods due to limitations and disadvantages conventional insecticides. Mating disruption has become an effective and economical method for controlling codling moth (1). The total pome fruit area treated with mating-disruption formulations worldwide in 2001 is estimated at 90,000 hectares. In the USA the estimated area is 48,000 hectares.

In the USA, codling moth has been one of the most difficult pests to control by organic methods. Without chemical insecticides, pest numbers can increase exponentially. Most organically acceptable alternatives for codling moth control do not provide effective nor economical control. These methods include: mass trapping, beneficial insects (including inundative release), microbial insecticides (Bt, virus), botanicals (ryania), sterile male release and parasitic nematodes. Sanitation (removal of infested fruit) and summer oils have provided better control but are still not adequate by themselves.

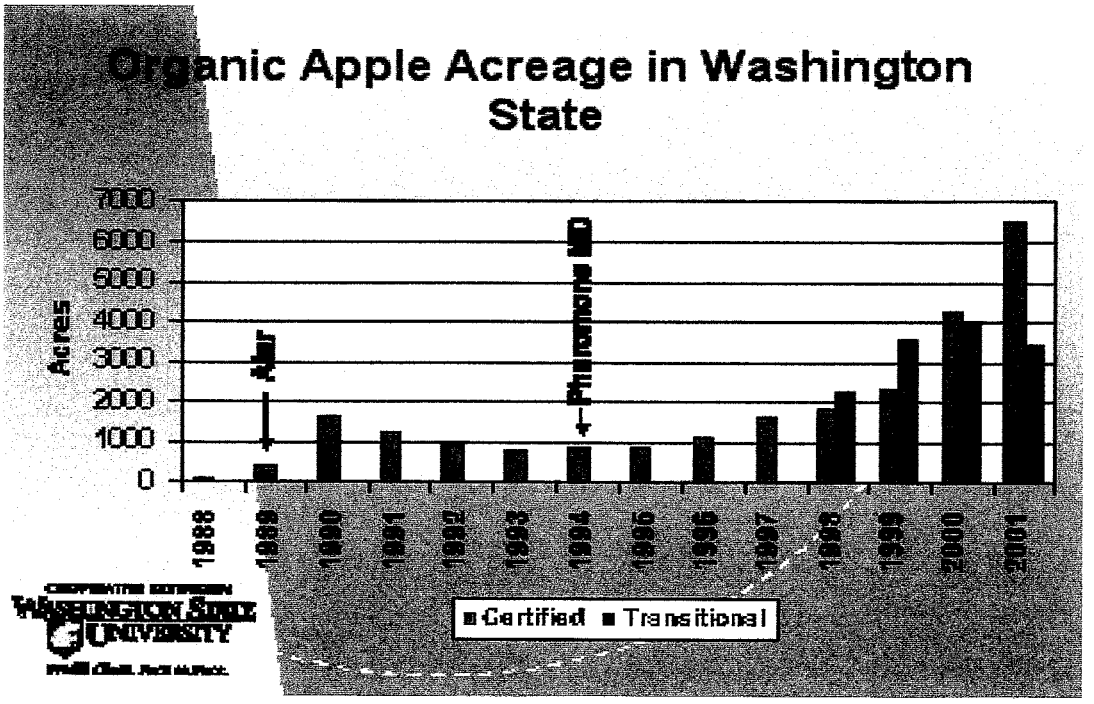
Codling Moth Control - Organic Options

- Mating Disruption
- Summer Oils
- Sanitation: remove infested fruit
- Sterile Males
- Trapping: larvae and adults
- *Bacillus thuringiensis* (Bt)
- Virus (CMGV)
- Nematodes
- Botanicals: ryania, rotenone, pyrethrum
- Parasites: *T. platneri, Ascogaster* sp.
- Predators: birds, beetles, spiders, ants, etc.

Mating disruption, either alone or in combination with other biological and cultural control methods, has proven to be the most effective tool for controlling codling moth in organic apples and pears. This technique is the basis for any codling moth management program in organic apples and pears – most other methods are used to supplement mating disruption.

Approximately 20,000 acres of organic apples and pears are grown in the USA (6). It is estimated that more than 95% of these acres are treated with some type of mating disruption formulation for codling moth control. In the USA it would be very difficult to grow an organic apple or pear for fresh market consumption without the use of mating disruption. Furthermore, with the development of mating disruption for codling moth there has been a steady increase in organic apple acreage in Washington State (6).

U.S. Organic Tree Fruit Acreage - 2001				
	Apple	Pear	Stone Fruit	All Fruit
WA	6540	1308	285	8436
CA	4529	842	3112	8662
AZ	2800	-	-	2830
CO	1535	100	155	1923
ID	503	-	3	506
OR	350	500	305	1180
Others	1015	48	78	1198
US Total	17,272	2798	3038	23,835
WA transition	3411	642	75	4408



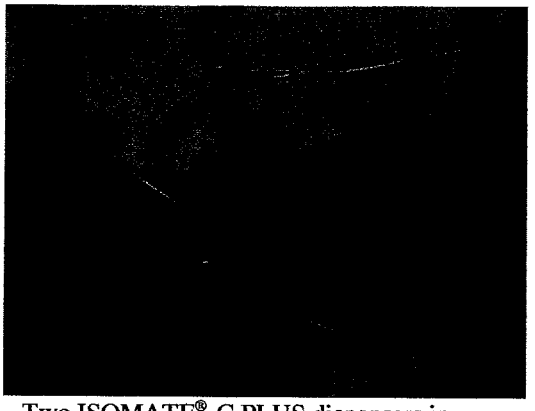
D. Granatstein, Center for Sustaining Agricultural and Natural Resources, WSU, Wenatchee, WA.

Pheromone Stability

Codlemone, the sex pheromone for the codling moth, is prone to degradation via exposure to heat, light and oxygen (7). Unprotected Codlemone lasts less than two hours in the field. The decomposition of the pheromone in commercial formulations can result in significant decrease in the effective longevity in the orchard. Small amounts of stabilizers, i.e. BHT, are added to protect the pheromone active ingredient from UV degradation and oxidation. The addition of these stabilizers can double the formulation's field life and thus greatly improve the economics of mating disruption. This has led to greater adoption mating disruption by growers.

CBI-deleted

In ISOMATE® formulations, these stabilizers are added to the pheromone contained with the plastic tubes. These tubes ("twist-ties" or "ropes") are applied by hand within the crop canopy. The pheromone dispensers do not come into contact with the fruit (or cotton fiber).



Two ISOMATE®-C PLUS dispensers in apples applied by hand.

ISOMATE®-C PLUS applied with plastic clip in walnuts.

ISOMATE®-CTT applied with "hoop" applicator in apples.

PB-ROPE L dispenser applied in cotton.

Some Other Uses of This Inert Ingredient

The inert ingredient is an antioxidant for food, animal feed, petrol products, synthetic rubbers, plastics, animal and vegetable oils, soaps. It is also an antiskinning agent in paints and inks. (8) The FDA has listed the accepted uses of this inert ingredient in its Food and Drugs Code of Federal Regulations (21 CFR). The references are listed below:

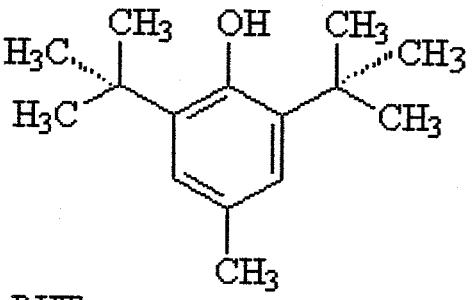
137.350	Enriched rice- In the case of enriched parboiled rice, butylated hydroxytoluene may be added as an optional ingredient in an amount not to exceed 0.0033 percent by weight of the finished food.
166.110	Margarine-Preservatives including but not limited to the following within these maximum amounts in percent by weight of the finished food: dodecyl gallates, BHT, BHA, ascorbyl palmitate, ascorbyl stearate, all individually or in combination, 0.02 percent.
172.110	BHA, food additive, may be safely used in food for human consumption with BHT as specified in 172.115.
172.115	BHT, food additive, may be safely used in specified foods as follows: dehydrated potato shreds, dry breakfast cereals, emulsion stabilizers for shortenings, potato flakes, potato granules, sweet potato flakes.
172.185	TBHQ, a food additive, may be safely used in foods as an antioxidant in combination with BHT.
172.615	Chewing gum base
173.340	Components of defoaming agents limited to use in processing beat sugar and yeast
175.105	Adhesives
175.125	Pressure-sensitive adhesives
175.300	Antioxidant of resinous and polymeric coatings
175.380	Xylene-formaldehyde resins condensed with 4,4'-isopropylidenediphenol-epichlorohydrin epoxy resins
175.390	Zinc-silicon dioxide matrix coatings

176.170	Components of paper and paperboard in contact with aqueous and fatty foods
176.210	Defoaming agents used in the manufacture of paper and paperboard
177.1010	Antioxidants and stabilizers of acrylic and modified acrylic plastics, semirigid and rigid
177.1210	Closures with sealing gaskets for food containers
177.1350	Ethylene-vinyl acetate copolymers
177.2260	Substances employed in fiber finishing in resin-bonded filters
177.2600	Antioxidants and antizonants (total not to exceed 5% by weight of rubber product) of rubber articles intended for repeated use
178.2010	Antioxidants and/or stabilizers for polymers
178.3570	Lubricants with incidental food contract may be safely used on machinery used for producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food.
179.45	The finishing film may contain BHT of the polyethylene film for packaging materials for use during the irradiation of prepackaged foods.
181.24	Substances classified as antioxidants when migrating from food-packaging material (limit of addition to food .005%).
182.3173	Chemical Preservatives-BHT

SOURCES AND DETAILED DESCRIPTION OF MANUFACTURING PROCEDURES:

This inert ingredient is listed in the Merck Index (Merck), with the following information:

Systematic Name	Butylated Hydroxytoluene
Synonyms	BHT; 2,6-Bis(1,1-dimethyl-ethyl)-4-methylphenol; 2,6-di-tert-butyl-p-cresol; 2,6-di-tert-butyl-4-methylphenol; Antracine 8; Tenox BHT; Ionol CP; Sustane; Dalpac; Impruvol; Vianol.
CAS Number	3896-11-5
Molecular Formula	$C_{15}H_{24}O$
Molecular Weight	220.34
Structure:	



BHT
butylated hydroxytoluene

Manufacturing Process

CBI-deleted

SUMMARY OF ANY PREVIOUS REVIEWS BY STATE OR PRIVATE CERTIFICATION AGENCIES:

This inert ingredient has been approved for use as a stabilizer in the following mating disruptant, end-use products that have been certified by the Washington Dept. of Agriculture (WSDA) Organic Food Program: Isomate®-C Plus, Isomate®-M 100, Isomate®-CM/LR and Isomate®-C TT. These four products meet the current WSDA organic standards. WSDA has acknowledged that these four products do not meet the USDA's NOP standards because of their inert ingredients. WSDA has petitioned the US EPA that these non-approved synthetic ingredients, i.e. BHT, be moved from EPA's List 3 to List 4. It was noted that BHT is an antioxidant, it is approved by the FDA, and it is on the GRAS list.

REGULATORY STATUS WITH EPA, FDA OR STATE AUTHORITIES:

This inert ingredient has been approved for use as a stabilizer in the following mating disruptant, end-use products under the registrant, Pacific Biocontrol Corporation, EPA Company No. 53575:

Pacific Biocontrol's Product Name	EPA Reg. No.	US EPA -Biopesticides and Pollution Prevention Division	California Dept of Pesticide Regulation
Isomate®-C Plus	53575-6	Yes	Yes
PB-Rope L	53575-15	Yes	Yes
Isomate®-M 100	53575-19	Yes	Yes
Isomate®-CM/LR Pheromone	53575-20	Yes	No
Isomate®-BAW Pheromone	53575-21	Yes	Yes
Isomate®-OBLR/PLR Plus	53575-24	Yes	Yes
Isomate®-C TT	53575-25	Yes	Yes
Isomate®-M Rosso	53575-26	Yes	Yes

Isomate®-C Plus is registered in 18 states, PB-Rope L is registered in four states, Isomate®-M 100 is registered in 19 states, Isomate®-CM/LR Pheromone is registered in two states, Isomate®-BAW Pheromone is registered in three states, Isomate®-C TT is registered in six states, and Isomate®-M Rosso is registered in one state. Isomate®-OBLR/PLR Plus was registered by the EPA in January 2002 and is registered in two states. Isomate®-C Plus has been registered in Canada under PCP No. 22899 since 1998.

The FDA approved this inert ingredient, BHT, under 21 CFR 172.115 (9), as a food additive permitted for addition to food for human consumption:

"The food additive BHT (butylated hydroxytoluene) alone or in combination with other antioxidants permitted in this Subpart B may be safely used in or on specified foods, as follows:

a) The BHT meets the following specification: Assay (total BHT) 99 percent minimum.
b) The BHT is used alone on in combination with BHA, as an antioxidant in foods as follows:

Food	Limitations (total BHA and BHT parts per million)
Dehydrated potato shreds	50
Dry breakfast cereals	50
Emulsion stabilizers for shortenings	200
Potato flakes	50
Potato granules	10
Sweet potato flakes	50

c) To assure safe use of the additive:
   1) The label of any market package of the additive shall bear, in addition to the other information required by the Act, the name of the additive.
   2) When the additive is marketed in a suitable carrier, in addition to meeting the requirement of paragraph (c)(1) of this section, the label shall declare the percentage of the addictive in the mixture." (9)

The Agency most familiar with these types of mating disruptant products is the US EPA. In 1995, the EPA formed BPPD (Biopesticides and Pollution Prevention Division) to manage and accelerate the regulatory process for biologically-based pesticide products. The goal of the new Division was to streamline the process of registering biological products and to provide a consistently high quality of service to the companies of these types of products. Under BPPD, there are two sub-divisions, Microbials and Biochemicals. Pheromones products are reviewed under the Biochemicals Sub-division.

The US EPA has assisted the regulatory relief for pheromones and other similar semio-chemicals by recognizing the difference between semiochemicals and conventional chemical pesticides.

"The Agency has assumed that pheromones and other similar semiochemicals are different from conventional synthetic pesticides, and has attempted to facilitate their registration with reduced data requirements and regulatory relief efforts." (10)

The US EPA had registered 20 pheromones as active ingredients as of November 1999, and more than 60 products had been registered with these active ingredients.

"As of November 1999, EPA has registered (licensed for sale) approximately 20 moth mating pheromones as pesticide active ingredients and more than 60 individual pesticide products containing these active ingredients." (11)

The first regulatory relief measures that the US EPA established for pheromone products were the tolerance exemptions for the following:

(1) In 1993, inert ingredients of semiochemical dispensers. (12)
(2) In 1994, arthropod pheromones in retrievably sized polymeric matrix dispensers. (13)
(3) In 1995, lepidopteran pheromones in any mode application. (10)

"Based on the information considered, the Agency concludes that tolerances are not necessary to protect the public health for the inert ingredients in the semiochemical dispenser products." (12)

"In the proposal, EPA set forth its reasons for determining that a tolerance for these pheromone products is not necessary to protect public health." (13)

"Lepidopteran pheromones that are naturally occurring compounds, or identical or substantially similar synthetic compounds, designated by an unbranched aliphatic chain (between 9 and 18 carbons) ending in alcohol, aldehyde or acetate functional group and containing up to 3 double bonds in the aliphatic backbone, are exempt from the requirement of a tolerance in or on all raw agricultural commodities." (10)

The EPA has recognized that pheromone products in retrievable sized, polymeric matrix dispensers pose minimal risk with their low use rates and has significantly eased the regulatory guidelines for registering these types of products.

"Most recently the Agency has recognized that a special category of pheromone products dispensed from larger sized polymeric matrices with low annual use rates represent minimal risk for dietary and environmental exposure and has greatly eased the burden to register these items." (10)

As indicated above, the first approved exemption from tolerance was for the inert ingredients of a semiochemical dispenser. This includes antioxidants like BHT.

"All inert ingredients of semiochemical dispenser products formulated with and/or contained in dispensers made of polymeric matrix materials (including the monomers, plasticizers, dispersing agents, antioxidants, UV protectants, stabilizers and other inert ingredients), are exempted from the requirement of tolerance when used as carriers in pesticide formulations for application to growing crops only." (12)

The US EPA has been using many methods to expedite the registrations of these types of products (lepidopteran pheromones in polymeric matrix dispensers). Product chemistry data is usually the only data that is needed for registration by the US EPA. Mammalian toxicity data has been waived. For these types of products, many times the ecological effects, environmental fate and ground water data is also waived.

"Recognizing the low toxicity (Toxicity categories III and IV) and low expected exposure to humans from contact with pheromones in point source applications (e.g., in solid matrix dispenser), the Agency has waived the requirement for certain mammalian toxicity studies, such as subchronic (90-day) oral and inhalation toxicity, immunotoxicity, and developmental toxicity. Due to the low use rate and target species specificity, the Agency has been using a variety of measures to facilitate the development of registration of pheromone products.

To expedite the registration of lepidopteran pheromone products, the Agency usually only considers product chemistry data, and if needed, inert clearance data for pesticidal used of these compounds on food crops." (14-Isomate-BAW Pheromone, Registration Eligibility Document, II. Overview, F. Data Requirements)

CHEMICAL ABSTRACT SERVICE (CAS) NUMBER OR OTHER PRODUCT NUMBER, SAMPLES OF LABELS:

CAS Number: 128-37-0

Attached in the appendix are the labels of Pacific Biocontrol's eight products (15), which are formulated with Sumilizer BHT-R as an antioxidant.

Product	EPA Reg. No.	Registration Date
Isomate®-C Plus	53575-6	July 1993
PB-Rope L	53575-15	October 1993
Isomate®-M 100	53575-19	April 1997
Isomate®-CM/LR Pheromone	53575-20	September 1997
Isomate®-BAW Pheromone	53575-21	September 1999
Isomate®-OBLR/PLR Plus	53575-24	January 2002
Isomate®-C TT	53575-25	March 2001
Isomate®-M Rosso	53575-26	May 2001

CBI-deleted

CBI-deleted

PHYSICAL PROPERTIES OF THE SUBSTANCE AND CHEMICAL MODE OF ACTION: INCLUDING ENVIRONMENTAL IMPACTS, INTERACTIONS WITH OTHER MATERIALS, TOXICITY AND PERSISTENCE, EFFECTS ON HUMAN HEALTH, EFFECTS TO SOIL ORGANISMS, CROPS OR LIVESTOCKS:

Physical Properties of BHT:

Properties	Results
Appearance	White Solid
Odor	Odorless
Boiling Point	265°C
Vapor Pressure	6.5mmHg (120°C)
Melting Point	70°C
Specific Gravity	1.048 (80°C)
Solubility in Water	Insoluble
Solubility	Soluble in methyl ethyl ketone, toluene, ethyl acetate, styrene monomer, DOP
Flash Point	127°C
Auto Ignition Point	420°C
Reactivity	None
Stability	Stable under ordinary conditions

Chemical Mode of Action:

This inert ingredient functions as an antioxidant.

Environmental Impacts:

The US EPA has recognized that these types of pheromone products are expected to have no adverse effects and minimal exposure since they are very specific to the insect that they are controlling and have a small release rate in the environment.

"IV. Assessing Risks to the Environment
Adverse effects on nontarget organisms (mammals, birds, and aquatic organisms) are not expected because these pheromones are released in very small amounts to the environment and act on a select group of insects." (12)

In the registration of Isomate®-BAW Pheromone, the US EPA did not require ecological effects, environmental fate and ground water data because of the product's use pattern and lack of exposure. Since the formulation is impregnated in a polyethylene dispenser and slowly released, there is expected to be little exposure and transport. Minimal to no exposure and risk was expected to non-target terrestrial and aquatic species.

"1. Ecological Effects Hazard Assessment
All Tier I ecological effects data requirements are waived based on the proposed use pattern and lack of exposure.

2. Environmental Fate and Ground Water Data
Environmental fate and groundwater data are not required for biochemical pesticides unless adverse effects on nontarget species are observed as a result of acute testing for ecological effects (Tier I).

3. Ecological Exposure and Risk Characterization
Because Isomate®-BAW Pheromone is enclosed in a solid matrix dispenser and is slowly released by volatization, transport and exposure is expected to be very limited. This system is generally accepted as posing minimal to no exposure and risk to non-target terrestrial and aquatic species." (14- III. Science Assessment, C. Environmental Assessment)

Sumitomo Chemical Co. lists the fish toxicity for Sumilizer BHT-R as LC_{50}=6.2ppm at 48 hours (Killfish).

Interaction with other materials:

BHT, as an antioxidant, is added to the pheromone active ingredients. BHT is incorporated entirely within the lumen of the dispenser and separated from the surface by the thickness of polymer. The only interaction of BHT will be with the active ingredients inside the dispenser. BHT will act as an antioxidant to help protect the active ingredients from oxidation.

TOXICITY AND PERSISTENCE, EFFECTS ON HUMAN HEALTH:

Fact Sheets are created for each new active ingredient that the US EPA registers. Instead of creating individual fact sheets for each new lepidopteran pheromone active ingredient, the EPA created a Generic Fact Sheet for all lepidopteran pheromones since they are very similar with low toxicity and low exposure. The EPA states in this Fact Sheet that during the more than 10 years of use with lepidopteran pheromones, there have been no reports of adverse effects. Therefore, no risk is expected to humans from use of these products.

"Based on low toxicity in animal testing, and expected low exposure to humans, no risk to human health is expected from the use of these pheromones. During more than 10 years of use of lepidopteran pheromones as pesticides, no adverse effects have been reported." (11)

For Pacific Biocontrol's Isomate®-BAW Pheromone, the US EPA determined that there is negligible exposure to handlers and the public, since it is contained in a dispenser and slowly released in small amounts. The dietary exposure is concluded as minimal based on the EPA's assessment of these types of compounds as volatile, with low application rates and from their metabolism.

"Because the active ingredients contained in Isomate®-BAW Pheromone are slowly released in very small amounts by dispenser, the potential for dermal, eye and inhalation exposure to pesticide handlers and to the general public is expected to be negligible. Further, the Agency has concluded that the potential for dietary exposure is expected to be minimal based on volatility if the compounds, the low application rates and known metabolism of similar compounds." (14-I. Executive Summary, b. Human Exposure) The US EPA also stated that the potential risk to humans is minimal, since there is low exposure by this type of product, and there are no significant toxicological concerns.

"The potential risks to humans are considered negligible based on low exposure and the lack of significant toxicological concerns. A determination has been made that no unreasonable adverse effects to the US population in general, and to infants and children in particular, will result from the use of this compound when label instructions are followed." (14-I. Executive Summary, c. Risk Assessment)

Under Occupational, Residential, School and Day Care Exposure and Risk Characterization for Pacific Biocontrol's Isomate®-BAW Pheromone, the US EPA determined that no adverse effects and minimal exposure and risk are expected based on the product's use pattern. All of Pacific Biocontrol's products are exempt from the Worker Protection Standard labeling. These formulations are contained within solid, polymeric matrix dispensers.

"Human exposure and risk to these compounds is expected to be minimal in occupational, residential, school and day care settings.

a. Occupational Exposure and Risk Characterization
Based on the use pattern, the potential for dermal, eye and inhalation exposure to pesticide handlers is expected to be negligible. No adverse health effects to workers are

expected from the use of this product. According to Regulation (PR) Notice 93-7, "Labeling Required by the Worker Protection Standard (WPS)," WPS does not apply to attractants used in insect traps. Since Isomate®-BAW Pheromone is to be used in solid matrix device, it is exempt from WPS labeling requirements." (14- III. Science Assessment, B. Human Health Assessment)

Under Drinking Water Exposure and Risk Characterization, the US EPA stated that exposure from the residues of this type of product in water is not expected based on the product's application method.

"Exposure is not expected from an accumulation of Isomate®-BAW Pheromone in the aquatic environment due to the application method. The Agency does not anticipate exposure to residues of Isomate®-BAW Pheromone in drinking water." (14- III. Science Assessment, B. Human Health Assessment)

The potential health effects for Sumilizer BHT-R are listed on Sumitomo's MSDS (16). The material has been evaluated for its toxicological properties and classified as GRAS by the FDA. The toxicological information from the MSDS is as follows:

Eye Effects:	Non-irritant based on test results in rabbits.
Skin Effects	Non-irritant based on test results in rabbits.
Acute Oral Effects:	The oral LD_{50} in rats is 2,340-2,530/kg. Practically non-toxic in normal industrial use.
Acute Inhalation Effects:	No data available.
Subchronic and Chronic Toxicity:	Material produced liver enlargement and hemorrhagic death after continuous oral administration in rats. It is recognized that these effects are not produced in humans. No adverse effects occurred during 12 months in dogs after oral administration of 45-244g/kg.
Carcinogenicity:	Not observed after continuous treatment of this material in the diet of rats and mice for 104 and 96 weeks. This material is listed Group 3 by IARC (not classifiable as to its carcinogenicity to humans). Not classified as carcinogenic by NTP or OSHA.
Mutagenicity:	Negative results in the Ames test, Rec-assay, and DNA-repair test in rat liver cells, in vitro chromosomal aberration test in CHL cells, in vivo micronucleus test, and dominant lethal test in mice. Some positive results in UDS test in rats and in vitro chromosomal aberration test in CHO cells.
Teratogenicity:	No teratogenic effects have been observed in rats and mice.

EFFECTS OF SOIL ORGANISMS, CROPS OR LIVESTOCKS:

From a clean safety record, the US EPA has stated that there is no risk in consuming food containing residues from these lepidopteran pheromone products. The Agency also allows the experimental use up to 250 acres with these types of products instead of the 10 acres allowed on conventional pesticides.

"The safety record for lepidopteran pheromones has allowed the Agency to conclude that consumption of food containing residues of the pheromone presents no risk. In addition, these pheromones can be used experimentally without a permit on up to 250 acres, versus the 10-acre limit imposed on other pesticides." (11)

Under Dietary Exposure and Risk Characterization of Pacific Biocontrol's Isomate®–BAW Pheromone, the US EPA determined that the dietary exposure is minimal since this type of product is contained in a dispenser and not applied to the crop.

"These compounds are incorporated into dispensers and are not directly applied to the growing plants. Therefore, dietary exposure to these compounds is expected to be minimal." (14-III. Science Assessment, B. Human Health Assessment)

Under Dietary Exposure and Risk Characterization, the US EPA has also determined from previously registered lepidopteran pheromones and their known chemical structure of long chain fatty acids that since they have low acute toxicity, low application rates and minimal exposure, then there will be no dietary hazard from residues on the treated crops.

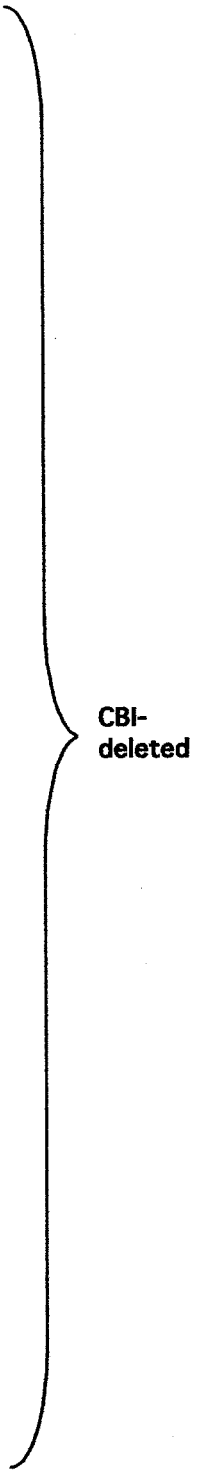
"The Agency has concluded that residues on treated crops are not a dietary hazard for the following reasons: low acute mammalian toxicity in lepidopteran pheromones registered to date, the known metabolism of long chain fatty acids, low application rates, and nominal human exposure due to application rate and to volatization." (14-III. Science Assessment, B. Human Health Assessment)

Under Ecological Risk Assessment, the US EPA stated that exposure is to be minimal to no risk to non-target terrestrial and aquatic species, since the product is contained in a dispenser and slowly released.

"Because Isomate®-BAW Pheromone is enclosed in a solid matrix dispenser and is slowly released by volatization, transport and exposure is expected to be very minimal to no exposure and risk to non-target terrestrial and aquatic species." (14- I Executive Summary, C. Risk Assessment)

BHT, as an antioxidant, is added to the pheromone active ingredients, which are impregnated in plastic dispensers and then hand applied to the crop. Since the dispensers are applied on branches or stems (i.e. Isomate®-C Plus, PB-Rope L, Isomate®–M 100, Isomate®-CM/LR Pheromone, Isomate®-OBLR/PLR Plus, Isomate®-C TT and Isomate®-M Rosso) or on stakes at the canopy level of the crop (i.e. Isomate®-BAW Pheromone), then there shouldn't be any contact with the soil organisms or livestocks. The ISOMATE® dispensers should have little or no contact with the crop itself.

CBI-deleted



SAFETY INFORMATION, INCLUDING MSDS AND A REPORT FROM THE NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH STUDIES (NIEHS):

The manufacturer of Sumilizer BHT-R that is formulated as an inert ingredient in mating disruptant products for Pacific Biocontrol Corp. is Sumitomo Chemical Co. Ltd., Tokyo, Japan. The MSDS (16) from the manufacturer is attached in the appendix.

The National Toxicology Program under the National Institute of Environmental Health Studies listed the known uses for BHT as "antioxidant for petroleum and food products; animal feed; food packaging" (17). BHT "was patented in 1947 and received approval for use as a food additive and preservative by the FDA in 1954. Since 1959, BHT has been generally recognized as safe (GRAS) for use in foods and is one of the most commonly used antioxidants in foods containing fats." (17) "A bioassay of BHT for possible carcinogenicity was conducted by administering the test chemical in feed to" (17) rats and mice. This two year study resulted in negative results for male rats, female rats, male mice and female mice at doses of 0, 3,000, 6,000 ppm/50 per group. (17) "It is concluded that under the conditions of this bioassay, BHT was not carcinogenic for rats or mice." (17)

The US EPA has recognized these types of products, with inert ingredients like BHT, as having low exposure and minimal risk, since they have low acute toxicity and low application rates, are contained in solid, polymeric matrix dispensers, are released slowly, and have little or no contact with the crop. Mating disruption is a non-toxic, behavioral method for the control of insects. It does not affect the crop, the grower, the environment, or other non-target insects.

RESEARCH INFORMATION, INCLUDING RESEARCH REVIEWS AND BIBLIOGRAPHIES:

A literature and Internet search was performed on the Internet. There is research on BHT in several areas. A number of studies have been conducted on the health benefits of BHT even in the field of alternative medicine. Because BHT is a common food additive, a number of studies have been done of the possible carcinogenic effects. Some of these studies have shown a positive correlation between consumption of BHT in large doses in lab rats and possible carcinogenic effects. One of these articles summarized the experiments with BHT and known carcinogens and stated "BHT acts as both a promoter and antipromoter of carcinogenesis, depending upon the experimental conditions. Carcinogenesis is usually decreased when the BHT is administered prior to or concurrent with the carcinogen (Ulland 1973, Goodman 1976, Clapp 1979, McCay 1980, King 1981, Williams 1983). When BHT is administered after carcinogenic exposure, the incidence of cancer is frequently increased (Peraino 1977, Witschi 1981, Imaida 1982, Williams 1983). The increases are greatest with hepatically metabolized carcinogens where large single doses of BHT are administered post-exposure. Compared to phenobarbital, another enzyme inducer, BHT is a "weak enhancer" and then "only at near-toxic doses" (Maeura 1984)." (18)

Because BHT can be both a promoter and anti-promoter of carcinogens, then "the type of carcinogens that people are exposed to will determine whether its net effect is to increase or decrease incidence of cancer. Because cruciferous vegetables (cabbage, cauliflower, brussels sprouts, broccoli) are known to induce liver enzymes similarly to BHT -- and also lower general cancer incidence epidemiologically -- we can infer that BHT is likely to lower net cancer risks. In the Soviet Union during the 50s and 60s, BHT (called ionol by the Soviets) was extensively studied as an anti-tumor compound (Emanuel 1963, 1973) culminating in its approval as a treatment for bladder cancer." (18)

An article published in the New York Times also talked about the benefits of BHT in fighting cancer. "Two widely used food preservatives increased levels of a natural cancer fighter in laboratory animals and appear to do the same in humans, a researcher says. Advocates of natural foods have long objected to the use of preservatives, but Dr. Andrew Dannenberg of Cornell Medical College found that the preservatives BHA and BHT 'revved up' the gene for an enzyme that helps destroy carcinogens before they lead to tumors. When the genes are cranked up, they produce more of the enzyme, providing better protection against cancer-causing substances in the environment, Dr. Dannenberg reported last month at the International Conference on Cancer Prevention at Rockefeller University in New York. The gene produces an enzyme called UDP-glucuronosyltransferase, or UGT. The study found elevated levels of the enzyme in the liver, kidneys and small intestines of rats fed higher doses of BHA and BHT than are normally found in foods, Dr. Dannenberg said. He then found preliminary evidence that the substances do the same thing in humans. Dr. Dannenberg said he had also found that sulforaphane, an anti-cancer agent recently isolated in broccoli, exerts its action partly in the same way, by energizing the gene for UGT."(18)

The most complete report found was conducted by the Joint FOA/WHO Expert Committee on Food Additives under Elizabeth Vavasour, Toxicological Evaluation Division, Health Canada. This report evaluated the biological data of the long-term effects of BHT. In only one of the number of studies that have been performed on rodents was there evidence of hepatocarcinogenic effects. "This study differed from those conducted previously in that the rats were exposed to BHT *in utero*, during the lactation period, and for a further 40 weeks after the standard 2-year exposure period." (19) IARC (International Agency for Research on Cancer) reviewed the findings of this study and concluded that carcinogenicity was very difficult to assess for humans. "It was difficult to draw conclusions about the observed incidence of liver lesions in the treated groups because of the large differences in survival between treatment and control groups. The carcinogenicity of BHT to humans could not be evaluated." (19)

Therefore, a new study was performed to investigate the hepatic changes in male rats, and it concluded that only at higher doses (above maximum tolerated doses) does BHT show to induce this type of change in rats. "BHT has been shown to induce hepatocellular necrosis and proliferation in male Wistar rats at doses higher than those used in either of these long-term studies and which exceeded the maximum tolerated dose." (19)

The Joint FOA/WHO Expert Committee on Food Additives has evaluated BHT for an acceptable daily intake for humans over the last 40 years. The most recent evaluation of BHT concluded from the possible hepatic changes in rats that the accepted daily intake for man is 0-0.3mg kb bw. "In view of the probable involvement of hepatic enzyme induction in the development of the hepatocellular damage associated with repeated doses of BHT, the Committee concluded that, in this case, enzyme induction was the most sensitive index of effects on the liver. A well-defined threshold was demonstrated at 100 mg/kg bw/day in the long-term study reviewed for the first time at this meeting, giving a NOEL of 25 mg/kg bw/day. Effects observed in the reproduction segments of the *in utero*/lifetime exposure studies were also taken into account in the derivation of this NOEL. The Committee used a safety factor of 100 to allocate an ADI of 0-0.3 mg/kg bw for BHT." (19)

The Committee also evaluated the national intake of BHT among 10 countries. BHT was unlikely to exceed the ADI on the basis of the estimated intakes except when the General Standard for Food Additives (GSFA) was assumed to be the maximum limit. These estimates were based on BHT being the only antioxidant in foods where it was permitted and contained in the foods at the maximum level permitted. However, when BHT was used in conjunction with other antioxidants, the amount of BHT should be lower. "The Committee recognized that the ADI for BHT is unlikely to be exceeded on the basis of the estimated intakes in the 10 countries for which data were available but that it might be exceeded when the proposed maximum limits in the GSFA are assumed. The Committee recognized that BHT is likely to be used in conjunction with other antioxidants, such as *tert*-butylated hydroquinone and BHA, which act synergistically with BHT. Consequently, the amount of BHT used in practice will be lower and it will be used in fewer foods than assumed in the estimates. All of the estimates except for that from Japan are based on the assumption that BHT is the only antioxidant in foods where use is permitted and that all such foods contain it at maximum permitted levels. The actual intakes of BHT will depend on the relative proportions of antioxidants used in foods and on the proportion of foods in any one category that contains the additive." (20)

Research has been conducted on BHT in food packaging. Research of the antioxidant in the LDPE film in the packaging of oatmeal indicated that BHT caused the least amount of change in the film after being in storage for 10 weeks. "Cereals in general, and particularly oatmeals, are considered rather sensitive to oxidation owing to their relatively high fat content. The addition of antioxidants can sometimes prolong the shelf-life of products. The aim of the present study was to investigate how the rate of lipid oxidation of a packaged oatmeal product was affected by the nature and level of antioxidants incorporated in an LDPE film structure. The stability of the product, which was determined by hexanal analysis using GC-MS and by electronic nose analysis, showed very small variations over the chosen storage period. No oxidation, as determined by hexanal levels in the oatmeal, was initiated during storage, but small variations in volatile profile were seen among the samples analyzed by the electronic nose. The product stored in the BHT-impregnated LDPE film had undergone the least change during 10 weeks of storage at 20°C." (21)

PETITION JUSTIFICATION STATEMENT – THAT STATES WHY THE SYNTHETIC SUBSTANCE IS NECESSARY, ALTERNATIVES THAT COULD BE USED, BENEFICIAL EFFECTS TO THE ENVIRONMENT, ETC:

Why the Synthetic Substance is Necessary:

Sumilizer BHT-R is an inert ingredient formulated in eight of Pacific Biocontrol's mating disruptant end-use products. This inert ingredient is a stabilizer that protects the pheromone formulation from oxidation when it is applied in the field.

Why Use Antioxidants?

Some materials are much more susceptible to degradation from oxygen and are more quickly damaged. Pheromones can be unstable and easily broken down by oxidation.

The presence of oxygen can start to break down the weak chemical bonds, which leads to deterioration. An antioxidant like BHT can prevent or slow down the disintegration of the pheromone by oxygen.

Longevity of the Dispenser

Mating disruption technology is applied prior to the moth's emergence, and the pheromone releases over a range of days depending on temperature. These dispensers are designed to provide longevity, eliminate the need for multiple applications and reduce the probability of uncontrolled mating between applications. Under certain conditions, a single application may provide season-long control.

Since application of these types of products is time-consuming and additional applications can be costly, the longevity of these dispensers is important to the grower and his pocketbook. Growers want convenience, cost-effectiveness and season-long control.

Possible Alternatives to Antioxidants:

A possible alternative to BHT is Vitamin E. Vitamin E is used in some of Pacific Biocontrol's products as an antioxidant and is accepted on EPA's List 4. Both Vitamin E and BHT have antioxidant properties. However, BHT also absorbs radicals while Vitamin E does not. Monoene acetate pheromones may be stabilized by a simple antioxidant such as Vitamin E. However, pheromones composed of dienes, like the conjugated dienes in Isomate-C Plus, require a stabilizer with both antioxidant and radical absorbing properties like BHT to prevent polymerization and isomerization. So Vitamin E alone is not sufficient.

Another alternative that Pacific Biocontrol has considered is formulating their products without this inert ingredient or with Vitamin E as the sole antioxidant. This type of product will still work, but without BHT, at a very shorten interval. Therefore, multiple applications will be needed for season-long control. This will force organic growers to use a product this is more costly and time-consuming and less effective in its control. A new product will also take time to field test and to register. The EPA estimated that the review time would be at least a year, and Pacific Biocontrol will need at least one full season to test the product experimentally.

Possible Alternatives to Mating Disruption Technology for Organic Use:

Most organically acceptable alternatives for mating disruption do not provide effective or economical control. These methods include: mass trapping, beneficial insects (including inundative release), microbial insecticides (Bt, virus), botanicals (ryania), sterile male release and parasitic nematodes. Sanitation (removal of infested fruit) and summer oils have provided better control but are still not adequate by themselves. Mating disruption, either alone or in combination with these types of biological and cultural controls, has proven to be an effective tool for controlling insects.

Beneficial Effects to the Environment:

Mating disruption is a non-toxic, behavioral method for the control of the insects, which does not affect the crop, the grower, the environment, or other non-target insects. Mating disruption is an essential tool for pest management in organic production. Organic production, especially in apples and pears, will be severely affected by the elimination of mating disruption. Therefore, it will be very difficult to grow organic apples, without mating disruption for control of the codling moth, when 95% of the acreage is treated with mating disruption.

The inert ingredient, BHT, is added to protect the insect pheromone active ingredients from oxidation. Without stabilizers, formulation longevity will be significantly reduced resulting in less effective, less economical control. There are no acceptable alternative inert stabilizers that provide adequate protection of the pheromones. The use of the inert stabilizer doubles the formulation's field life and thus greatly improves the economics of mating disruption.

CBI-deleted

ISOMATE®'s dispensers have little or no contact with crop fruit or fiber.

The US EPA has the most familiarity with these type of pheromone products as it has registered more than 20 lepidopteran active ingredients and more than 60 products containing those active ingredients. The Agency has recognized the difference between semiochemicals and conventional chemical pesticides and facilitated regulatory relief to ease the burden of registering these types of products.

The first regulatory relief measure that the US EPA established was the tolerance exemption for inert ingredients of semiochemical dispensers. This included UV stabilizers like BHT. In addition, the US EPA recognized that these lepidopteran pheromone products are expected to have no adverse effects and minimal exposure because they have low acute toxicity and low application rates, are contained in a dispenser and slowly released, and have no contact with the crop.

The US EPA has eight registered products under Pacific Biocontrol with Sumilizer BHT–R as an inert ingredient.

Note: ISOMATE® is a registered trademark for pheromone products manufactured by Shin-Etsu Chemical Co. PB-ROPE L is used in cotton for the control of the pink bollworm. Pacific Biocontrol Corporation holds the US EPA registrations of the ISOMATE® and PB-ROPE L products. Pacific Biocontrol sells the ISOMATE® and PB–ROPE L products in the USA and Canada.

References:

(1) Thomson, D.R., Gut, L.J., and Jenkins, J.W. (1999) "Pheromones for Insect Control: Strategies and Successes", *Biopesticides: Use and Delivery*, (Hall, F.R. and Menn, J.J., eds.), Humana Press Inc, New Jersey, pp. 385-412.

(2) Antilla, L., Whitlow, M., Staten, R.T., El-Lissy, O., and Myers, F. (1996) "An Integrated Approach to Area Wide Pink Bollworm Management in Arizona", Proceedings, Beltwide Cotton Conference, Nashville, TN, pp. 1083-1085.

(3) Jenkins, J.W., Doane, C.C., Schuster, D.J., McLaughlin, J.R. and Jimenez, M.J. (1990) "Development and Commercial Application of Sex Pheromone for Control of the Tomato Pinworm", *Behavior-modifying Chemicals for Insect Management: Applications of Pheromones and Other Attractants*, (Ridgway, R.L., Silverstain, R.M, and Inscoe, M.N., eds.), Marcel Dekker Inc. New York, pp. 269 – 279.

(4) Trumble, J.T. and Alvarado-Rodriguez, B. (1993) "Development and Economic Evaluation of an IPM program for Fresh Market Tomato Production in México", *Agriculture, Ecosystems and Environment*, (Eisevier Science Publishers B.V.), Amsterdam, 43, pp. 1-18.

(5) Benbrook, C.M., Groth, E., Halloran, J.M., Hansen, M.K., and Marquardt, S. (1996) " The Pear Pest Management System Continuum in California: A Case Study", *Pest Management at the Crossroad*, Consumers Union, Yonkers, New York, pp. 191-195.

(6) Granatstein, D. (2002) "Recent Trends in Organic Tree Fruit Production: 2001", www.trfrec.wsu.edu.

(7) Ideses, R. and Shani, A. (1988) "Chemical Protection of Pheromones Containing an Internal Conjugated Diene System from Isomerization and Oxidation", *Journal of Chemical Ecology*, 14, pp. 1657-1669.

(8) (1989)"1548. Butylated Hydroxytoluene", *The Merck Index*, (Budavari, S., O'Neil, M.J. Smith, A., and Heckelman, P.E., eds.), Merck & Co., Inc., New Jersey, p. 238

(9) (1992) "21CFR 172.115 BHT", *Code of Federal Regulations: Food and Drugs: 21: Parts 170 to 199*, Office of the Federal Register National Archives and Records Administration, Washington, p. 26

(10) Hutton, P. (1995) "Lepidopteran Pheromones: Tolerance Exemption", *Federal Register*, 60, 168, pp. 45060-45062.

(11) Steinwand, B. (2001) "Generic Factsheet for Lepidopteran Pheromone", http://www.epa.gov/oppbppd1/biopesticides/factsheets/fs-generic_lep.htm.

(12) Welch, C. (1993) "Inert Ingredients of Semiochemical Dispensers; Tolerance Exemption", *Federal Register*, 58, 234, pp. 64493-64494.

(13) Hutton, P. (1994) "Arthropod Pheromones: Tolerance Exemption", *Federal Register*, 59, 61, pp. 14757-14759.

(14) US EPA OPP BPPD (1999) "Registration Eligibility Document Isomate®-BAW Pheromone", (PC Codes 117203 and 119409), pp. 1-23.

(15) Pacific Biocontrol Corporation (2001) Labels of Isomate®-C Plus, PB-Rope L, Isomate®-M 100, Isomate®-CM/LR Pheromone, Isomate®-BAW Pheromone, Isomate®-OBLR/PLR Plus, Isomate®-C TT and Isomate®-M Rosso

(16) Sumitomo Chemical Co., Ltd. (1998) Material Safety Data Sheet of Sumilizer BHT-R, *MSDS No. MM1113-US*, pp. 1-5.

(17) National Toxicology Program, "Butylated Hydroxytoluene Factsheet", "TR-150 Bioassay of Butylated Hydroxytoluene (BHT) for Possible Carcinogenicity (CAS No. 128-37-0)";" Target Organs and Levels of Evidence, NTP Technical Report Number 150", National Toxicology Program under National Institute of Environmental Health Studies (NIEHS), http://ntp-server.niehs.nih.gov/htdocs/Results_status/ResstatB/10575-K.html, http://ntp-server.niehs.nih.gov/htdocs/LT-studies/tr150.html, http://ntp-server.niehs.nih.gov/htdocs/levels/tr150levels.html.

(18) Fowkes, S.W. (1984) "Carcinogenicity", Cognitive Enhancement Research Institute, http://www.smartbodyz.com/herpes-bht-2.htm

(19) Vavasour, E. "Evaluation of National Intake Assessments of Butylated Hydroxytoluene (BHT)", *WHO Food Additives Series*, 35, http://www.inchem.org/documents/jecfa/jecmono/v35je02.htm

(20) International Program on Chemical Safety, World Health Organization (1999) "Evaluation of National Intake Assessments of Butylated Hydroxytoluene (BHT)", *WHO Food Additives Series*, 42, http://www.inchem.org/documents/jecfa/jecmono/v42je25.htm

(21) Wessling, C. (2001) "Antioxidant ability of BHT-and alpha-tocopherol-impregnated LDPE film in packaging of oatmeal", *Journal of the Science of Food and Agriculture*, 81, 2, pp. 194-201

BIOCONTROL®

ISOMATE®-C PLUS

ACTIVE INGREDIENTS:	
(E, E)-8, 10-Dodecadien-1-ol	53.0 %
Dodecanol	29.7 %
Tetradecanol	6.0 %
INERT INGREDIENTS	11.3 %
TOTAL	100.0 %

Keep out of reach of children

WARNING

PRECAUTIONARY STATEMENTS
HAZARDS TO HUMANS AND DOMESTIC ANIMALS

WARNING

Causes skin irritation. May cause eye injury. Do not get in eyes, on skin, or on clothing. Wear eye protection (such as goggles or safety glasses) and gloves when handling. Do not touch eyes while handling or attaching the dispensers. Wash thoroughly with soap and water after handling.

ENVIRONMENTAL HAZARDS

Do not discharge into lakes, streams, ponds or public waters unless in accordance with NPDES permit. For guidance contact your regional office of the Environmental Protection Agency.

PACIFIC BIOCONTROL CORPORATION
14615 NE 13th Court, Suite A
Vancouver, WA 98685 U.S.A.
Telephone (360) 571-2247
1-800-999-8805

MADE IN JAPAN

NET CONTENTS:
400 Dispenser Units
One dispenser contains 0.008 fl oz or 205 mg
Total content of package: 3.2 fl oz or 82 gm

EPA Est. No: 47265-JP-01
EPA Reg. No: 53575-6

STORAGE AND DISPOSAL

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

Pesticide Storage	Store unopened package at temperatures below 40°F in a dry location. Product may be stored in cold storage facilities used for food storage.
Pesticide Disposal	Pesticide that cannot be used according to label instructions must be disposed of according to applicable Federal, State and Local procedures.
Container Disposal	Dispose of empty dispensers by burning or burying with prunings in winter. If burned, stay out of smoke. Foil envelopes can be disposed of as household refuse.
General	Consult Federal, State or Local disposal authorities for approved alternative procedures.

DIRECTIONS FOR USE

IT IS A VIOLATION OF FEDERAL LAW TO USE THIS PRODUCT IN A MANNER INCONSISTENT WITH ITS LABELING.

It is *critical* that ISOMATE-C PLUS is applied as directed.

1. Crop	Apple, pear, walnut, quince, prune, plum, peach, pecan and nectarine.
2. Pest	Codling moth *(Cydia pomonella)*, hickory shuckworm *(Cydia caryana)*.
3. Rate	400 dispensers per acre or 1000 dispensers per hectare. Apply double rate of dispensers to edges of orchard.
4. Application	Apply dispensers securely to lateral branches in upper third of tree canopy. Can be applied efficiently from moving trailer.
5. Timing	Apply prior to moth emergence in spring. Dispensers release pheromone for 120-140 days. Reapply dispensers to crops with long field seasons (i.e. more than 120 days).
6. Precautions	Isomate-C Plus suppresses codling moth and hickory shuckworm from mating. However, if a major source of mated female moths of these species is present adjacent to the treated field, migration of these moths may significantly reduce the level of control achieved. Sources are likely to be unsprayed apple, pear, quince, walnut, plum, crabapple trees or other host species within 300 yards of the treated field. This can be overcome by: a. Treatment of **entire blocks** and not just sections of large conventionally treated fields that frequently serve as sources of mated females. b. Treatment of infestation sources with Isomate-C Plus (e.g. a strip at least 50 yards wide nearest the treated field). c. Treatment of infestation source with an effective insecticide. Other pests must be monitored on a regular schedule so that timely intervention with conventional insecticides is possible.

WARRANTY AND LIMITATION OF DAMAGES

All statements concerning the use of this product apply only when used as directed. The Manufacturer makes no warranties, expressed or implied, concerning this product or its use, which extend beyond the description on the label. Read all directions carefully.

BIOCONTROL®

PB-ROPE L

ACTIVE INGREDIENTS:

(Z, Z)-7, 11-Hexadecadien-1-yl Acetate	46.7 %
(Z, E)-7, 11-Hexadecadien-1-yl Acetate	44.1 %
INERT INGREDIENTS	9.2 %
TOTAL	100.0 %

Keep out of reach of children
CAUTION

PRECAUTIONARY STATEMENTS
HAZARDS TO HUMANS AND DOMESTIC ANIMALS

CAUTION

STATEMENT OF PRACTICAL TREATMENT

Avoid contact with eyes. In case of contact, immediately flush with water. Get medical attention if irritation persists. Wash hands with soap and water after handling.

ENVIRONMENTAL HAZARDS

Do not discharge into lakes, streams, ponds or public waters unless this product is specifically identified and addressed in a NPDES permit. For guidance contact your regional office of the Environmental Protection Agency.

PACIFIC BIOCONTROL CORPORATION
14615 NE 13th Court, Suite A
Vancouver, WA 98685 U.S.A.
Telephone (360) 571-2247
1-800-999-8805

MADE IN JAPAN

NET CONTENTS:
500 Dispenser Units
One dispenser contains 0.0056 fl oz or 147 mg
Total content of package: 2.8 fl oz or 73.5 gm

EPA Est. No: 47265-JP-01
EPA Reg. No: 53575-15

STORAGE AND DISPOSAL

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

Pesticide Storage	Store in original unopened package at temperatures below 40°F in a dry location.
Pesticide Disposal	Pesticide that cannot be used according to label instructions must be disposed of according to applicable Federal, State and Local government procedures. Contact the State pesticide or EPA Hazardous Waste representative at nearest EPA regional office.
Container Disposal	Dispose of containers in a sanitary landfill, or by incineration, or, if allowed by State or Local authorities, by burning. If burned, stay out of smoke.

DIRECTIONS FOR USE

IT IS A VIOLATION OF FEDERAL LAW TO USE THIS PRODUCT IN A MANNER INCONSISTENT WITH ITS LABELING.

It is *critical* that PB-ROPE L is applied as directed.

1. Crop	Cotton.
2. Pest	Pink bollworm *(Pectinophora gossypiella).*
3. Rate	100-200 dispensers per acre or 250-500 dispensers per hectare.
4. Application	i. Twist dispensers loosely around the main stem of cotton near the bottom of the plant. ii. Apply immediately prior to moth emergence in the field or adjoining area. iii. Dispensers must be applied uniformly throughout the treated acreage to obtain a reduction in mating.
5. Timing	Attach dispensers at pinsquare.
6. Precautions	PB-Rope L suppresses mating of pink bollworms. However, if a major source of mated female pink bollworm moths is present in adjacent areas, migration of these moths may significantly reduce the level of control achieved. Sources are likely to be cotton (sprayed or unsprayed) or other host species within 300 yards of the treated field. This may be counteracted by: a. Treatment of **entire blocks** and not just sections of large conventionally treated fields. b. Treatment of infestation sources with PB-Rope L (e.g. a strip at least 250 yards wide nearest the treated field). c. Treatment of infestation sources with an effective insecticide. Supplementary applications of insecticide are advised when PB-Rope L is used to control very high populations of pink bollworms. Other pests must be monitored so that timely intervention with insecticides is possible.

WARRANTY AND LIMITATION OF DAMAGES

All statements concerning the use of this product apply only when used as directed. The Manufacturer makes no warranties, expressed or implied, concerning this product or its use, which extend beyond the description on the label. Read all directions carefully.

BIOCONTROL®

ISOMATE®-M 100

ACTIVE INGREDIENTS:

Z-8-Dodecen-1-yl Acetate	88.5 %
E-8-Dodecen-1-yl Acetate	5.7 %
Z-8-Dodecen-1-ol	1.0 %
INERT INGREDIENTS	4.8 %
TOTAL	100.0 %

Keep out of reach of children

CAUTION

STATEMENT OF PRACTICAL TREATMENT:

IF ON SKIN: Wash with plenty of soap and water. Get medical attention.

IF IN EYES: Flush eyes with plenty of water. Get medical attention if irritation persists.

PRECAUTIONARY STATEMENTS
HAZARDS TO HUMANS AND DOMESTIC ANIMALS

CAUTION

Harmful if absorbed through skin. Causes eye irritation. Avoid contact with skin, eyes or clothing. Wash thoroughly with soap and water after handling.

ENVIRONMENTAL HAZARDS

Do not apply directly to water, to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of this product. Do not discharge into lakes, streams, ponds or public waters unless in accordance with NPDES permit. For guidance contact your regional office of the Environmental Protection Agency.

PACIFIC BIOCONTROL CORPORATION
14615 NE 13th Court, Suite A
Vancouver, WA 98685 U.S.A.
Telephone (360) 571-2247 or 1-800-999-8805

MADE IN JAPAN

NET CONTENTS:
400 Dispenser Units
One dispenser contains 0.0094 fl oz or 243.8 mg
Total content of package: 3.75 fl oz or 97.5 gm

EPA Est. No: 47265-JP-01
EPA Reg. No: 53575-19

STORAGE AND DISPOSAL

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

Pesticide Storage	Store in original unopened package at temperatures below 40°F in a dry location. Product may be stored in cold storage facilities used for food storage.
Pesticide Disposal	Pesticide that cannot be used according to label instructions must be disposed of according to applicable Federal, State and Local government procedures. Contact the State pesticide or EPA Hazardous Waste representative at nearest EPA regional office.
Container Disposal	Dispose of dispensers in sanitary landfill or by incineration, or if allowed by State and Local authorities, by burning. If burned, stay out of smoke. Foil envelopes can be disposed of as household refuse.

DIRECTIONS FOR USE

IT IS A VIOLATION OF FEDERAL LAW TO USE THIS PRODUCT IN A MANNER INCONSISTENT WITH ITS LABELING.

It is *critical* that ISOMATE-M 100 is applied as directed.

1. Crop	Peach, nectarine, almond, apricot, plum, apple, quince and macadamia.
2. Pest	Oriental fruit moth *(Grapholita molesta)*, macadamia nut borer *(Cryptophlebia ombrodelta)*, koa seed worm *(Cryptophlebia illepida)*.
3. Rate	100-150 dispensers per acre or 250-375 dispensers per hectare (0.9 fl oz or 23.4 gm a.i. per application).
4. Application	Apply dispensers in at least upper third of tree, preferably within 2-3 feet of treetop. Apply dispensers within canopy and on branches to maximize shade protection. Apply dispensers securely on lateral branches. Dispensers twisted too tightly may girdle branches. Can be applied efficiently from moving trailer or with a pole applicator.
5. Timing	Apply prior to moth emergence in the spring. Dispensers release pheromone for up to 90 days. In crops with long field seasons or in orchards with high pest populations, a second application is recommended. If subsequent applications are required, apply prior to the start of subsequent flights.
6. Precautions	Isomate-M 100 suppresses oriental fruit moth from mating. However, if a major source of mated female moths of this species is present adjacent to the treated field, migration of these moths may significantly reduce the level of control achieved. Sources are likely to be unsprayed peach, nectarine, almond, apricot, plum, apple, quince and macadamia trees or other host species within 300 yards of the treated field. This can be overcome by: a. Treatment of **entire blocks** and not just sections of large conventionally treated fields that frequently serve as sources of mated females. b. Treatment of infestation sources with Isomate-M 100 (e.g. a strip at least 50 yards wide nearest the treated field). c. Treatment of infestation source with an effective insecticide. d. Treatment of 4-6 rows along border of pheromone treated orchard with insecticide. Other pests must be monitored on a regular schedule so that timely intervention with conventional insecticides is possible.

WARRANTY AND LIMITATION OF DAMAGES

All statements concerning the use of this product apply only when used as directed. The Manufacturer makes no warranties, expressed or implied, concerning this product or its use, which extend beyond the description on the label. Read all directions carefully.

BIOCONTROL®

ISOMATE®-CM/LR PHEROMONE

ACTIVE INGREDIENTS:	
Z-11-Tetradecenyl acetate	45.8 %
E, E-8, 10-Dodecadien-1-ol	39.2 %
Dodecanol	6.6 %
Tetradecanol	1.0 %
INERT INGREDIENTS	7.4 %
TOTAL	100.0 %

Keep out of reach of children

CAUTION

STATEMENT OF PRACTICAL TREATMENT:

IF ON SKIN: Wash with plenty of soap and water. Get medical attention if irritation persists.

IF IN EYES: Flush eyes with plenty of water. Get medical attention if irritation persists.

PRECAUTIONARY STATEMENTS
HAZARDS TO HUMANS AND DOMESTIC ANIMALS

CAUTION

Harmful if absorbed through skin. Causes eye irritation. Avoid contact with skin, eyes or clothing. Wash thoroughly with soap and water after handling.

ENVIRONMENTAL HAZARDS

Do not apply directly to water, to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of this product. Do not discharge into lakes, streams, ponds or public waters unless in accordance with NPDES permit. For guidance contact your regional office of the Environmental Protection Agency.

PACIFIC BIOCONTROL CORPORATION
16010 NE 36th Avenue, Suite A
Ridgefield, WA 98642 U.S.A.
Telephone (360) 574-9726
1-800-999-8805

MADE IN JAPAN

NET CONTENTS:
400 Dispenser Units

EPA Est. No: 47265-JP-01
EPA Reg. No: 53575-20

One dispenser contains 0.01 fl oz or 264 mg
Total content of package: 4.1 fl oz or 105 gm

STORAGE AND DISPOSAL

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

Pesticide Storage	Store in original unopened package at temperatures below 40°F in a dry location. Product may be stored in cold storage facilities used for food storage.
Pesticide Disposal	Pesticide that cannot be used according to label instructions must be disposed of according to applicable Federal, State and local government procedures. Contact the State pesticide or EPA Hazardous Waste representative at nearest EPA regional office.
Container Disposal	Dispose of dispensers in sanitary landfill or incineration, or if allowed by State and local authorities, by burning. If burned, stay out of smoke. Foil envelopes can be disposed of as household refuse.

DIRECTIONS FOR USE

IT IS A VIOLATION OF FEDERAL LAW TO USE THIS PRODUCT IN A MANNER INCONSISTENT WITH ITS LABELLING.

It is *critical* that ISOMATE-CM/LR is applied as directed.

1. Crop	Apple and pear.
2. Pest	Codling moth *(Cydia pomonella)*, oblique banded leafroller *(Choristoneura rosaceana)*, pandemis leafroller *(Pandemis pyrusana)*.
3. Rate	400 dispensers per acre or 1000 dispensers per hectare (3.9 fl oz or 102 gm a.i. per application). Apply double rate of dispensers to edges of orchard.
4. Application	Apply dispensers in at least upper third of tree, preferably within 2-3 feet of treetop. Apply dispensers within canopy and on branches to maximize shade protection. Apply dispensers securely on lateral branches. Dispensers twisted too tightly may girdle branches. Can be applied efficiently from moving trailer or with a pole applicator.
5. Timing	Apply prior to codling moth emergence in the spring. Dispensers release pheromone for 100 -120 days. In crops with long field seasons (i.e. more than 120 days) or in orchards with high pest populations, a second application is recommended. If subsequent applications are required, apply prior to the start of subsequent flights.
6. Precautions	Isomate-CM/LR suppresses codling moth and leafroller moth from mating. However, if a major source of mated female moths of these species is present adjacent to the treated field, migration of these moths may significantly reduce the level of control achieved. Sources are likely to be unsprayed apple, pear, peach, apricot, plum, and cherry trees or other host species within 300 yards of the treated field. This can be overcome by: a. Treatment of **entire blocks** and not just sections of large conventionally treated fields that frequently serve as sources of mated females. b. Treatment of infestation sources with Isomate-CM/LR (e.g. a strip at least 50 yards wide nearest the treated field). c. Treatment of infestation source with an effective insecticide. d. Treatment of 4-6 rows along border of pheromone treated orchard with insecticide. Other pests must be monitored on a regular schedule so that timely intervention with conventional insecticides is possible.

WARRANTY AND LIMITATION OF DAMAGES

All statements concerning the use of this product apply only when used as directed. The Manufacturer makes no warranties, expressed or implied, concerning this product or its use, which extend beyond the description on the label. Read all directions carefully.

BIOCONTROL®

ISOMATE®-BAW PHEROMONE

ACTIVE INGREDIENTS:

(Z,E)-9,12-Tetradecadien-1-yl Acetate	55.0 %
Z-9-Tetradecen-1-ol	24.8 %
OTHER INGREDIENTS	20.2 %
TOTAL	100.0 %

Keep out of reach of children

CAUTION

STATEMENT OF PRACTICAL TREATMENT:

IF ON SKIN: Wash with plenty of soap and water. Get medical attention if irritation persists.

IF IN EYES: Flush eyes with plenty of water. Get a physician if irritation persists.

PRECAUTIONARY STATEMENTS
HAZARDS TO HUMANS AND DOMESTIC ANIMALS

CAUTION

Causes moderate eye irritation. Avoid contact with skin, eyes or clothing. Wash thoroughly with soap and water after handling.

ENVIRONMENTAL HAZARDS

Do not apply directly to water, to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of this product. Do not discharge into lakes, streams, ponds or public waters unless in accordance with NPDES permit. For guidance contact your regional office of the Environmental Protection Agency.

PACIFIC BIOCONTROL CORPORATION
14615 NE 13th Court, Suite A
Vancouver, WA 98685 U.S.A.
Telephone (360) 571-2247
1-800-999-8805

MADE IN JAPAN

EPA Est. No: 47265-JP-01
EPA Reg. No: 53575-21

NET CONTENTS:
500 Dispenser Units
One dispenser contains 0.0097 fl oz or 252.65 mg
Total content of package: 4.86 fl oz or 126.3 gm

STORAGE AND DISPOSAL

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

Pesticide Storage	Store in original unopened package at temperatures below 40°F in a dry location. Only unopened or unbroken dispenser packages may be stored in cold storage facilities used for food storage. Care must be taken to avoid contamination of food or feed items.
Pesticide Disposal	Pesticide that cannot be used according to label instructions must be disposed of according to applicable Federal, State and Local government procedures. Contact the State pesticide or EPA Hazardous Waste representative at nearest EPA regional office.
Container Disposal	Dispose of dispensers in sanitary landfill or by incineration, or if allowed by State and Local authorities, by burning. If burned, stay out of smoke. Foil envelopes can be disposed of as household refuse.

DIRECTIONS FOR USE

IT IS A VIOLATION OF FEDERAL LAW TO USE THIS PRODUCT IN A MANNER INCONSISTENT WITH ITS LABELING.

It is *critical* that ISOMATE-BAW is applied as directed.

1. Crop	Alfalfa, asparagus, beans, beets, cabbage, celery, cole crops, cotton, cucumbers, lettuce, onions, peanuts, peas, peppers, soybeans, strawberries, sweet potatoes, tomatoes, tobacco.
2. Pest	Beet armyworm *(Spodoptera exigua)*.
3. Rate	Minimum of 100 dispensers per acre (0.9 fl oz or 23.9 gm a.i. per acre) or 250 dispensers per hectare. Maximum of 200 dispensers per acre (1.8 fl oz or 47.9 gm a.i. per acre) or 500 dispensers per hectare. Do not exceed 150 gm a.i. per acre per year.
4. Application	Attach dispensers to stakes placed uniformly within the treated field. Stakes should be at the canopy level of the crop. Avoid placing dispensers in contact with the soil. At 100 dispensers per acres, stakes should be placed approximately every 20 feet apart. Treat all border rows. Increase the number of dispensers on upwind side of the fields or along borders adjacent to other beet armyworm hosts. Higher rates may be needed in smaller fields or in windy conditions.
5. Timing	Monitor with pheromone traps and crop inspection. Apply product prior to adult flight, early enough to disrupt mating communication within the field. Consult your local pest control advisor for proper timing. Dispenser releases pheromone for 60-90 days depending on temperature.
6. Precautions	Isomate-BAW suppresses mating between beet armyworms. However, if a major source of mated female moths of this species is present adjacent to the treated field, migration of these moths may significantly reduce the level of control achieved. A common source of mated females is unsprayed host species within 300 yards of the treated field. This can be overcome by: a. Treatment of **entire blocks** and not just sections of large conventionally treated fields that frequently serve as sources of mated females. b. Treatment of infestation sources with Isomate-BAW (e.g. a strip at least 50 yards wide nearest the treated field). c. Treatment of infestation source with an effective insecticide. Supplementary applications of insecticide are advised when Isomate-BAW is used to control very high populations of beet armyworm, and when beet armyworm larvae are imported into the field on transplants. All pests must be monitored so that timely intervention with insecticides is possible.

WARRANTY AND LIMITATION OF DAMAGES

All statements concerning the use of this product apply only when used as directed. The Manufacturer makes no warranties, expressed or implied, concerning this product or its use, which extend beyond the description on the label. Read all directions carefully.

BIOCONTROL®

ISOMATE®-OBLR/PLR PLUS

ACTIVE INGREDIENTS:
Z-11-Tetradecen-1-yl Acetate..... 88.97 %
OTHER INGREDIENTS..... 11.03 %

TOTAL.....100.00 %

226.82 mg active ingredients per dispenser

Keep out of reach of children

CAUTION

FIRST AID

If on Skin or Clothing	• Take off contaminated clothing. • Rinse skin immediately with plenty of water for 15-20 minutes. • Call a poison control center or doctor for treatment advice.
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. You may also contact 1-800-999-8805 for further questions.

PRECAUTIONARY STATEMENTS
HAZARDS TO HUMANS AND DOMESTIC ANIMALS

CAUTION

Harmful if absorbed through skin. Causes moderate eye irritation. Avoid contact with skin, eyes or clothing. Wash thoroughly with soap and water after handling.

ENVIRONMENTAL HAZARDS

Do not apply directly to water, to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of this product. Do not discharge into lakes, streams, ponds or public waters unless in accordance with NPDES permit. For guidance contact your regional office of the Environmental Protection Agency.

PACIFIC BIOCONTROL CORPORATION
14615 NE 13th Court, Suite A, Vancouver, WA 98685 U.S.A.
Telephone (360) 571-2247 or 1-800-999-8805

MADE IN JAPAN
EPA Est. No: 47265-JP-01
EPA Reg. No: 53575-24

NET CONTENTS: 400 Dispenser Units
One dispenser contains 0.0099 fl oz or 255.5 mg
Total content of package: 3.95 fl oz or 102.2 gm

STORAGE AND DISPOSAL
Do not contaminate water, food or feed by storage or disposal

Pesticide Storage	Store in original unopened package at temperatures below 40°F in a dry location. Only unopened or unbroken dispenser packages may be stored in cold storage facilities used for food storage. Care must be taken to avoid contamination of food or feed items.
Pesticide Disposal	Wastes resulting from this product may be disposed of on site or at an approved waste disposal facility.
Container Disposal	Dispose of dispensers in sanitary landfill or by incineration, or if allowed by State and Local authorities, by burning. If burned, stay out of smoke. Foil envelopes can be disposed of as household refuse.

DIRECTIONS FOR USE
It is a violation of Federal law to use this product in a manner inconsistent with its labeling.
It is *critical* that ISOMATE-OBLR/PLR PLUS is applied as directed.

1. Crop	Apple, pear, apricot, cherry, peach and plum.
2. Pest	Obliquebanded leafroller *(Choristoneura rosaceana)* and pandemis leafroller *(Pandemis pyrusana)*.
3. Rate	Minimum of 200 dispensers per acre (1.75 fl oz or 45.36 gm a.i. per acre) or 500 dispensers per hectare. Maximum of 400 dispensers per acre or 1000 dispensers per hectare (3.5 fl oz or 90.73 gm a.i. per acre). Apply double rate of dispensers to edges of orchard. Do not exceed 150 gm a.i. per acre per year
4. Application	Apply dispensers securely to lateral branches in upper third of tree canopy, preferably within 2-3 feet of treetop. Dispensers twisted too tightly may girdle branches. Can be applied efficiently from a moving trailer or with a pole applicator.
5. Timing	Apply prior to leafroller emergence in the spring. Dispensers release pheromone for up to 150 days depending on temperature. In crops with long field seasons (i.e. more than 150 days), a second application is recommended. If subsequent applications are required, apply prior to the start of subsequent flights. Consult your local pest control advisor for proper timing.
6. Note	Isomate-OBLR/PLR Plus suppresses the obliquebanded and pandemis leafrollers from mating. However, if a major source of mated female moths of these species is present adjacent to the treated field, migration of these moths may significantly reduce the level of control achieved. Sources are likely to be unsprayed apple, pear, peach, apricot, plum, prune and cherry trees or other wild plant host species within 300 yards of the treated field. This can be reduced by: a. Treatment of **entire blocks** and not just sections of large conventionally treated fields that frequently serve as sources of mated females. b. Treatment of infestation sources with Isomate-OBLR/PLR Plus (e.g. a strip at least 50 yards wide nearest the treated field). c. Treatment of infestation source with an effective insecticide. d. Treatment of 4-6 rows along border of pheromone treated orchard with insecticide. Supplementary applications of insecticide are advised when Isomate-OBLR/PLR Plus is used to control high populations of leafrollers. All pests must be monitored so that timely intervention with insecticides is possible.

WARRANTY AND LIMITATION OF DAMAGES

All statements concerning the use of this product apply only when used as directed. The Manufacturer makes no warranties, expressed or implied, concerning this product or its use, which extend beyond the description on the label. Read all directions carefully.

Revision Date: February 2002

BIOCONTROL®

ISOMATE®-C TT

ACTIVE INGREDIENTS:	
(E, E)-8, 10-Dodecadien-1-ol	53.0 %
Dodecanol	29.7 %
Tetradecanol	6.0 %
OTHER INGREDIENTS	11.3 %
TOTAL	100.0 %

382.4 mg active ingredients per dispenser

Keep out of reach of children

WARNING

FIRST AID

If on Skin or Clothing
- Take off contaminated clothing.
- Rinse skin immediately with plenty of water for 15-20 minutes.
- Call a poison control center or doctor for treatment advice.

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. You may also contact 1-800-999-8805 for further questions.

PRECAUTIONARY STATEMENTS
HAZARDS TO HUMANS AND DOMESTIC ANIMALS

WARNING

Harmful if absorbed through skin. Causes eye irritation. Avoid contact with skin, eyes or clothing. Wash thoroughly with soap and water after handling.

ENVIRONMENTAL HAZARDS

Do not apply directly to water, to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of this product. Do not discharge into lakes, streams, ponds or public waters unless in accordance with NPDES permit. For guidance contact your regional office of the Environmental Protection Agency.

PACIFIC BIOCONTROL CORPORATION
14615 NE 13th Court, Suite A, Vancouver, WA 98685 U.S.A.
Telephone (360) 571-2247 or 1-800-999-8805

MADE IN JAPAN
EPA Est. No: 47265-JP-01
EPA Reg. No: 53575-25

NET CONTENTS: 400 Dispenser Units
One dispenser contains 0.017 fl oz or 431.4 mg
Total content of package: 6.81 fl oz or 172.56 gm

STORAGE AND DISPOSAL

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

Pesticide Storage	Store in original unopened package at temperatures below 40°F in a dry location. Product may be stored in cold storage facilities used for food storage.
Pesticide Disposal	Pesticide that cannot be used according to label instructions must be disposed of according to applicable Federal, State and Local government procedures. Contact the State pesticide or EPA Hazardous Waste representative at nearest EPA regional office.
Container Disposal	Dispose of dispensers in sanitary landfill or by incineration, or if allowed by State and Local authorities, by burning. If burned, stay out of smoke. Foil envelopes can be disposed of as household refuse.

DIRECTIONS FOR USE

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

It is *critical* that ISOMATE-C TT is applied as directed.

1. Crop	Apple, pear, walnut, quince, peach, prune, plum, peach, pecan and nectarine.
2. Pest	Codling moth *(Cydia pomonella)*, hickory shuckworm *(Cydia caryana)*.
3. Rate	200 dispensers per acre or 500 dispensers per hectare (3.0 fl oz or 76.5 gm a.i. per application). Apply double rate of dispensers to edges of orchard. Do not exceed 150 gm a.i. per acre per year.
4. Application	Apply dispensers in upper third of tree, preferably within 2-3 feet of treetop. Apply dispensers within canopy. Apply dispensers securely on branches. Can be applied efficiently from moving trailer or with a pole applicator.
5. Timing	Apply prior to codling moth emergence in the spring. Dispensers release pheromone for 120-140 days depending on temperature. In crops with long field seasons (i.e. more than 120 days), a second application is recommended. If subsequent applications are required, apply prior to the start of subsequent flights. Consult your local pest control advisor for proper timing.
6. Note	Isomate-C TT suppresses codling moth and hickory shuckworm from mating. However, if a major source of mated female moths of these species is present adjacent to the treated field, migration of these moths may significantly reduce the level of control achieved. Sources are likely to be unsprayed apple, pear, quince, walnut, plum, crabapple trees or other host species within 300 yards of the treated field. This can be overcome by: a. Treatment of **entire blocks** and not just sections of large conventionally treated fields that frequently serve as sources of mated females. b. Treatment of infestation sources with Isomate-C TT (e.g. a strip at least 50 yards wide nearest the treated field). c. Treatment of infestation source with an effective insecticide. d. Treatment of 4-6 rows along border of pheromone treated orchard with insecticide. Supplementary applications of insecticide are advised when Isomate-C TT is used to control very high populations of codling moth or hickory shuckworm. All pests must be monitored so that timely intervention with insecticides is possible.

WARRANTY AND LIMITATION OF DAMAGES

All statements concerning the use of this product apply only when used as directed. The Manufacturer makes no warranties, expressed or implied, concerning this product or its use, which extend beyond the description on the label. Read all directions carefully.

BIOCONTROL®

ISOMATE®-M ROSSO

ACTIVE INGREDIENTS:	
Z-8-dodecen-1-yl acetate	88.5 %
E-8-dodecen-1-yl acetate	5.7 %
Z-8-dodecen-1-ol	1.0 %
OTHER INGREDIENTS	4.9 %
TOTAL	100.0 %

Keep out of reach of children

CAUTION

FIRST AID

If on Skin or Clothing	• Take off contaminated clothing. • Rinse skin immediately with plenty of water for 15-20 minutes. • Call a poison control center or doctor for treatment advice.
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. You may also contact 1-800-999-8805 for further questions.

PRECAUTIONARY STATEMENTS
HAZARDS TO HUMANS AND DOMESTIC ANIMALS

CAUTION

Harmful if absorbed through skin. Causes eye irritation. Avoid contact with skin, eyes or clothing. Wash thoroughly with soap and water after handling.

ENVIRONMENTAL HAZARDS

Do not apply directly to water, to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of this product. Do not discharge into lakes, streams, ponds or public waters unless in accordance with NPDES permit. For guidance contact your regional office of the Environmental Protection Agency.

PACIFIC BIOCONTROL CORPORATION
14615 NE 13th Court, Suite A
Vancouver, WA 98685 U.S.A.
Telephone (360) 571-2247 or 1-800-999-8805

MADE IN JAPAN
EPA Est. No: 47265-JP-01
EPA Reg. No: 53575-26

NET CONTENTS: 400 Dispenser Units
One dispenser contains 0.01 fl oz or 264.3 mg
Total content of package: 4.06 fl oz or 105.7 gm

STORAGE AND DISPOSAL

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

Pesticide Storage	Store in original unopened package at temperatures below 40°F in a dry location. Product may be stored in cold storage facilities used for food storage.
Pesticide Disposal	Pesticide that cannot be used according to label instructions must be disposed of according to applicable Federal, State and Local government procedures. Contact the State pesticide or EPA Hazardous Waste representative at nearest EPA regional office.
Container Disposal	Dispose of dispensers in sanitary landfill or by incineration, or if allowed by State and Local authorities, by burning. If burned, stay out of smoke. Foil envelopes can be disposed of as household refuse.

DIRECTIONS FOR USE

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

It is *critical* that ISOMATE-M ROSSO is applied as directed.

1. Crop	Peach, nectarine, almond, apricot, plum, apple, quince and macadamia.
2. Pest	Oriental fruit moth *(Grapholita molesta)*, macadamia nut borer *(Cryptophlebia ombrodelta)*, koa seed worm *(Cryptophlebia illepida)*.
3. Rate	200 dispensers per acre or 500 dispensers per hectare (1.94 fl oz or 50.5 gm a.i. per application). Do not exceed 150 gm a.i. per acre per year.
4. Application	Apply dispensers in upper third of tree, preferably within 2-3 feet of treetop. Apply dispensers within canopy and on branches to maximize shade protection. Apply dispensers securely on lateral branches. Dispensers twisted too tightly may girdle branches. Can be applied efficiently from moving trailer or with a pole applicator.
5. Timing	Apply prior to moth emergence in the spring. Dispensers release pheromone for up to 120 days depending on temperature. In crops with long field seasons (i.e. more than 120 days), a second application is recommended. If subsequent applications are required, apply prior to the start of subsequent flights. Consult your local pest control advisor for proper timing.
6. Precautions	Isomate-M Rosso suppresses orient fruit moth, macadamia nut borer and koa seed worm from mating. However, if a major source of mated female moths of these species is present adjacent to the treated field, migration of these moths may significantly reduce the level of control achieved. Sources are likely to be unsprayed peach, nectarine, almond, apricot, plum, apple, quince and macadamia trees or other host species within 300 yards of the treated field. This can be overcome by: a. Treatment of **entire blocks** and not just sections of large conventionally treated fields that frequently serve as sources of mated females. b. Treatment of infestation sources with Isomate-M Rosso (e.g. a strip at least 50 yards wide nearest the treated field). c. Treatment of infestation source with an effective insecticide. d. Treatment of 4-6 rows along border of pheromone treated orchard with insecticide Supplementary applications of insecticide are advised when Isomate-M Rosso is used to control very high populations of oriental fruit moth, macadamia nut borer or koa seed worm. All pests must be monitored so that timely intervention with insecticides is possible.

WARRANTY AND LIMITATION OF DAMAGES

All statements concerning the use of this product apply only when used as directed. The Manufacturer makes no warranties, expressed or implied, concerning this product or its use, which extend beyond the description on the label. Read all directions carefully.

MATERIAL SAFETY DATA SHEET

1. CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

Product Name :Sumilizer BHT-R
Chemical Name :2,6-Di-tert-butyl-p-cresol

MANUFACTURER :

Sumitomo Chemical Co.,Ltd.
27-1,Shinkawa,2-Chome,
Chuo-ku, Tokyo 104, Japan

EMERGENCY TELEPHONE NUMBERS :

Sumitomo Chemical Co.,Ltd.
TEL +81-3-5543-5641(Japan)
FAX +81-3-5543-5916(Japan)

2. COMPOSITION/INFORMATION ON INGREDIENTS

COMPONENT	CAS No.	%	OSHA PEL	ACGIH TLV
#Sumilizer BHT-R	128-37-0	100	10mg/m^3	10mg/m^3

#Hazardous with the meaning of 29 C.F.R.Part 1910.1200.

3. HAZARDS IDENTIFICATION

EMERGENCY OVERVIEW :White crystalline powder that is no immediate hazard.
Avoid contact with skin and eyes. Avoid breathing dust.

POTENTIAL HEALTH EFFECTS:

INHALATION :No known effect.

EYE CONTACT :Mechanical irritation will probably develop after contact with this material.

SKIN CONTACT :Non-irritating.

INGESTION :Practically non-toxic in normal industrial use.

CHRONIC :Listed in Group 3(not classifiable as to their carcinogenicity to humans) by IARC.

MSDS continued on next page.(US)

4. EMERGENCY AND FIRST AID MEASURES

INHALATION :If exposure to dust causes irritation or distress, remove subject to fresh air. Give oxygen or artificial respiration if needed. Get medical attention.

SKIN CONTACT :Immediately flush skin with plenty of water. Remove clothing. Get medical attention if irritation develops or persists. Wash clothing before reuse.

EYE CONTACT :Immediately flush eyes with plenty of water for at least 15 minutes,and contact a physician.

INGESTION :If swallowed, immediately get medical attention.

5. FIRE-FIGHTING MEASURES AND EXPLOSION HAZARD DATA

FLASH POINT :127℃

AUTOIGNITION TEMPERATURE :420℃

DUST EXPLOSION LIMITS :Lower :15g/m3

EXTINGUISHING MEDIA :Use carbon dioxide or dry chemical for small fires; universal foam or water spray for large fires.

SPECIAL FIRE-FIGHTING PROCEDURES :Fire fighters should be provided with positive-pressure self-contained breathing apparatus and other protective equipment.

UNUSUAL FIRE and EXPLOSION HAZARDS :Not known.

HAZARDOUS DECOMPOSITION PRODUCTS :May generate CO when heated to burning.

6. ACCIDENTAL RELEASE MEASURES

GENERAL : Eliminate all ignition source.
Consult an expert on the disposal of recovered material. Ensure disposal is in compliance with government requirements and ensure conformity of local disposal regulations. Notify the appropriate authorities immediately. Take all additional action necessary to prevent and remedy the adverse effects of the spill.

LAND SPILL : Sweep up or shovel into sealable containers and then wash out with water. Prevent washings from entering waterways.

7. HANDLING AND STORAGE

PRECAUTIONS :Avoid contact with skin and eyes. Avoid breathing dust.
Use with adequate ventilation. Ground and bond containers when transferring material.
Store out of direct sunlight .Keep away from all ignition source.
Keep containers closed when not in use.

8. EXPOSURE CONTROLS AND PERSONAL PROTECTION

ENGINEERING CONTROLS (VENTILATION) : Use local ventilation at places where dust can be released into the workplace air. Keep dust concentrations below the recommended TLV.

ACGIH TLV for =10 mg/m^3
OSHA PEL for =10mg/m^3

PERSONAL PROTECTION

RESPIRATORY :Not required for occasional handling if adequate ventilation is available.
A respirator is recommended for prolonged handling or exposure.

PROTECTIVE GLOVES : Wear rubber gloves.

EYE PROTECTION : Wear safety goggles or equivalent eye protection.

OTHER : Wear appropriate protective clothing to prevent skin contact.

WORK/HYGIENIC PRACTICES : Always clean protective equipment and workplace.

9. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE	:White crystalline powder	ODOR	:Odorless
MELTING POINT	:70℃	SPECIFIC GRAVITY	:1.048(20℃/4℃)
BOILING POINT	:265℃	SOLUBILITY in water	:Practically insoluble
VAPOR DENSITY	:Not known	pH	:Not known
PERCENT VOLATILE	:Not known	VAPER PRESSURE	:6.5mmHg(120℃)

10. STABILITY AND REACTIVITY

STABILITY : Stable on normal condition. HAZARDOUS POLYMERIZATION : Will not occur.

CONDITIONS TO AVOID :High temperature

INCOMPATIBILITY :Not known

HAZARDOUS DECOMPOSITION PRODUCTS : May generate CO when heated to burn.

MSDS continued on next page.(US)

11. TOXICOLOGICAL INFORMATION

ACUTE INHALATION EFFECTS :No data available.

SKIN EFFECT :This material is classified as non-irritant based on the test results in rabbits according to the OSHA standard.(1)

EYE EFFECT :This material is classified as non-irritant based on the test results in rabbits according to the OSHA standard.(1)

ACUTE ORAL EFFECTS :The oral LD_{50} in rats is 2,340~2,530mg/kg. (1)

SUBCHRONIC AND CHRONICTOXICITY :
This material produce liver enlargement and hemorrhagic death after continuous oral administration in rats. It is recognized that these effects are not produced in humans.(1)
No adverse effects are occurred after oral administration of total 45~244g/kg during 12 months in dogs. (1)

CARCINOGENICITY:
Carcinogenicity had not been observed after continuous treatment of this material in diet for 104 and 96 weeks in rats and mice, respectively.(1)
This material is listed in Group3(not classifiable as to their carcinogenicity to humans) by IARC.
NTP or OSHA have not classified it as carcinogenicity.

MUTAGENICITY :Negative results have been reported in Ames test,Rec-assay,DNA-repair test in rat liver cells, in vitro chromosomal aberration test in CHL cells, in vivo micronucleus test and dominant lethal test in mice.
Some positive results have been reported in UDS test in rats and in vitro chromosomal aberration test in CHO cells. (1)

TERATOGENICITY: No teratogenic effects have been observed in rats and mice. (1)

REPRODUCTION :Developmental delay of offspring was observed in reproduction studies in rats and mice.(1)

This material had been evaluated for its toxicological properties and classified as GRAS by FDA. (9CFR§182.3173)

12. ECOLOGICAL INFORMATION

FISH ACUTE TOXICITY :LC50 is 6.2ppm(48hr) in killifish. (1)
BIODEGRADABILITY :Not biodegradability. (1)

13. DISPOSAL CONSIDERATIONS

To be incinerated by adequate method.Dispose in accordance with federal, state and local regulations. The owner of the materials responsible for proper waste disposal.

MSDS continued on next page.(US)

14. TRANSPORT INFORMATION

Ground and bond containers when transferring material. Keep containers tightly closed during transport.

15. REGULATORY INFORMATION (not meant to be all inclusive)

TSCA (Toxic Substance Control Act) : Listed on TSCA

16. OTHER INFORMATION

REVISION SUMMARY :Revised due to amendment of contents in section 9 on 31/March/1998.
Revised due to amendment of contents in section 11 on 16/December/1998.

REFERENCES :(1)Technical information of Sumitomo Chemical Co., Ltd.

The information is believed to be accurate and represents the best information currently available to us. However, no guarantee or warranty of any kind, expressed or implied, is made with respect to the information contained herein.

End of MSDS (US)

Beneficial Effects to the Environment:

Mating disruption is a non-toxic, behavioral method for the control of the insects, which does not affect the crop, the grower, the environment, or other non-target insects. Mating disruption is an essential tool for pest management in organic production. Organic production, especially in apples and pears, will be severely affected by the elimination of mating disruption. Therefore, it will be very difficult to grow organic apples, without mating disruption for control of the codling moth, when 95% of the acreage is treated with mating disruption.

The inert ingredient, BHT, is added to protect the insect pheromone active ingredients from oxidation. Without stabilizers, formulation longevity will be significantly reduced resulting in less effective, less economical control. There are no acceptable alternative inert stabilizers that provide adequate protection of the pheromones. The use of the inert stabilizer doubles the formulation's field life and thus greatly improves the economics of mating disruption.

This inert ingredient is incorporated entirely within the lumen of the dispenser and is separated from the surface by the thickness of the polymer. However, in the study with Isomate®-C Plus under constant temperature of 40° for one month, only 6.78% was shown to diffuse the surface of the dispenser. Therefore, BHT is being released gradually from the surface of the dispenser. ISOMATE®'s dispensers have little or no contact with crop fruit or fiber. CBI

The US EPA has the most familiarity with these type of pheromone products as it has registered more than 20 lepidopteran active ingredients and more than 60 products containing those active ingredients. The Agency has recognized the difference between semiochemicals and conventional chemical pesticides and facilitated regulatory relief to ease the burden of registering these types of products.

The first regulatory relief measure that the US EPA established was the tolerance exemption for inert ingredients of semiochemical dispensers. This included UV stabilizers like BHT. In addition, the US EPA recognized that these lepidopteran pheromone products are expected to have no adverse effects and minimal exposure because they have low acute toxicity and low application rates, are contained in a dispenser and slowly released, and have no contact with the crop.

The US EPA has eight registered products under Pacific Biocontrol with Sumilizer BHT–R as an inert ingredient.

Note: ISOMATE® is a registered trademark for pheromone products manufactured by Shin-Etsu Chemical Co. PB-ROPE L is used in cotton for the control of the pink bollworm. Pacific Biocontrol Corporation holds the US EPA registrations of the ISOMATE® and PB-ROPE L products. Pacific Biocontrol sells the ISOMATE® and PB–ROPE L products in the USA and Canada.

References:

(1) Thomson, D.R., Gut, L.J., and Jenkins, J.W. (1999) "Pheromones for Insect Control: Strategies and Successes", *Biopesticides: Use and Delivery*, (Hall, F.R. and Menn, J.J., eds.), Humana Press Inc, New Jersey, pp. 385-412.

(2) Antilla, L., Whitlow, M., Staten, R.T., El-Lissy, O., and Myers, F. (1996) "An Integrated Approach to Area Wide Pink Bollworm Management in Arizona", Proceedings, Beltwide Cotton Conference, Nashville, TN, pp. 1083-1085.

(3) Jenkins, J.W., Doane, C.C., Schuster, D.J., McLaughlin, J.R. and Jimenez, M.J. (1990) "Development and Commercial Application of Sex Pheromone for Control of the Tomato Pinworm", *Behavior-modifying Chemicals for Insect Management: Applications of Pheromones and Other Attractants*, (Ridgway, R.L., Silverstain, R.M, and Inscoe, M.N., eds.), Marcel Dekker Inc. New York, pp. 269 – 279.

(4) Trumble, J.T. and Alvarado-Rodriguez, B. (1993) "Development and Economic Evaluation of an IPM program for Fresh Market Tomato Production in México", *Agriculture, Ecosystems and Environment*, (Eisevier Science Publishers B.V.), Amsterdam, 43, pp. 1-18.

(5) Benbrook, C.M., Groth, E., Halloran, J.M., Hansen, M.K., and Marquardt, S. (1996) " The Pear Pest Management System Continuum in California: A Case Study", *Pest Management at the Crossroad*, Consumers Union, Yonkers, New York, pp. 191-195.

(6) Granatstein, D. (2002) "Recent Trends in Organic Tree Fruit Production: 2001", www.trfrec.wsu.edu.

(7) Ideses, R. and Shani, A. (1988) "Chemical Protection of Pheromones Containing an Internal Conjugated Diene System from Isomerization and Oxidation", *Journal of Chemical Ecology*, 14, pp. 1657-1669.

(8) (1989)"1548. Butylated Hydroxytoluene", *The Merck Index*, (Budavari, S., O'Neil, M.J. Smith, A., and Heckelman, P.E., eds.), Merck & Co., Inc., New Jersey, p. 238

(9) (1992) "21CFR 172.115 BHT", *Code of Federal Regulations: Food and Drugs: 21: Parts 170 to 199*, Office of the Federal Register National Archives and Records Administration, Washington, p. 26

(10) Hutton, P. (1995) "Lepidopteran Pheromones: Tolerance Exemption", *Federal Register*, 60, 168, pp. 45060-45062.

(11) Steinwand, B. (2001) "Generic Factsheet for Lepidopteran Pheromone", http://www.epa.gov/oppbppd1/biopesticides/factsheets/fs-generic_lep.htm.

(12) Welch, C. (1993) "Inert Ingredients of Semiochemical Dispensers; Tolerance Exemption", *Federal Register*, 58, 234, pp. 64493-64494.

(13) Hutton, P. (1994) "Arthropod Pheromones: Tolerance Exemption", *Federal Register*, 59, 61, pp. 14757-14759.

(14) US EPA OPP BPPD (1999) "Registration Eligibility Document Isomate®-BAW Pheromone", (PC Codes 117203 and 119409), pp. 1-23.

(15) Pacific Biocontrol Corporation (2001) Labels of Isomate®-C Plus, PB-Rope L, Isomate®-M 100, Isomate®-CM/LR Pheromone, Isomate®-BAW Pheromone, Isomate®-OBLR/PLR Plus, Isomate®-C TT and Isomate®-M Rosso

(16) Sumitomo Chemical Co., Ltd. (1998) Material Safety Data Sheet of Sumilizer BHT-R, *MSDS No. MM1113-US*, pp. 1-5.

(17) National Toxicology Program, "Butylated Hydroxytoluene Factsheet", "TR-150 Bioassay of Butylated Hydroxytoluene (BHT) for Possible Carcinogenicity (CAS No. 128-37-0)";" Target Organs and Levels of Evidence, NTP Technical Report Number 150", National Toxicology Program under National Institute of Environmental Health Studies (NIEHS), http://ntp-server.niehs.nih.gov/htdocs/Results_status/ResstatB/10575-K.html, http://ntp-server.niehs.nih.gov/htdocs/LT-studies/tr150.html, http://ntp-server.niehs.nih.gov/htdocs/levels/tr150levels.html.

(18) Fowkes, S.W. (1984) "Carcinogenicity", Cognitive Enhancement Research Institute, http://www.smartbodyz.com/herpes-bht-2.htm

(19) Vavasour, E. "Evaluation of National Intake Assessments of Butylated Hydroxytoluene (BHT)", *WHO Food Additives Series*, 35, http://www.inchem.org/documents/jecfa/jecmono/v35je02.htm

(20) International Program on Chemical Safety, World Health Organization (1999) "Evaluation of National Intake Assessments of Butylated Hydroxytoluene (BHT)", *WHO Food Additives Series*, 42, http://www.inchem.org/documents/jecfa/jecmono/v42je25.htm

(21) Wessling, C. (2001) "Antioxidant ability of BHT-and alpha-tocopherol-impregnated LDPE film in packaging of oatmeal", *Journal of the Science of Food and Agriculture*, 81, 2, pp. 194-201

21

Pheromones for Insect Control

Strategies and Successes

D. R. Thomson, L. J. Gut, and J. W. Jenkins

1. Historical Perspective

There is general agreement among government agencies, research institutions, industry, grower organizations, and the public that there is a need to reduce reliance on broad-spectrum insecticides by accelerating efforts to incorporate ecologically sound technologies into agricultural pest-management programs. The development and implementation of pest control technology based on behavior-controlling chemicals, or semiochemicals, offers a unique opportunity to move in this direction. Semiochemicals are chemical messages that organisms use to communicate with each other. Among the semiochemicals, insect sex pheromones have probably received the most attention from the scientific, regulatory, and agricultural communities. Sex pheromones are chemical messages between individuals of the same species, which facilitate mating. By their nature, pheromones are highly specific and their use for insect control would not disrupt other biological interactions within a cropping system.

The first published identification of an insect sex pheromone was that of the silkworm moth *Bombyx mori* L. *(1)*. During the 1960s, the pheromones of 11 other insects were identified *(2)*. Research on sex pheromones increased in subsequent years, as efforts were made to utilize these materials for managing insects. Currently, the pheromones of more than 1600 species have been identified *(3,4)*. The production of synthetic copies of sex pheromones has led to the development and widespread commercial use of sex-pheromone traps for monitoring and trapping insect pests in many sectors, including agriculture, forestry, government detection and quarantine programs, and consumer protection.

From: *Methods in Biotechnology, vol. 5: Biopesticides: Use and Delivery*
Edited by: F. R. Hall and J. J. Menn

of action, may be able to slow or prevent the development of resistance by reducing exposure to insecticides. Resistance to pheromones has not been documented in the field, and, at least in some species, is unlikely, given the broad response to blend ratios *(17)*. Therefore, mating-disruption products should have longer life expectancy, and may help preserve the dwindling supply of effective conventional insecticides.

Mating-disruption technology controls the target pest by manipulating certain aspects of sexual behavior *(18)*. Pheromones are nontoxic to natural enemies. In contrast, conventional insecticides are generally broad-spectrum, killing both pest and nonpest species. Greater reliance on pheromone technologies in agricultural crops will increase the potential for the biological control of secondary pests by allowing for crop environments that can sustain high populations of predators and parasitoids. Enhanced biological control often corresponds to a reduction in the number of insecticide applications for control of secondary insects *(19–21)*, resulting in savings for the grower *(22)*.

The above discussion reviewed some of the factors that have favored the success of mating-disruption technology in the marketplace. The focus of the remainder of this chapter is to discuss how specific advances in critical areas have enhanced the development, implementation, and adoption of mating-disruption technologies in agricultural systems. It does not review the principles of mating disruption, nor does it attempt to discuss the many successful uses of this technology. These have been carefully reviewed in other publications *(18,23–26)*. The following critical issues will be discussed regarding the use of mating-disruption technology in two agricultural systems: biology and pest status; identification, formulation, and delivery system; and applied research, extension, and economics. We have selected two pest management systems, PBW in cotton and codling moth (CM) *Cydia pomonella*, in pome fruits, to illustrate how these critical issues have been addressed, and to discuss their overall impact on mating disruption product development and commercial adoption.

PBW and CM represent two very different challenges for mating-disruption technologies. Worldwide, mating disruption is used most extensively for the control of PBW, a serious pest of an annual row crop that is grown on large acreages. In 1996, tenders were awarded in Egypt to supply mating-disruption products for approx 200,000 ha of cotton *(14)*. The CM system provides a model for the successful development and adoption of this technology in horticultural crops, and provides an assessment of the technology in a much different cropping situation, with the added challenges of a large crop canopy, larger pest complexes, and lower damage thresholds because of high crop value. Mating-disruption technology is also extensively used to control CM in pome fruit in the United States *(20,27)*, Italy *(28)*, and South Africa *(29)*. Worldwide in 1997, it is estimated that mating-disruption technologies were used to control CM on between 24,000 and 28,000 ha of pome fruit (Thomson, personal communication).

2. Case Studies

2.1. Pink Bollworm

2.1.1. Biology and Pest Status

The PBW is the most serious pest of cotton, *Gossypium hirsutum* L. and *G. barbadense* L, worldwide, including North and South America, Spain, Greece, Egypt, Pakistan, India, China, and Australia. In the desert southwestern United States, this pest infests approx 200,000 ha of cotton, including the highest yielding areas of Arizona and the Imperial Valley of California *(30)*. In 1997, there were an additional approx 54,000 ha of cotton infested just south of the United States border in Mexico (R. Staten, personal communication).

Throughout its range, PBW is usually the primary economic pest of cotton. Cotton bolls are the preferred site for oviposition. Upon hatching, first-instar larvae quickly enter squares or bolls. Injury is caused when larvae cut and stain fiber and feed in seeds within the developing cotton bolls. Larval damage also permits the development of decay from microorganisms. Infestations can lower quality of lint and seed, and yield reductions of 30% or higher are not uncommon *(31)*.

Quarantine programs, cultural practices *(32)*, sterile moth releases *(33,34)*, and conventional chemical insecticides have been used to manage PBW populations. Control with conventional insecticides is difficult, because larvae are well protected within cotton squares and bolls. Insecticide applications are therefore normally targeted at nocturnally active adults. Insecticide costs for PBW control are high. Gonzales *(35)* reported that insecticide costs during the period 1978–1988 averaged $640/ha/yr. Furthermore the reliance on conventional chemical insecticides has led to outbreaks of secondary pests *(36)* and development of resistance *(37)*. Because of the problems associated with traditional chemotherapy, PBW has been a prime candidate for development of alternative control methods. Development of mating-disruption technology for this pest began more than 25 yr ago *(38)*.

2.1.2. Critical Issues in Identification, Formulation, and Delivery System

PBW sex pheromone, gossyplure, was first identified by Hummel et al. *(39)* and Bier et al. *(40)*. Compared to many insect pheromones, gossyplure (Z:Z and Z:E 7,11 hexadecadienyl acetate 50:50) is relatively simple to synthesize, and is stable in the environment *(41)*. Soon after its identification, work began on formulations and delivery systems *(7,10,42)*. In 1978, the first mating-disruption product was registered by EPA for PBW control *(10)*.

Table 1
Mating-Disruption Products Registered by USEPA, 1997

Pest	Abbreviation	Year of first registration	Registrations	Companies
Pink bollworm	PBW	1978	9	4
Codling moth	CM	1991	5	4
Tomato pinworm	TPW	1982	5	3
Oriental fruit moth	OFM	1987	3	3
Gypsy moth	GM	1983	2	2
Peach twig borer	PTwB	1995	2	2
Peach tree borer	PTB	1995	1	1
Tufted apple bud moth	TABM	1994	1	1
Grape berry moth	GBM	1990	1	1
Omnivorous leafroller	OLR	1996	1	1
Leafrollers	LR[a]	1997	1	1
Other (aphids, mites)			2	2

[a]Obliquebanded, blackheaded fireworm, pandemis.

The potential of using sex pheromones to control insect pests was first demonstrated 30 yr ago *(5)*. This and other early research *(6–9)* demonstrated that the release of large amounts of synthetic sex pheromone into the atmosphere of a crop could interfere with mate location, thereby controlling the pest by delaying or preventing mating. Despite the success demonstrated in these studies, the first commercial mating-disruption product registered in the United States for the control of an insect pest was not until 1978, when the pheromone of the pink bollworm (PBW) *Pectinophora gossypiella* (Saunders), was registered *(10)*. Currently, there are over 30 mating-disruption products registered by the US Environmental Protection Agency (EPA) for control of more than 12 pests (**Table 1**). Several factors have enhanced the development and commercial use of mating-disruption technology for the control of insects.

In the United States and elsewhere, regulations governing the registration and field application of conventional insecticides have become more restrictive. The cost and time to register these materials has increased substantially, and is viewed as a barrier to the development of new products *(11)*. Similar concerns associated with new data requirements for reregistration have made many products economically marginal to their registrants, resulting in their removal from the market. For the manufacturers of mating-disruption products, the registration process was also considered an impediment to the development of technology *(12)*. The time and the costs to register these narrow-spectrum products could not be justified by small market size.

The EPA has implemented many changes in the regulatory process to accelerate the development and registration of pheromone-based control technologies *(13,14)*. The EPA established a toxicology-based, tiered testing requirement for pheromones and other biochemical products. Longer-term toxicology studies are required if adverse results are obtained in the initial acute tests. Further, the EPA exempted from the requirement of tolerance all inert ingredients of pheromone products formulated in dispensers made of polymeric matrix materials. This exemption applied to dispensers large enough to be retrieved from the field, and enabled companies to continuously improve their formulations without the need to seek EPA approval *(14)*. In 1994, the EPA issued a general exemption from tolerance requirements for all arthropod-pheromone active ingredients, again in large, polymeric materials, and when applied at <370 g active ingredient/ha/yr. In addition, the EPA increased the amount of area that could be treated with pheromone products without an Experimental Use Permit (EUP) from 4 to 100 ha. In 1995, the EPA expanded regulatory relief to include sprayable pheromone formulations, by issuing tolerance exemptions for all lepidopteran pheromones, and increasing the acreage cutoff to 100 ha for these formulations as well. In order to better manage the registration of pheromones and other biochemical technologies, the EPA established the Biopesticide and Pollution Prevention Division. Together, these changes have provided pheromone products with a distinct advantage in the registration process, and facilitated and accelerated their introduction into the marketplace.

In 1993, the EPA published the Worker Protection Standard for Agricultural Pesticides (40 CFR, Part 170). Mandatory compliance with all the requirements became effective April 15, 1994. The new regulations were designed to protect farm workers and pesticide handlers from health hazards associated with pesticide exposure. The new regulations increased the restricted entry intervals, enhanced training, required greater notification and posting, and provided for the establishment of decontamination sites. Compliance with the new regulations has made farm worker management more difficult and expensive in agricultural crops in which conventional insecticides are routinely applied. Fortunately, the EPA exempted pheromones from these regulations. As a result, in agricultural crops in which mating-disruption technology is used alone, or in conjunction with the limited use of insecticides, the management of agricultural workers is simplified and less expensive, giving mating-disruption technology another advantage in the marketplace.

It is well over 50 yr since the discovery and commercialization of DDT. The success of DDT and other insecticides in killing insect pests was followed closely by control problems associated with insecticide resistance *(15)*. Currently, there are over 504 insect pests known to be resistant to insecticides, and the number continues to increase *(16)*. Mating disruption, with its unique mode

Table 2
Pink Bollworm Infestation and Control Cost in Parker Valley Pink Bollworm Pheromone Program

Year	Bolls inspected	Larvae found	% Infestation	Cost/ha ($)
1989	26,879	6282	23.35	—
1990	34,726	3442	9.91	17.20
1991	35,477	507	1.42	17.20
1992	30,064	261	0.86	22.00
1993	25,200	0	0.00	9.00
1994	16,109	32	0.20	11.52
1995	16,520	63	0.38	13.10
1996	45,597	1200	2.63	20.36

Source: Arizona Cotton Research and Protection Council.

munication). Higher-than-normal populations entered diapause at the end of 1995, and mild overwintering conditions contributed to larval survival. Furthermore, population pressure was aggravated in early 1996 by uncontrolled PBW infestations coming from volunteer cotton plants which had grown undetected in nearby wheat fields. As a result, population pressure was uncommonly high in 1996, and approx 10% of the cotton fields within the program experienced infestation of at least 10%, despite repeated mating disruption and insecticide applications, at a cost of more than $120/ha. However, 90% of the fields in the program were relatively clean, and the average infestation of 2.63% should not indicate a failure of mating disruption to control the pest.

Although the Parker Valley Program may be considered a successful example of mating disruption, it also illustrates the tenuous nature of area-wide programs. Although mating disruption has been biologically and economically effective, the organized program did not continue in 1997. Instead, growers reduced pheromone-protected acres and increased use of transgenic Bt-cotton. This change may be attributed to some of the problems experienced in the pheromone program during 1996, and to the relatively inexpensive and low-maintenance transgenic cotton technology. Grower cost for Bt-cotton is approx $80/ha, significantly lower than the cost of PBW control to program growers in 1996. The use of transgenic cotton more than doubled in Arizona between 1996 and 1997. It is estimated that approx 144,000 ha of cotton were planted in Arizona during 1997, and that nearly 50% of this area was planted to transgenic Bt-cotton varieties. At present, transgenic cotton appears to be very effective in controlling PBW infestations, and, compared to mating disruption, easier to implement. However, there is serious concern that widespread use of the technology will place extreme pressure on insects to develop resistance to

Bt toxin *(65)*. Furthermore, research indicates that the resistance factor is an incompletely dominant trait (A. C. Bartlett, personal communication). Unfortunately, the greater the degree of dominance, the less likely a high-dose strategy as presently employed is going to succeed (T. J. Dennehy, personal communication). Despite these concerns, the planting of trangenic cotton has reduced the use of PBW mating disruption in the desert southwest United States. Mating disruption is now limited to refugia fields, or, in a much smaller amount, to supplemental application on top of Bt-cotton fields. Termination of the Parker Valley Pheromone Project is unfortunate, because the area-wide program employed many of the criteria necessary for successful implementation of mating disruption:

1. Early initiation at pinsquare stage cotton, while populations are relatively low;
2. Intensive monitoring with pheromone traps and frequent plant inspections;
3. Use of economic thresholds;
4. Careful adherence to effective rates and uniform pheromone distribution;
5. Proper timing to avoid gaps between application;
6. Reduction of immigration of mated females; and
7. Judicious use of other control measures, including standard insecticides.

Unfortunately, these criteria are often not used in less organized programs.

There are now various methods available for management of PBW in the desert southwest United States. Mating disruption has been an effective and commercially viable method for controlling this pest for nearly 20 yr. Although recent introduction of transgenic cotton has decreased the use of mating disruption, in the future this technique could be combined with other methods, such as sterile-male releases, short-season cotton and transgenic varieties to combat infestations, and manage resistance development to Bt-toxins. PBW mating disruption is likely to increase in other countries, where transgenic cotton is not yet available and PBW remains a serious pest.

2.2. Codling Moth

2.2.1. Biology and Pest Status

CM is a key pest of pome fruits in North and South America, South Africa, Australia, and Europe. Eggs are laid on the twigs or leaves adjacent to the fruit, or directly on the fruit. Upon hatching, larvae tunnel into the fruit. Failure to control this pest can result in very high levels of crop loss, with at least 80% wormy fruit at harvest *(66)*. CM is primarily controlled throughout the world by one or more applications of broad-spectrum, primarily organophosphorous, insecticides. These compounds, if used correctly, generally provide commercially acceptable levels of control, with damage to fruit kept below 1%. Unfortunately, organophosphorous compounds are highly toxic to natural enemies

The earliest research on PBW mating disruption was conducted with hand-applied devices *(7,38)*. Commercial success coincided with development of mechanically applied formulations. These involved mixture of slow-release dispensers, such as plastic microtube fibers and laminated flakes, with stickers. The mixtures were applied by specialized equipment attached to aircraft or tractors *(10,43,44)*. These formulations had greater commercial acceptance compared to hand-applied dispensers, especially among cotton growers in the southwestern United States.

A series of studies led to the development of an attracticide formulation. Nocturnal observations of PBW moth mating behavior, in fields treated with mechanically applied formulations, demonstrated that males approach and contact the pheromone-laden dispensers. Furthermore, moth scales could be found in the stickers surrounding the dispensers *(45)*. These observations led to incorporation of small amounts of insecticide in the stickers used to adhere the pheromone dispensers to crop foliage. Control was an outcome of males contacting the sticker and absorbing a lethal dose of insecticide *(46,47)*. Butler and Las *(48)* demonstrated that applications of attracticide for PBW control did not adversely impact beneficial insects in the field. This attracticide approach is thought to be more robust than mating disruption alone, resulting in greater efficacy under higher population pressure *(49)*. Even though some researchers have not seen significant advantage of this approach over classical mating disruption *(50)*, use of attracticide has been adopted by growers, because it allows reduced rates of synthetic pheromone, as low as 100 mg active ingredient (ai)/ha/d, and lower costs. Presently, most commercial applications of PBW mating disruption in the United States use the attracticide approach.

In certain markets, hand-applied dispensers are acceptable or even preferable. These include areas where labor is readily abundant and inexpensive, where mechanical application equipment is not available, and in crops where proper dispenser placement is difficult to achieve with broadcast application equipment. For PBW and other pests, long-lived pheromone dispensers have been developed that protect labile pheromone-active ingredients, and provide effective release for extended periods of field exposure *(51–53)*. These formulations can be easily applied to plant foliage, and may provide season-long population suppression from a single application. Furthermore, they eliminate possible gaps in coverage during which mating may occur. This is a frequently observed problem with shorter-lived formulations. Season-long dispensers are loaded with higher rates of pheromone, typically 147–165 mg, and are applied at 250–500 dispensers/ha. Recent measurements, using field-portable electroantennograms of pheromone movement from high-load dispensers, indicate concentrations sufficient to affect PBW mating behavior may be transported by wind up to 100 m from the emitting source *(54)*. These observations suggest pheromone dispensers could provide effective mating disruption when placed at much more widely spaced intervals.

The major disadvantage of all types of mechanically or hand-applied formulations is the difficulty of application or the need for specialized mechanical equipment. The equipment required for mechanical applications is not available in most foreign markets, and hand-application, although possible, is often not preferred. Hand labor may be scarce or expensive, and application of sticky formulations can be undesirable, especially if insecticides are added. These drawbacks helped encourage the development of sprayable, microencapsulated formulations for PBW mating disruption *(55,56)*. Commercial microencapsulated formulations are now available for several important insect pests in the United States. For PBW, microencapsulated formulations are applied at 5.0–12.5 g/ai/ha, and may last up to 14 d per application, depending on temperature. They are most often used during periods of little rainfall. At lower rates, microencapsulated formulations are often applied concurrently with standard rates of conventional insecticides, as a bioirritant strategy designed to increase pest exposure to the insecticide *(57)*.

2.1.3. Critical Issues in Implementation: Applied Research, Extension, and Economics

The many contributions to pheromone identification and formulation development discussed in the previous section have resulted in the establishment of mating-disruption technology as an effective and economical method for PBW management *(18,31,33,58,59)*. One of the most successful demonstrations of the use of mating disruption for PBW control has been conducted by the Arizona Cotton Research and Protection Council (ACRPC). During 1990–1996, ACRPC conducted a pheromone-based control program in cotton planted in the Parker Valley along the Colorado River *(60)*. Acreage ranged between 9575 ha and 11,430 ha. PBW control consisted of mating disruption or attracticide, and the judicious application of conventional insecticides. Control measures were applied based on intensive monitoring using pheromone-baited traps (1 trap/4 ha) and plant inspection. In 1989, in the year prior to the program, average boll infestation in the valley was more than 23% (**Table 2**). After the first year of the program, PBW-infested bolls were reduced by more than 50%. By the fourth year, 1993, no larvae were detected in more than 25,000 bolls inspected, at a cost to the grower of only $55.60/ha. PBW infestation increased slightly in 1994 and 1995, then in 1996, boll infestation increased substantially, to 2.63%. This caused concern among growers and program organizers.

Success of PBW mating disruption is inversely density-dependent. Many studies illustrate the importance of early application while populations are still low *(7,61–64)*. The decline in efficacy in the Parker Valley Project might be attributed to high population densities during 1996 (L. Antilla, personal com-

included hollow fiber tapes *(93)*, rubber tubing *(94)*, laminate flakes *(94)*, and polyethylene-tube dispensers *(95)*. Since 1991, the EPA has registered four dispensing systems for the control of CM, including polyethylene tubes, bag-type membranes, plastic coils, and laminate flakes. No broadcast formulations have been registered. Results from early research trials were often poor, and probably related to formulation and delivery problems *(79)*. Formulations and delivery systems have improved, but control problems still occur with currently registered commercial formulations. If codlemone is the sole component of CM pheromone, then control problems related to formulation and delivery system are probably caused by photochemical degradation *(96,97)* and/or longevity of release from the dispenser *(97)*.

The impact of photochemical degradation on dispenser performance was investigated for the polyethylene tube formulation. McDonough et al. *(96)* reported that as much as 61% of the pheromone was lost through photochemically induced degradation, and only 39% through evaporation. Codlemone is a conjugated diene alcohol, and, like other similar chemicals, is prone to photochemical degradation via exposure to heat, light, and oxygen *(98,99)*. Degradation occurs via isomerization of the double bond *(100)*, and oxidation to peroxides and furans *(97,99)*. The amount of codlemone degradation in polyethylene-tube dispensers, first used commercially, effectively decreased the longevity and necessitated two applications of dispensers per season *(96)*. Unfortunately, the cost of the dispensers precluded a second application *(22)*, and many attempted to get season-long control with a single application of dispensers. This approach often resulted in control problems late in the season. In response, new formulations of the polyethylene-tube dispenser have been developed, with substantially less photodegradation. The effective field life has increased by close to 50%, from 75–90 d to 120–140 d, depending on temperature (D. Thomson, personal communication). A field life of 140 d can provide season-long control in more temperate pome fruit-growing regions. Overall, the improved performance of polyethylene-tube dispensers has improved the efficacy of CM-mating disruption later in the season, resulting in a better rate of success, leading to enhanced adoption by growers.

A related problem with current delivery systems has been inadequate field life, because of factors other than chemical degradation. Field life is a function of the pheromone load and its rate of release from the dispenser. The loading rate is easily controlled in the lab; however, release rate in the field (assuming no photodegradation) is complex, and is affected by the physical and chemical characteristics of the dispenser, and environmental factors, such as temperature and wind. Ideally, dispensers should show a flat release rate at a constant temperature over the expected field life. However, release rates change strongly, relative to temperature, and weakly, relative to wind velocity (M. Suckling, personal communication). Thus, dispensers are likely to have shorter field lives in areas with high temperatures, or when conditions in a cooler region are unusually warm. This variation in release rates caused by temperature difference makes it difficult to determine the effective field life of a dispenser. Many commercial formulations have shown dramatic decreases in release rate following field exposure *(101)*. The result has been inadequate amounts of pheromone released into the orchard later in the season. However, some manufacturers have improved their products, resulting in substantial improvements in performance *(101)*.

Currently available commercial formulations employ either a single- or multiple-application strategy. These strategies are designed to ensure the adequate release of pheromone during the mating period of up to three CM generations. Control problems have occurred when dispensers have run out of pheromone earlier than expected, leaving gaps when there is no pheromone dispensed. Dispenser manufacturers must carefully determine the expected field life of their products, to ensure proper use and performance in the field.

2.2.3. Critical Issues in Implementation: Applied Research, Education, and Economics

We believe CM mating disruption has succeeded because the research, extension, and agricultural communities have tried to integrate pheromones into pest management programs, rather than simply adopt another technology for CM control. By implementing a pheromone-based pest management strategy, many of the limitations to the efficacy and acceptance of pheromones imposed by the orchard-cropping system have been mitigated. These include highly variable environmental conditions, a low tolerance for fruit damage, a complex CM mating system, and a diverse arthropod community.

Early on, it was recognized that the best opportunities for CM control were in orchards where physical characteristics and environmental conditions, including topography, size and shape, canopy structure, and wind allowed for uniform distribution of pheromone. Relatively flat and even canopied sites have served as the primary candidates for CM mating disruption; sites with steep slopes or large numbers of missing trees have generally been avoided.

The borders of disrupted orchards have been especially vulnerable to CM *(20,78)*. Two processes have contributed to the development of border infestations. Mated females immigrate from adjacent orchards that are not treated with pheromone. In addition, it is suspected that pheromone concentrations are lower on the borders than the interior, thus increasing the likelihood of males locating females and mating on the borders. Growers have judiciously protected the orchard perimeter by treating with insecticides, a 2× rate of pheromone, or a combination of the two. Border problems can also be remedied by

of most pests, and are a major factor limiting the success of biological control in pome fruit. CM resistance to organophosphate insecticides has been detected in parts of the western United States *(67,68)*, and in South Africa (M. Addison, personal communication). Up to 12 sprays per season are common in South Africa, and, despite this intensive program, some orchards suffer at least 30% fruit injury at harvest *(29)*. Increasing difficulty in controlling this pest has certainly provided a sense of urgency in research efforts to develop new CM-control technologies.

In North America, considerable research has been directed toward the development of more selective controls for CM, including the insect-growth regulators (IGRs; *69–71*). In Europe, IGRs have been used commercially for years. They are generally considered to be nontoxic to natural enemies, and have become an important component of apple pest management programs *(28)*. However, Riedl and Zelger *(72)* have reported high levels of resistance in some regions to one class of growth-regulating compounds: the chitin synthesis inhibitors. In addition to the potential for resistance, field trials conducted in North America and Europe have indicated that IGRs are not as efficacious as organophosphates in controlling CM (J. Brunner, personal communication). Therefore, it is unlikely that IGRs will be an effective and reliable stand-alone replacement for organophosphates. Other tactics tested in North America, including CM granulosis virus *(73)* and mass release of sterile males *(74,75)*, have been demonstrated to be relatively soft on beneficial insects and mites. However, these have either been too expensive, remain unregistered, or have not been effective enough to obtain widespread commercial acceptance in the United States.

The use of sex pheromones for mating disruption has long shown promise as a control for CM *(76–79)*, but only recently has it become widely adopted *(27,28,80,81)*. The total area of pome fruit production around the world treated with various mating-disruption technologies has grown from an estimated 1500 ha in 1991 to at least 24,000 ha in 1997. The results have generally been good. However, control problems still occur that may be related to issues in pheromone identification, formulation, and delivery system. Control problems have also been attributed to environmental conditions, high population densities, immigration, or improper use, and extensive research and education is still required to improve the level of control and enhance use of the technology.

2.2.2. Critical Issues in Identification, Formulation, and Delivery System

Lab and field research on mating disruption by industry and university scientists in Australia, Canada, Europe, Japan, and the United States over the past 20 yr has provided the foundation for the commercial development of pheromone technology for the control of CM *(79)*. Roelofs et al. *(82)* identified (E,E)-8,10-

dodecadien-1-ol (codlemone) as the major component of CM sex pheromone. Evidence for secondary components in the CM pheromone was reported 10 yr later by Bartell and Bellas *(83)*. Subsequent research supported this finding, with dodecanol-1 and tetradecanol-1 identified as the important secondary components *(84–86)*. Rothchild et al. *(87)* conducted research on a blend of codlemone, dodecanol, and tetradecanol for mating disruption of CM, and subsequently patented its use. On the basis of this new information, a major manufacturer incorporated the three components, codlemone, dodecanol, and tetradecanol, in their formulation. The identification of secondary compounds, and their subsequent use in a commercial formulation, was important, because many of the early trials employing dispensers loaded only with codlemone had demonstrated inconsistent and often poor results *(79)*. It was a widely held assumption that mating disruption worked best when the complete blend was used *(88)*. However, many products appeared on the market in both Europe and the United States with only codlemone in the formulation.

To clarify the importance of secondary components, McDonough et al. *(89)* looked at the behavioral responses of CM males in a wind tunnel to sources emitting codlemone, or a mixture of codlemone, dodecanol, and tetradecanol, and were unable to show differences. In small orchard plots, McDonough and colleagues further demonstrated that inhibition of male attraction to virgin-female-baited pheromone traps was the same whether traps were in an environment containing elevated levels of codlemone alone, or codlemone plus dodecanol and tetradecanol *(90)*. McDonough et al. *(91)* concluded that male CMs were sensitive only to the major component, E,E8-10-dodecadien-1-ol. He suggested that control problems often seen in pheromone-treated orchards were probably related to the photodegradation of the pheromone in the dispenser, and not the result of an incomplete blend.

The weight of the scientific evidence indicates that dodecanol and tetradecanol are not of critical importance to the efficacy of CM mating disruption. However, their exact role remains unclear. There is still an active search for additional components in the CM pheromone (G. Gries, personal communication). If additional components are eventually identified, perhaps their inclusion in commercial formulations will enhance control of CM. McDonough et al. *(91)* reported that, in small plots, dispensers loaded with an equilibrium blend of codlemone and its geometric isomers resulted in a higher level of disorientation of males to virgin-female traps than dispensers loaded with high-purity codlemone. These findings have not yet been thoroughly tested in the field to determine their importance to the efficacy of CM mating disruption.

CM pheromone has been formulated and tested for efficacy in both broadcast and retrievable dispensers. Sprayable formulations have included microcapsules *(92)* and chopped hollow fibers *(77)*. Retrievable formulations have

Brunner *(20)* determined that the effective use of this high-load trapping system required replacing lures every 3 wk during the first CM generation, and every 2 wk during subsequent generations. CM pheromone traps used in conventionally managed orchards have typically been placed at midcanopy or lower, because this position has resulted in good moth capture, and they are easier to maintain than traps placed higher in the canopy. However, when high-load lure-baited pheromone traps have been used in mating-disrupted orchards, they have been more sensitive when placed in the upper part of the canopy *(106)*. Trapping programs have been most effective when they are used in conjunction with visual inspection of fruit for CM damage. Routine examinations of fruit in the upper canopy, along orchard borders, in susceptible varieties, on the tops of slopes, near prop or bin piles, and near fruit-packing operations has proved invaluable for early detection of CM fruit damage when a disruption program is failing.

The most important impediment to the commercial acceptance of pheromone-based integrated pest management (IPM) is the higher costs relative to insecticide-based pest management. Thirty-nine people employed in the field of insect pest management in Washington and California were surveyed for their opinions on the cost of CM mating-disruption technology relative to the cost of conventional insecticides *(107)*. Fifty-one percent stated that they found the cost of mating-disruption technology to be high compared to conventional insecticides; 31 and 8% indicated that they found the cost to be very high and extremely high, respectively. Only 8% of the respondents indicated that the cost of CM mating disruption was reasonable. In small plot trials conducted in pear orchards over a 6-yr period in California, the average cost of a pheromone-based IPM program was 40% higher than a standard insecticide program *(108)*. Two applications of mating disruption dispensers are required to control CM in California, thereby substantially increasing costs. A study conducted in Washington State found that the cost of a pheromone-based IPM program, when adjusted for material, labor, and machinery costs, was $133/ha higher than a conventional insecticide program *(22)*. The disparity in these costs provides a strong disincentive to adopt a pheromone-based IPM system approach.

Orchard systems are highly complex, with several hundred arthropod species having the potential to reach pest status *(109,110)*. The successful deployment of CM mating disruption, and the subsequent reduction or elimination of insecticides to control CM, often creates orchard environments where some of these species can rapidly increase to economically damaging levels *(20)*. In 1993, a 485-ha apple orchard in central Washington State was successfully treated in its entirety with tube-type dispensers. Supplemental insecticides were only applied to border areas. Forty-eight sex pheromone traps captured over 6000 obliquebanded leafroller, *Choristoneura rosaceana*, males. In 1994, the orchard was again treated with tube-type dispensers and, again, supplemental insecticides were only applied to local border areas. Trap captures of obliquebanded leafroller increased fourfold, to over 24,000. Increased applications of insecticides were required to prevent serious economic injury to the fruit. This example clearly illustrates how the successful use of CM mating disruption can increase the control problems of certain secondary pests, and thus provide a disincentive to the adoption of the technology. The costs associated with the increased use of insecticides for leafroller control, or the economic losses because of fruit injury can outweigh the benefits and savings derived from the biological control of other secondary pests. The aforementioned impediments to the adoption of a pheromone-based IPM systems approach present serious obstacles to the commercial success of mating-disruption technology for CM. Yet, despite these obstacles, CM mating disruption is increasingly being adopted by apple and pear growers around the world, because of a combination of good results and the increased difficulty of using broad-spectrum insecticides for the control of CM.

Using CM mating disruption in a large, contiguous area is considered a better strategy than in small, individual orchards. The initiation of the USDA-sponsored CAMP has enhanced the status of mating-disruption technology as viable alternative to conventional insecticides, especially when pheromones are applied in an area-wide approach. The objective of the program is to reduce the use of organophosphate insecticides to control CM by 80% over a 5-yr period *(111)*. The CAMP adopted an IPM approach utilizing mating-disruption technology, judicious and timely applications of insecticides, biological control, and sanitation. In 1995, five project sites were selected in California, Oregon, and Washington. The size of each site and number of growers involved varied between locations. The results have been quite promising during the first 2 yr of the project, with the number of insecticides applied for CM control and fruit injury at harvest declining substantially. For example, at the Howard Flat CAMP site in north central Washington, cullage because of CM fruit injury averaged 0.8% in the year prior to the project, and dropped to 0.01% after 3 yr of area-wide mating disruption. Seventy percent of blocks showed no detectable damage. Organophosphate sprays targeted against CM at Howard Flat have decreased from an average of 2.8 to 0.8 for the season. The number of CAMP sites, and their size, has grown considerably, from five sites, totaling 1300 ha in 1995, to 10 sites, totaling 3970 ha in 1997. It is hoped that the success of the area-wide approach will encourage growers to work cooperatively, ensuring the application of mating-disruption technology over wide areas to improve efficacy.

3. Future Needs and Trends

Since the first isolation and identification of an insect pheromone more than 35 yr ago, pheromones have been used successfully to control several impor-

the area-wide application of mating-disruption technology. Recently, an area-wide approach to control CM with mating disruption and other suitable technologies was initiated in western North America. In 1995, the USDA, in association with cooperating universities in California, Oregon, and Washington, initiated the Codling Moth Area-wide Management Program (CAMP). The excellent results achieved at all CAMP sites has increased awareness about the benefits of the area-wide application of mating-disruption technology.

The CM mating system has posed some special challenges to achieving control with mating-disruption technology. Adults mate shortly after emergence in the spring, and mating activity is concentrated in the upper-third of the canopy *(102)*. Thus, both the timing of application and the positioning of dispensers within the canopy can dramatically affect the efficacy of mating disruption. For example, Weissling and Knight *(103)* demonstrated that significant levels of mating occurred in the upper-half of the tree, when dispensers were placed at a mid-canopy height of 1.8 m. However, little or no mating occurred in the tree when dispensers were placed high in the canopy, at 3.6 m (trees were about 4.2 m tall). The best control of CM with mating disruption has been achieved when dispensers are placed high in the orchard canopy (0.6 m from the top of the tree). In the absence of an education or training program, dispensers have frequently been placed too low in the canopy (approx 1.8 m). Commercially, it is the experience of the authors and others *(28,29)* that low dispenser placement has resulted in many CM control problems.

Improvements in methods for applying CM disruption products has greatly facilitated the use of this technology. In orchards with canopy heights >3 m, proper placement of dispensers during the first few years of commercial use could not be accomplished from the ground. Applying dispensers with the assistance of ladders was time-consuming (up to 12.5 h/ha), and added considerably to the already high cost of control. Superior Ag (Yakima, WA) recently introduced a very good method for applying tube and laminate dispenser types, using a combination of a telescoping pole and a clip to which the dispenser is attached. Some manufacturers have engineered their dispensers with a clip already attached. Application entails pushing a clip holding a dispenser onto a selected branch and leaving it there when the pole is twisted and pulled away. It takes <5 h to treat 1 ha of apples with this technique.

The population density of CM prior to treatment has been cited as a key factor determining the efficacy of mating disruption *(77,80,95)*. Commercially acceptable levels of control, in which pheromones are used without supplemental insecticides, have consistently been achieved only when initial CM densities are very low. The strong interaction between density and efficacy has encouraged some critical modifications in use patterns for this tactic. Gut et al. *(20)* and Thomson *(27)* reported that, under conditions of low pest pressure, a tube-type dispenser, applied at rates of 1000 dispensers/ha or 500 dispensers/ha, provided the same level of control. Growers have adopted the cost-saving strategy of lowering application rates if CM densities are known to be low. Moderate or high initial pest densities has resulted in serious control failures *(20,27–29)*.

High population densities occur when and where the control of CM with conventional insecticides has not been effective because of resistance or poor application techniques. Concerns about using mating-disruption technology under conditions of high pest pressure have been mitigated by adopting a broad-based pest-management program. Many organic growers have implemented a program that includes pheromone, botanical insecticides, mineral oil, and sanitation. An area-wide project initiated in 1993 has used a combination of mating-disruption technology and the judicious use of insecticides to combat CM resistance to azinphosmethyl in northern California pear orchards *(104)*. Mating-disruption technology has been supplemented with insecticides only when extensive monitoring indicates it is necessary. As a result, the use of organophosphate insecticides decreased dramatically. The impact of reduced exposure to azinphos-methyl to resistance is still being examined. A similar approach has been the normal practice in Washington apple orchards, particularly during the first year of a mating-disruption program. Essentially, early-season sprays, in conjunction with mating-disruption technology, are used to ensure that CM densities are low and, thus, controllable. Even orchards with a history of poor control with insecticides and, therefore, high populations, have become primary targets for pheromone-based management. Evidence shows that a combination of mating-disruption technology and a few insecticide treatments is more efficacious than a conventional program of season-long application of insecticides. Over the past 2 yr, many orchards with known high populations of CM have applied tube-type dispensers at 500 dispensers/ha instead of the label rate of 1000 dispensers/ha and then supplemented with 1–3 applications of insecticides. To date, the results have been excellent.

For the past 20 yr, monitoring CM with pheromone traps has been a standard management practice in orchards throughout the world. Trapping systems can be used to determine when to apply insecticides, and whether population densities are high enough to warrant treatment *(105)*. Monitoring CM adults has proved to be more difficult in orchards treated with mating-disruption products. Failure to capture moths in a pheromone trap baited with a standard lure containing 1 mg of codlemone has been found to be an unreliable indicator of successful disruption *(20)*. Charmillot *(78)* showed that the sensitivity of CM pheromone traps in disrupted orchards could be increased by using lures containing higher amounts of codlemone. Further research in the western United States has led to the adoption of a 10-mg red septa-baited pheromone trap as an important component of pheromone-based CM control programs. Gut and

sales of mating disruption products do not provide the same financial returns on investment as conventional insecticides, and may explain, in part, why so few large agrochemical companies have developed mating-disruption technology, and why many suppliers of agricultural chemicals have not actively promoted the use of mating-disruption technology. Pheromones have been referred to as the chemical equivalent of parasites and predators. Like beneficial organisms, pheromones are target-specific, exploit certain aspects of insect behavior, and often result in reduced use of insecticides. In general, they have been developed by small companies, or by industries not normally associated with agricultural chemicals. Ironically, a major impediment to the acceptance of pheromones may be their successful use in IPM programs.

In summary, the adoption by growers of pheromone-based IPM programs will depend on how well the systems can meet grower concerns about efficacy and cost. A better understanding of how and why the technology works will enable the design of more cost-effective technology. The integration of mating-disruption technology into reliable and predictable pest-management programs will enhance adoption. Therefore, the development of monitoring and sampling techniques, in conjunction with economic thresholds, will be essential to accurately assess the biological relationships between key and secondary insects and their natural enemies, to ensure cost-effective control of all pests in the system.

Acknowledgments

We thank the Arizona Cotton Research and Protection Council for permission to use their data from the Parker Valley Program. Thanks also to Tim Dennehy, Robert Staten, Larry Antilla, Alan Bartlett, and Hasan Bolkan for providing information and making suggestions.

References

1. Butenandt, A., Beckman, R., Stamm, D., and Hecker, E. (1959) Uber den Sexuallockstoff des Seidenspinner *Bombyx mori*, Reidarstellung und Konstitution. *Zeitschrift Naturforschung B.* **14,** 283,284.
2. Tamaki, Y. (1988) Pheromones of the lepidoptera, in *CRC Handbook of Natural Pesticides, vol. 4: Pheromones* (Morgan, E. D. and Mandava, N. B., eds.), CRC, Boca Raton, FL, pp. 35–93.
3. Arn, H., Toth, M., and Priesner, E., eds. (1992) *List of Sex Pheromones of Lepidoptera and Related Attractants*, 2nd ed., International Organization for Biological Control, Montfavet, France.
4. Mayer, M. S. and McLaughlin, J. R., eds. (1991) *Handbook of Insect Pheromones and Attractants*, CRC, Boca Raton, FL.
5. Gaston, L. K., Shorey, H. H., and Sario, S. A. (1967) Insect population control by the use of sex pheromones to inhibit orientation between the sexes. *Nature* **213,** 1155.

6. Mitchell, E. R., Copeland, W. W., Sparks, A. N., and Sekul, A. A. (1974) Fall armyworm: disruption of pheromone communication with synthetic acetates. *Environ. Entomol.* **3,** 778–780.
7. Shorey, H. H., Kaae, R. S., and Gaston, L. K. (1974) Sex pheromones of Lepidoptera. Development of a method for pheromonal control of *Pectinophora gossypiella* in cotton. *J. Econ. Entomol.* **67,** 347.
8. Taschenberg, E. F., Cardé, R. T., and Roelofs, W. L. (1974) Sex pheromone mass trapping and mating disruption for control of redbanded leafroller and grape berry moths in vineyards. *Environ. Entomol.* **3,** 239–242.
9. Rothchild, G. H. L. (1975) Control of oriental fruit moth (*Cydia molesta* [Busck] [Lepidoptera, Tortricidae]) with synthetic female pheromone. *Bull. Ent. Res.* **65,** 473–490.
10. Brooks, T. W., Doane, C. C., and Staten, R. T. (1979) Experience with the first commercial pheromone communication disruptive for suppression of an agricultural pest, in *Chemical Ecology: Odour Communication in Animal* (Ritter, F. J., ed.), Elsevier/North Holland, Amsterdam, pp. 375–388.
11. Kirsch, P. K. (1988) Pheromones: their potential role in control of agricultural pests. *Am. J. Alternative Agriculture* **3,** 83–97.
12. Jellinek, S. D. and Gray, E. C. (1992) Opportunities for expediting the U.S. regulatory process for pheromones and other semiochemicals, in *Insect Pheromones and Other Behaviour-Modifying Chemicals* (Ridgeway, R. L., Inscoe, M., and Arn, H., eds.), BCPC Monograph 51, Surrey, UK, pp. 110–122.
13. Hutton, P. O. (1993) New regulatory strategies for pheromones: EPA policies, interpretations and rules, in *Pest Management: Biologically Based Technologies* (Lumsden, R. D. and Vaughn, J. L., eds.), American Chemical Society, Washington, DC, pp. 388–390.
14. Kirsch, P. K. (1997) Amazing daze: confusion brings change, in *Technology Transfer in Mating Disruption* (Witzgall, P. and Arn, H., eds.), IOBC wprs Bulletin vol. 20 (1), Montfavet, France, pp. 249–256.
15. Georghiou, G. P. (1994) Principles of insecticide resistance management. *Phytoprotection* **75,** 51–59.
16. Georghiou, G. P. and Lagunes-Tejeda, A. (1991) The occurrence of resistance to pesticides in arthropods. U.N. Food and Agriculture Organization, Rome, AGPP/MISC/91-1, p. 318.
17. Haynes, K. F. and Baker, T. C. (1988) Potential for evolution of resistance to pheromones. *J. Chem. Ecol.* **14,** 1547–1560.
18. Cardé, R. T. and Minks, A. K. (1995) Control of moth pests by mating disruption: successes and constraints. *Ann. Rev. Entomol.* **40,** 559–585.
19. Van Steenwyk, R. A., Toscano, N. C., Ballmer, G. R., Kido, K., and Reynolds, H. T. (1975) Increase of *Heliothis* spp. in cotton under various insecticide treatment regimes. *Environ. Entomol.* **4,** 993–996.
20. Gut, L. J. and Brunner, J. F. (1994) Pheromone-mediated control of codling moth in Washington apple orchards. *Good Fruit Grower* **45,** 35–48.
21. Trumble, J. T. and Alvarado-Rodriguez, B. (1993) Development and economic evaluation of an IPM program for fresh market tomato production in Mexico. *Agric. Ecosyst. Environ.* **43,** 267–284.

tant pest species. However, their use has not advanced as far as many have hoped or predicted. The reasons for this are varied and often debated.

There has been considerable progress over the past decade in pheromone isolation and identification, and formulations and delivery systems. Accurate identification, increased stability, and longevity, in addition to more uniform release rates following field exposure, have enhanced the performance of dispensers. However, the mechanisms of how mating disruption controls insects is not well understood in many insect/cropping systems, even where the technology is used successfully. Therefore, the amount of pheromone needed per hectare, and the best method to deliver it, are seldom accurately known. As a result, it is difficult to design and commercialize cost-effective technology, and most end users consider mating-disruption technology to be both risky and expensive.

The development of the portable electroantennogram *(112)* has enabled instantaneous measurement of pheromone concentrations within crop canopies *(113,114)*. Previous technology was only able to measure time-averaged concentrations *(115)*, which did not take into account the instantaneous fluctuations in concentrations or plume structures relative to changes in temperature or wind speed. However, the new sampling apparatus is complex, expensive, and difficult to use, restricting the number of samples that can be taken at a point in time. With limited samples, the dynamics of pheromone movement, relative to the physical structure of the canopy and environmental conditions, is difficult to elucidate. Advances in this area, in conjunction with a better understanding of how pheromone concentrations within the canopy impact the flight and mating behavior of the target pest, should enhance understanding of how the mating-disruption technique works. Armed with this information, more efficacious technology should be designed and delivered it to the end user in a more economical manner.

The effectiveness of mating-disruption technologies has largely been determined by the biological limitations imposed by the pest and cropping system. It is apparent that pests vary in their susceptibility to pheromone-mediated control. Some species, such as PBW, tomato pinworm *(116)*, and peach tree borer *(117)*, seem to be highly sensitive to their pheromones, and control can be achieved even under relatively high population density. Other species, such as CM, appear to be moderately responsive to synthetic pheromone and more difficult to control by mating disruption. In this group of species, there seems to be a strong interaction between population density and efficacy. Mating disruption often can only be used successfully over the long term if periodically combined with one or more insecticide treatments.

Currently, commercial disruption products are most cost-effective in situations in which conventional insecticides are not performing well, or their availability is restricted. In these cases, the high cost of using mating disruption can be justified. On many farms, however, pests are easily controlled with one or two applications of fairly inexpensive insecticides. These farms are less likely to switch to a more expensive control program until these insecticides are lost, or the indirect benefits of using mating-disruption technology can be shown to outweigh the increase in costs.

High costs, difficulty of application, and the need to control several pest species have limited the acceptance of hand-applied dispensers. In response, sprayable formulations are now being tested or used commercially for a number of insect pests, including obliquebanded leafroller (J. Brunner, personal communication), oriental fruit moth, *Cydia molesta* (L. Gut, personal communication), and PBW *(55,56)*. Recently, new dispenser technology termed "puffers" *(118)* or "metered semiochemical timed release system (MSTRS)" *(119)* has been tested for the control of a number of insects. These devices are applied at very low rates of 2–5 dispensers/ha. If efficacious, these new technologies could greatly reduce the costs of application, and thereby enhance adoption of mating-disruption technology.

The use of mating-disruption technology for control of key pests must be compatible with management strategies for the rest of the pest complex. Frequently, the use of mating-disruption technology, and the subsequent reduction in insecticides for a primary pest, results in outbreaks and necessitates control of secondary pests. Growers are keenly aware of the potential added costs. The pheromone industry must respond with technology that is capable of disrupting several species. In addition, other control tactics must be developed that are selective and compatible with mating disruption.

Efforts to develop and commercialize mating-disruption products have largely focused on pests with a high market potential. This is the same strategy that has effectively led to the development of most conventional insecticides. However, it may not be the best scenario for the pheromone industry. The chemical industry has largely been able to provide controls for minor pests by spinning off broad-spectrum chemistries that were primarily developed for control of economically important pests in major crops. Generally, mating-disruption products control only a single pest species, thus precluding the spin-off approach. The uniqueness of mating-disruption technologies is that the release device is of paramount importance. Once an effective device is developed, it potentially can be used to control any target pest that is amenable to disruption. Manufacturers of disruption products should consider developing and providing products for both major and minor pest species. This approach would be similar to one taken by another segment of the pheromone industry—manufacturers of lures for traps, which provide many products based on only a few release devices.

The implementation of pheromone-based pest-management programs is often accompanied by reductions in the sales of conventional insecticides. The

52. Staten, R. T., Flint, H. M., Weddle, R. C., Hernandez, E., and Yamamoto, A. (1987) Pink bollworm (Lepidoptera: Gelechiidae): large-scale field trials with a high-rate gossyplure formulation. *J. Econ. Entomol.* **80,** 1267–1271.
53. Jenkins, J. W., Antilla, L., and Miller, E. (1995) Mating disruption of the pink bollworm with three commercial formulations. *Proceedings, Beltwide Cotton Conferences*, vol. 2, San Antonio, TX, 4–7 January, pp. 989,990.
54. Färbert, P., Koch, U. T., Färbert, A., Staten, R. T., and Cardé, R. T. (1997) Pheromone concentrations measured with EAG in cotton fields treated for mating disruption of *Pectinophora gossypiella* (Lepidoptera: Gelechiidae). *Environ. Entomol*, **26,** 1105–1116.
55. Flint, H. M. and Merkle, J. R. (1984) Studies on disruption of sexual communication in the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), with microencapsulated gossyplure or its component ZZ-isomer. *Bull. Entomol. Res.* **74,** 25–32.
56. Hall, D. R. and Marrs, G. J. (1989) Microcapsules, in *Insect Pheromones in Plant Protection* (Jutsum, A. R. and Gordon, R. F. S., eds.), Wiley, Chichester, UK, pp. 199–248.
57. Weatherston, I. (1990) Principles of design of controlled-release formulations, in *Behavior-Modifying Chemicals for Insect Management: Applications of Pheromones and Other Attractants* (Ridgeway, R. L., Silverstein, R. M., and Inscoe, M. N., eds.), Marcel Dekker, New York, pp. 93–112.
58. Baker, T. C., Staten, R. T., and Flint, H. M. (1990) Use of pink bollworm pheromone in the southwestern United States, in *Behavior-Modifying Chemicals for Insect Management: Applications of Pheromones and Other Attractants* (Ridgeway, R. L., Silverstein, R. M., and Inscoe, M. N., eds.), Marcel Dekker, New York, pp. 417–436.
59. Campion, D. G., Critchley, B. R., and McVeigh, L. J. (1989) Mating disruption, in *Insect Pheromones in Plant Protection* (Jutsum, A. R. and Gordon, R. F. S., eds.), Wiley, Chichester, UK, pp. 89–119.
60. Antilla, L., Whitlow, M., Staten, R. T., El Lissy, O., and Myers, F. (1996) An integrated approach to areawide pink bollworm management in Arizona, in *Proceedings, Beltwide Cotton Conferences*, Nashville, TN, pp. 1083–1085.
61. Doane, C. C., and Brooks, T. W. (1981) Research and development of pheromones for insect control with emphasis on the pink bollworm, in *Management of Insect Pests with Semiochemicals* (Mitchell, E. R., ed.), Plenum, New York, pp. 285–303.
62. Critchley, B. R., Campion, D. G., McVeigh, L. J., Hunter-Jones, P., Hall, D. R., Cork, A., et al. (1983) Control of pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), in Egypt by mating disruption using an aerially applied microencapsulated pheromone formulation. *Bull. Ent. Res.* **73,** 289–299.
63. Stone, N. D., Gutierrez, A. P., Getz, W. M., and Norgaard, R. (1986) Pink bollworm control in southwestern desert cotton. III. Strategies for control: an economic simulation study. *Hilgardia* **54,** 42–56.

64. Staten, R. T., Miller, E., Grunnet, M., and Andress, E. (1988) The use of pheromones for pink bollworm management in cotton, in *Proceedings, Beltwide Cotton Production Research Conference*, New Orleans, LA, pp. 206–209.
65. Tabashnik, B. E. (1997) Seeking the root of insect resistance to transgenic plants. *Proc. Natl. Acad. Sci. USA* **94,** 3488–3490.
66. Myburgh, A. C. (1980) Infestation potential of the codling moth. *Deciduous Fruit Grower* **10,** 368–377.
67. Varela, L. G., Welter, S. C., Jones, V. P., Brunner, J. F., and Riedl, H. (1993) Monitoring and characterization of insecticide resistance in codling moth (Lepidoptera: Tortricidae) in four western states. *J. Econ. Entomol.* **86,** 1–10.
68. Knight, A. K., Brunner, J. F., and Alston, D. (1994) Survey of azinphosmethyl resistance in codling moth in Washington and Utah. *J. Econ. Entomol.* **87,** 285–292.
69. Burts, E. C. (1983) Effectiveness of a soft-pesticide program on pear pests. *J. Econ. Entomol.* **76,** 936–941.
70. Westigard, P. H. (1979) Codling moth: Control on pears with diflubenzuron and effects on non-target pest and beneficial species. *J. Econ. Entomol.* **72,** 552–554.
71. Westigard, P. H., Gut, L. J., and Liss, W. J. (1986) Selective control program for the pear pest complex in southern Oregon. *J. Econ. Entomol.* **79,** 250–257.
72. Riedl, H. and Zelger, R. (1994) Erste Ergebnisse der Untersuchungen zur Resistenz des Apfelwicklers gegenüber Diflubenzuron. *Obstbau Weinbau* **31,** 107–109.
73. Westigard, P. H. and Hoyt, S. C. (1988) Codling moth (Lepidoptera: Olethreutidae): Evaluation of the granulosis virus for control in Pacific Northwest apple and pear orchards. *Melanderia* **46,** 14–18.
74. Proverbs, M. D., Newton, J. R., and Logan, D. M. (1966) Orchard assessment of the sterile male technique for control of codling moth, *Carpocapsa pomonella* (L.) (Lepidoptera: Olethreutidae). *Can. Entomol.* **98,** 90–95.
75. Dyck, V. A., Graham, S. H., and Bloem, K. (1992) Implementation of the sterile insect release program to eradicate the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), in *British Columbia Symposium: Management of Insect Pests: Nuclear and Related Molecular and Genetic Techniques.* International Atomic Energy Agency, Vienna, p. 23.
76. Rothschild, G. H. L. (1982) Suppression of mating in codling moths with synthetic sex pheromone and other compounds, in *Insect Suppression with Controlled Release Pheromone Systems*, vol. 2 (Kydonieus, A. F. and Beroza, M., eds.), CRC, Boca Raton, FL, pp. 117–134.
77. Moffitt, H. R. and Westigard, P. H. (1984) Suppression of the codling moth (Lepidoptera: Tortricidae) population on pear in southern Oregon through mating disruption with sex pheromone. *J. Econ. Entomol.* **77,** 1513–1519.
78. Charmillot, P. J. (1990) Mating disruption technique to control codling moth in western Switzerland, in *Behavior-Modifying Chemicals for Pest Management* (Ridgeway, R. L., Silverstein, R. M., and Inscoe, M. N., eds.), Marcel Dekker, New York, pp. 165–182.
79. Vickers, R. A. and Rothchild, G. H. L. (1991) Use of sex pheromones for control of codling moth, in *World Crop Pests, vol. 5, Tortricid Moths: Their Biology,*

22. Williamson, E. R., Folwell, R. J., Knight, A. L., and Howell, J. F. (1996) Economics of employing pheromones for mating disruption of the codling moth, *Carpocapsa pomonella*. *Crop Protection* **15,** 473–477.
23. Bartell, R. J. (1982) Mechanisms of communication disruption by pheromone in the control of Lepidoptera: a review. *Physiol. Entomol.* **7,** 353–364.
24. Cardé, R. T. and Minks, A. K. (1997) *Insect Pheromone Research. New Directions*. Chapman and Hall, New York.
25. Jutsum, A. R. and Gordon, R. F. S. (1989) *Insect Pheromones in Plant Protection*. Wiley, Chichester, UK.
26. Wyatt, T. (1997) Putting pheromones to work: paths forward for direct control, in *Insect Pheromone Research: New Directions* (Cardé, R. T. and Minks, A. K., eds.), Chapman and Hall, New York, pp. 445–459.
27. Thomson, D. (1997) Confusion amongst codling moth fellows continues: a commercial perspective on the implementation of codling moth mating disruption in North America, in *Technology Transfer in Mating Disruption* (Witzgall, P. and Arn, H., eds.), IOBC wprs Bulletin, vol. 20 (1), Montfavet, France, pp. 57–63.
28. Waldner, W. (1997) Three years of large-scale control of codling moth by mating disruption in the South Tyrol, Italy, in *Technology Transfer in Mating Disruption* (Witzgall, P. and Arn, H., eds.), IOBC wprs Bulletin, vol. 20 (1), Montfavet, France, pp. 35–44.
29. Barnes, B. N. and Blomefield, T. L. (1997) Goading growers towards mating disruption: the South African experience with *Grapholitha molesta* and *Cydia pomonella* (Lepidoptera, Tortricidae), in *Technology Transfer in Mating Disruption* (Witzgall, P. and Arn, H., eds.), IOBC wprs Bulletin, vol. 20 (1), Montfavet, France, pp. 45–56.
30. Williams, M. R. (1996) Cotton insect losses 1995. *Proceedings, Beltwide Cotton Production Research Conference,* Nashville, TN, pp. 670–689.
31. Doane, C. C., Haworth, J. K., and Dougherty, D. G. (1983) NoMate PBW, a synthetic pheromone formulation for wide area control of the pink bollworm, in *Proceedings of the 10th International Congress of Plant Protection*, Brighton, UK.
32. Chu, C. C., Henneberry, T. J., Weddle, R. C., Natwick, E., Carson, J., Valenzuela, C., Birdsall, S., and Staten, R. (1996) Reduction of pink bollworm (Lepidoptera: Gelechiidae) populations in the Imperial Valley, California, following mandatory short-season cotton management systems. *J. Econ. Entomol.* **89,** 175–182.
33. Staten, R. T., Antilla, L., and Walters, M. L. (1995) Pink bollworm management: prospects for the future, in *Proceedings, Beltwide Cotton Conferences*, vol. 1, San Antonio, TX, 4–7 January, pp. 153–156.
34. Walters-Geyer, M., Sequeira, R. A., Paradice, D. B., Fowler, G. C., Miller, C., and Staten, R. T. (1994) Cotton pest management: a knowledge-based system to handle information input overload. *AI Applications* **8,** 1–20.
35. Gonzales, R. (1990) Comparison of pest control costs in Mexicali Valley and Imperial Valley, in *Proceedings, International Cotton Pest Work Committee*, Mazatlan, Mexico, 8–9 November, pp. 131–138.
36. Werner, F. G., Moore, L., and Watson, T. F. (1979) Arizona cotton insects. University of Arizona Cooperative Extension Service Bulletin, A23 R.

37. Burrows, T. M., Sevacherian, V., Moffit, L. J., and Baritelle, J. L. (1984) Economics of pest control alternatives for Imperial Valley cotton. *CA Agric.* **38,** 15,16.
38. McLaughlin, J. R., Shorey, H. H., Gaston, L. K., Kaae, R. S., and Stewart, F. D. (1972) Sex pheromone of Lepidoptera. XXXI. Disruption of sex pheromone communication in *Pectinophora gossypiella* with hexalure. *Environ. Entomol.* **1,** 645–650.
39. Hummel, H. E., Gaston, L. K., Shorey, H. H., Kaae, R. S., Byrne, K. J., and Silverstein, R. M. (1973) Clarification of the chemical status of the pink bollworm sex pheromone. *Science* **181,** 873–875.
40. Bierl, B. A., Beroza, M., Staten, R. T., Sonnet, P. E., and Adler, V. E. (1974) The pink bollworm sex attractant. *J. Econ. Entomol.* **67,** 211–216.
41. Yamamoto, A. and Ogawa, K. (1989) Chemistry and commercial production of pheromones and other behaviour-modifying chemicals, in *Insect Pheromones in Plant Protection* (Jutsum, A. R. and Gordon, R. F. S., eds.), Wiley, Chichester, UK, pp. 128–148.
42. Gaston, L. K., Kaae, R. S., Shorey, H. H., and Sellers, D. (1977) Controlling the pink bollworm by disrupting sex pheromone communication between adult moths. *Science* **196,** 904,905.
43. Henneberry, T. J., Gillespie, J. M., Bariola, L. A., Flint, H. M., Lingren, P. D., and Kydonieus, A. F. (1981) Gossyplure in laminated plastic formulations for mating disruption and pink bollworm control. *J. Econ. Entomol.* **74,** 376–381.
44. Kydonieus, A. F., Gillespie, J. M., Barry, M. W., Welch, J., Henneberry, T. J., and Leonhardt, B. A. (1981) Formulations and equipment for large volume pheromone applications by aircraft, in *Insect Pheromone Technology: Chemistry and Applications* (Leonhardt, B. A. and Beroza, M., eds.), ACS Symposium Series **190,** 175–191.
45. Miller, E., Staten, R. T., Nowell, C., and Gourd, J. (1990) Pink bollworm (Lepidoptera: Gelechiidae): point source density and its relationship to efficacy in attracticide formulations of gossyplure. *J. Econ. Entomol.* **83,** 1321–1325.
46. Haynes, K. F. and Baker, T. C. (1985) Sublethal effects of permethrin on the chemical communication system of the pink bollworm moth, *Pectinophora gossypiella*. *Arch. Insect Biochem. Physiol.* **2,** 283–293.
47. Haynes, K. F., Li, W.-G., and Baker, T. C. (1986) Control of the pink bollworm moth (Lepidoptera: Gelechiidae) with insecticides and pheromones (attracticide): Lethal and sublethal effects. *J. Econ. Entomol.* **79,** 1466–1471.
48. Butler, G. D. and Las, A. S. (1983) Predaceous insects: effect of adding permethrin to the sticker used in gossyplure applications. *J. Econ. Entomol.* **76,** 1448–1451.
49. Conlee, J. K. and Staten, R. T. (1981) US Patent 4671010.
50. Beasley, C. A. and Henneberry, T. J. (1984) Combining gossyplure and insecticides in pink bollworm control. *CA Agric.* **38,** 22–24.
51. Flint, H. M., Merkle, J. R., and Yamamoto, A. (1985) Pink bollworm (Lepidoptera: Gelechiiddae): field testing a new polyethylene tube dispenser for gossyplure. *J. Econ. Entomol.* **78,** 1431–1436.

108. Weddle, Hansen, and Associates (1994) Management of codling moth in Bartlett Pears in California: A preliminary analysis of the relative costs of insecticide- and pheromone-based IPM strategies. Research Reports for California Bartlett Pears. California Pear Advisory/Pear Pest Management Research Fund.
109. Meszaros, Z. (1984) Results of faunistical and floristical studies in Hungarian apple orchards (Apple ecosystem research no. 26). *Acta Phytopathol. Acad. Sci. Hungar.* **19,** 91–176.
110. Gut, L. J., Liss, W. J., and Westigard, P. H. (1991) Arthropod community organization and development in pear. *Environ. Manage.* **15,** 83–104.
111. Codling Moth Areawide Management Program (1995) Pilot test implementation and research progress reports, Year 1—1995 Oregon State University, Corvallis, OR, pp. 1–36.
112. Sauer, A. E., Karg, G., and Koch, U. T. (1990) Measurement of pheromone concentrations for the improvement of mating disruption. OILB-SROP/IOBC-WPRS Congress: Pheromones in Mediterranean Pest Management. Granada, Spain, 10–15 September.
113. Karg, G., Suckling, D. M., and Rumbo, E. R. (1992) Measurement of sex pheromone in orchard air using electroantennogram apparatus, in *Proceedings of the 45th New Zealand Plant Protection Conference,* pp. 304–306.
114. Karg, G. and Sauer, A. E. (1995) Spatial distribution of pheromone in vineyards treated for mating disruption of the grape vine moth *Lobesia botrana* measured with electroantennograms. *J. Chem. Ecol.* **21,** 1299–1314.
115. Otahshi, M., Uchijima, Z., and Yamamoto, A. (1991) Relationship between aerial concentration of a synthetic sex pheromone and mating in the disruption field of the smaller tea tortrix, *Adoxophyes* sp. (Lepidoptera: Tortricidae). *Jpn. J. Appl. Ent. Zool.* **35,** 207–211.
116. Jenkins, J. W., Doane, C. C., Schuster, D. J., McLaughlin, J. R., and Jimenez, M. J. (1990) Development and commercial application of sex pheromone for control of the tomato pinworm, in *Behavior-Modifying Chemicals for Insect Management: Applications of Pheromones and Other Attractants* (Ridgeway, R. L., Silverstein, R. M., and Inscoe, M. N., eds.), Marcel Dekker, New York, pp. 269–280.
117. McLaughlin, J. R., Doolittle, R. E., Gentry, C. R., Mitchell, E. R., and Tumlinson, J. H. (1976) Response to pheromone traps and disruption of pheromone communication in the lesser peachtree borer and the peachtree borer (Lepidoptera: Sesiidae). *J. Chem. Ecol.* **2,** 73–81.
118. Shorey, H. and Gerber, R. G. (1996) Use of puffers for disruption of sex pheromone communication among navel orangeworm moths (Lepidoptera: Pyralidae) in almonds, pistachios, and walnuts. *Environ. Entomol.* **25,** 1154–1157.
119. Baker, T. C., Mafra-Neto, A., Dittl, T., and Rice, M. E. (1997) A novel controlled-release device for disrupting sex pheromone communication in moths, in *Technology Transfer in Mating Disruption* (Witzgall, P. and Arn, H., eds.), IOBC wprs Bulletin, vol. 20 (1), Montfavet, France, pp. 141–150.

Natural Enemies and Control (Van der Geest, L. P. S., ed.), Elsevier, Amsterdam, pp. 339–370.

80. Barnes, M. M., Millar, J. G., Kirsch, P. A., and Hawks, D. C. (1992) Codling moth (Lepidoptera: Tortricidae) control by dissemination of synthetic female sex pheromone. *J. Econ. Entomol.* **85,** 1274–1277.
81. Knight, A. K. (1995) The impact of codling moth (Lepidoptera: Tortricidae) mating disruption on apple pest management in Yakima Valley, Washington. *J. Entomol. Soc. Br. Columbia* **92,** 29–38.
82. Roelofs, W. A., Comeau, A., Hill, A., and Milicevic, G. (1971) Sex attractant of the codling moth: characterization with electroantennogram technique. *Science* **174,** 297–299.
83. Bartell, R. J. and Bellas, T. E. (1981) Evidence for naturally occurring secondary compounds of the codling moth female sex pheromone. *J. Aus. Ent. Soc.* **20,** 197–199.
84. Einhorn, J., Witzgall, P., Audemard, M., Boniface, B., and Causse, R. (1986) Constituants secondaires de la pheromone sexuelle du carpocapsae des pommes, *Cydia pomonella* L. (Lepidoptera: Tortricidae). II. Premiere approche des effets comportementaux. *C. R. Acad. Sci. Paris* **302,** 263–266.
85. Arn, H., Guerin, P. M., Buser, H. R., Rauscher, S., and Mani, E. (1985) Sex pheromone blend of the codling moth, *Cydia pomonella*: evidence for a behavioral role of dodecan-1-ol. *Experientia* **41,** 1482–1484.
86. Bartell, R. J., Bellas, T. E., and Whittle, C. P. (1988) Evidence for biological activity of two further alcohols in the sex pheromone of female *Cydia pomonella* (L.) (Lepidoptera: Tortricidae). *J. Aust. Entomol. Soc.* **27,** 11,12.
87. Rothchild, G. H. L., Whittle, C. P., Bellas, T. E., and Vickers, R. A. (1988) Improved pheromone formulation. International Patent. World Intellectual Property Organization. International Publication Number, W/88/03755.
88. Minks, A. K. and Cardé, R. T. (1988) Disruption of pheromone communication in moths: is the natural blend really most efficacious? *Entomol. Exp. Appl.* **49,** 25–36.
89. McDonough, L. H., Davis, H. G., Chapman, P. S., and Smithhisler, C. (1993) Response of male codling moth (*Cydia pomonella*) to components of conspecific-female sex pheromone glands in flight tunnels. *J. Chem. Ecol.* **19,** 1737–1747.
90. McDonough, L. H., Davis, H. G., Chapman, P. S., and Smithhisler, C. (1994) Codling moth (*Cydia pomonella*): Disruptants of sex pheromonal communication. *J. Chem. Ecol.* **20,** 171–181.
91. McDonough, L. H., Davis, H. G., Chapman, P. S., and Smithhisler, C. (1995) Codling moth (*Cydia pomonella*), (Lepidoptera: Tortricidae): Is its sex pheromone multicomponent? *J. Chem. Ecol.* **21,** 1065–1071.
92. Moffit, H. R. (1974) Confusion with a sex pheromone as a means of controlling codling moth. IOBC Working Party Meeting on Biological Control of Codling Moth and *Adoxophyses*, Wadenswil, 26–29 November.
93. Mani, E., Arn, H., and Wildbolz, T. (1978). Inhibition of moth catch by evaporating the pheromone of the codling moth. *Mitteilungen der Bioloischen Bundesanstalt fur Land-und Fortwirtschaft* (Berlin-Dahlem) **180,** 50–52.

94. Charmillot, P. J. and Bloesch, B. (1987) La technique de confusion sexuelle: un moyen specifique de lutte contre le carpocapsae *Cydia pomonella* L. *Revue Suisse de Viticulture, Aboriculture et Horticulture* **19,** 129–138.
95. Howell, J. F., Knight, A. L., Unruh, T. R., Brown, D. F., Krysan, J. L., Sell, C. R., and Kirsch, P. A. (1992) Control of codling moth in apple and pear with sex pheromone-mediated mating disruption. *J. Econ. Entomol.* **85,** 918–925.
96. McDonough, L. H., Aller, W. C., and Knight, A. K. (1992) Performance characteristics of a commercial controlled release dispenser of sex pheromone for codling moth control by mating disruption. *J. Chem. Ecol.* **18,** 2177–2189.
97. Millar, J. G. (1995) Degradation and stabilization of E8,E10-dodecadienol, the major component of the sex pheromone of the codling moth (Lepidoptera: Tortricidae). *J. Econ. Entomol.* **88,** 1425–1432.
98. Ideses, R., Shani, A., and Klug, J. T. (1982) Cyclic peroxide: an isolable intermediate in singlet oxidation of pheromones to the furan system. *Chem. Ind.* **12,** 409,410.
99. Ideses, R. and Shani, A. (1988) Chemical protection of pheromones containing an internal conjugated diene system from isomerization and oxidation. *J. Chem. Ecol.* **14,** 1657–1669.
100. Brown, D. F., Knight, A. K., Howell, J. F., Sell, C. R., Krysan, J. L., and Weiss, M. (1992) Emission characteristics of a polyethylene pheromone dispenser for mating disruption of codling moth (Lepidoptera: Tortricidae). *J. Econ. Entomol.* **85,** 910–917.
101. Millar, J. G., McElfresh, J. S., and Rice, R. E. (1997) Technological problems associated with use of insect pheromones in insect management, in *Technology Transfer in Mating Disruption* (Witzgall, P. and Arn, H., eds.), IOBC wprs Bulletin Vol. 20 (1), Montfavet, France, pp. 19–26.
102. Howell, J. F. (1990) Reproductive biology, in *World Crop Pests, vol. 5: Tortricid Moth: Their Biology, Natural Enemies and Control* (Van der Geest, L. P. S., ed.), Elsevier, Amsterdam, pp. 157–174.
103. Weissling, T. J. and Knight, A. K. (1995) Oviposition and calling behavior of codling moth in the presence of codlemone. *Ann. Entomol. Soc. Am.* **89,** 142–147.
104. Welter, S. (1995) Areawide management, insecticide resistance, codling moth pheromone mating disruption. Research Project for California Bartlett Pears, California Pear Advisory Board, Sacramento, CA.
105. Riedl, H., Croft, B. A., and Howitt, A. J. (1976) Forecasting codling moth phenology based on pheromone trap catches and physiological time models. *Can. Entomol.* **108,** 449–460.
106. Barrett, B. A. (1995) Effect of synthetic pheromone permeation on captures of male codling moth (Lepidoptera: Tortricidae) in pheromone and virgin female moth-baited traps at different tree heights in small orchard blocks. *Environ. Entomol.* **24,** 1201–1206.
107. Weddle, Hansen, and Associates (1993) Barriers to the implementation of mating disruption for the control of codling moth. Research Reports for California Bartlett Pears. California Pear Advisory/Pear Pest Management Research Fund.

AN INTEGRATED APPROACH TO AREAWIDE PINK BOLLWORM MANAGEMENT IN ARIZONA

L. Antilla and M. Whitlow
Arizona Cotton Research and Protection Council
Tempe, AZ
R. T. Staten
USDA, Animal and Plant Health Inspection Service
Phoenix, AZ
O. El Lissy and F. Myers
Texas Boll Weevil Eradication Foundation
San Angelo, TX

Abstract

A multiyear areawide pink bollworm suppression program was conducted in Parker Valley, Arizona from 1990-1995. Two pheromone systems, the Shin Etsu or Mitsubishi PBW Rope and the Ecogen NoMate Attract N' Kill hollow fiber, represented the principal control components along with limited chemical insecticides. Population dynamics were monitored season long utilizing a standardized trapping program of one trap per ten acres of cotton.

Pheromone applications began annually at the six true leaf stage of cotton. During the 1990 and 1991 seasons, one rope application on 50% of the cotton acreage and four fiber/insecticide combinations were automatically applied on a 12-14 day schedule on the remaining acreage. Following 1991, the amount of rope utilized dropped progressively to zero in 1994. Fiber application protocol was modified over time from four automatic applications to treatments based exclusively on trap triggers.

A strict cultural control program of crop destruction and tillage was also instituted to reduce overwintering PBW populations. Weekly grower meetings provided updated program statistics and maintained program continuity. The six year program met or exceeded all stated objectives, namely:

1. Larval infestation rates in bolls were progressively reduced from 23.35 percent pre-program to 0.38 percent in 1995. This demonstrated the efficacy of pheromone technology.
2. The use of conventional insecticides for PBW control in Parker Valley was reduced from more than 216,000 cumulative acres treated per season pre-program to 2,047 in 1995.
3. PBW populations in bolls were maintained far below acceptable economic threshold of 5-10% through out the program.
4. The cost of PBW control was reduced from an historical average in excess of $70.00 per acre to an average of $28.33 per acre for the last three years of the program.

Introduction

Since 1965, the pink bollworm, *Pectinophora gossypiella* (Saunders), has been a major economic pest of cotton throughout Arizona and southern California. In an effort to combat this insect, growers have been forced to rely heavily upon chemical insecticides to suppress pink bollworm populations and maintain acceptable yields. This process has not only proven to be very costly, but has also produced an array of detrimental side effects:

a. The potential development of resistance to various chemicals, i.e., pyrethroids.
b. Secondary pest problems associated with increased pesticide usage.
c. Increasing environmental concerns related to the use of pesticides.

Further complications have resulted due to lack of continuity produced when growers apply various control methods, (or none at all), on differing time schedules. This allows for pink bollworm population reservoirs to build and regularly re-infest otherwise relatively clean areas throughout the state. The above represents a variety of factors that called for the development of alternate strategies. In support of this, during the fall of 1989, a demonstration program was designed to utilize pheromone technology as the main component in a large scale uniformly managed pink bollworm suppression program in Parker, Arizona. The goal of the program was to reduce pesticide use and grower costs through suppression of pink bollworm populations leading to eradication as technology is perfected.

The time frame for duration of the demonstration program was initially set at three years. A progressive reduction in pink bollworm populations over the period was determined to represent an acceptable criterion for program evaluation. Due to the success of the effort, the program has been continued for six years through 1995. It should be noted that the Parker program was designed to be a demonstration project and not a research effort. Untreated checks or control fields were not to be part of the evaluation process. Rather program assessments were to be made by comparing pink bollworm population trends in Parker Valley over the duration of the three year suppression effort. All comparisons were adjusted to equivalent heat unit accumulations. Preliminary program data has been previously reported. (Staten et al. 1995, Staten et al. 1996 in press) This present report will serve to provide complete documentation of program protocol, data and results to date.

Program Objectives

Initially in 1990-1991, the program set out to demonstrate the effectiveness of pheromone technology, reduce the use of conventional insecticides and provide pink bollworm suppression below economic thresholds on 90% of the fields in Parker Valley through August 15th.

Objectives were modified for 1992-1995 to extend pheromone treatments through September 15th. Initial objectives listed above including the minimization of cost inputs were maintained throughout the six year program.

Methods and Materials

Due to the dynamic nature of the Parker Program and because of ongoing efforts to analyze and improve technologies, the project was not conducted according to an irrevocable set of pre-established guidelines. Program strategies evolved over the course of the three year demonstration and beyond. As a precursor to full scale program initiation, 1989 was selected as the year to gather baseline data to be used for future program analysis. Forty-five cotton fields averaging approximately forty acres each were randomly selected in subsets of fifteen each from the north, middle and south portions of Parker Valley. An eighty boll sample was taken from each field on a weekly basis beginning the week of July 23rd and running through September 17, 1989. The samples were comprised of forty bolls from each of two diagonally opposed quadrants on week one, (i.e., NE and SW quads) and from the remaining two quadrants (i.e., NW and SE) on week two. The process was then reversed for alternating sampling periods.

The samples, consisting of green bolls which could be depressed slightly with thumb pressure, were taken by walking a loop near the corners of each quadrant. No bolls were taken from the outside four rows of cotton. Only after proceeding further into the field, did field personnel begin harvesting bolls.

All boll samples were then placed in labeled paper bags and returned to the field office where they were cracked and examined by technicians under table mounted 3X florescent ring light illuminated magnifying lenses. Findings of the survey were reported as the percent of larvae recovered from the total weekly boll sample. (Table 1)

Population Sampling

Surveillance activities in 1990 consisted of trapping all cotton fields in Parker Valley at the rate of one trap per field or forty acres, whichever was smaller. Standard Delta sticky traps were used (Foster et al. 1977). Traps were placed and run outside fields until layby, then moved at least fifty feet inside of field edges. Lure consisted of red rubber septa impregnated with 5mg load of gossyplure, a USDA standard.

Traps were serviced three times per week beginning with the week ending April 21, 1990. Multiple weekly servicing was carried through the programs treatment

3. To reduce pink bollworm populations below economic thresholds.
Historically, University of Arizona economic threshold recommendations or pink bollworm treatment have ranged from 5-10% infested bolls. Program objectives for 1990 and 1991 were clearly met with 3.6 and 0.1 percent infestation levels documented through August 18th and 17th for 1990 and 1991 respectively on more than 90% of program fields. Modified objectives extending program activities through September 15th produced maximum weekly boll infestation levels of 0.6, 0.0, 0.1 and 0.6 percent for 1992-1995 respectively.

4. To reduce the cost of pink bollworm control.
An historical review of Arizona Department of Agriculture treatment documents verified that Parker growers spent a minimum of $70 per season on pre-program pink bollworm control. Some spent much more. Since ACRPC personnel assumed season long management in 1992, average grower costs per acre for pink bollworm control have been successfully reduced to 55.00, 23.00, 29.00 and 33.00 for the period of 1992-1995. This in turn has resulted in a dramatic reduction of traditional insecticide use from a minimum of 216,000 acre equivalent per year pre-program to 2,047 acre equivalents in 1995 (Table 2).

In conclusion, it is evident that all program objectives have been met or exceeded. Based on the extensive data that has been gathered over a six year period and more importantly, the Parker growers' acceptance and vote of confidence, the program must be considered a success.

Acknowledgments

The authors appreciate the outstanding efforts of Lola Serrano, Don Struckmeyer, Marilyn Penrose, Shirley Haller and Letitia Tamulis of the Arizona Cotton Research & Protection Council in carrying out the control strategies. This program was made possible only by the leadership and support of the Parker Valley Growers Committee.

References

Baker, T.C., R.T. Staten and H.M. Flint. 1990. Use of pink bollworm pheromone in the southwestern United States. In Behavior Modifying Chemicals for Insect Management. eds. R.L. Ridgway, R.M. Silverstein and M.N. Inscoe, pp 417-436. CRC Press, Boca Raton, Florida.

Flint, H.M., J.R. Merkle and A. Yamamoto. 1985. Pink bollworm (Lepidoptera:-Gelechiidae): field testing a new polyethylene tube dispenser for gossyplure. Journal of Economic Entomology 78: 1431-1436.

Foster, R.N., R.T. Staten and E. Miller. 1977. Evaluation of traps for pink bollworm. Journal of Economic Entomology. 70:289-291

Leggett, J.E. Osama El Lissy and Larry Antilla. 1994. Pink Bollworm Moth Catches with Perimeter and In-Field Gossyplure Baited Delta Traps. Southwestern Entomologist, Vol. 19. No. 2: 147-155.

Staten, R.T., O. El-Lissy and L. Antilla. 1996 in press. Successful area-wide program to control pink bollworm by mating disruption. To be published in Pheromone Research: New Directions, R.T. Carde and A.K. Minks eds, Chapman and Hall, New York.

Staten, R.T., L. Antilla and M. Walters. 1995. Pink bollworm management: prospects for the future. In 1995 Proceedings Beltwide Cotton Conferences. San Antonio, TX January 4-7, 1995, pp. 153-156.

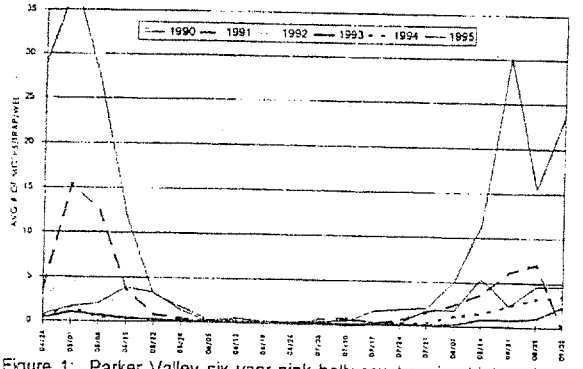


Figure 1: Parker Valley six year pink bollworm trapping history, Lapaz County, AZ 1995.

Table 1: Parker Valley seven year pink bollworm larval infestation summary, Lapaz County, AZ 1995.

Year	Total Bolls Collected	Total Larvae Recovered	Percent Larvae Recovered
1989	26,879	6,282	23.35
1990	34,726	3,442	9.91
1991	35,477	507	1.42
1992	30,064	261	0.86
1993	25,200	0	0.00
1994	16,109	32	0.19
1995	16,520	63	0.38

Table 2: Parker Valley pink bollworm program summary, Lapaz County, AZ 1995.

Year	Total Acres	Program Duration	Cumulative Acres Insecticide Treatments	Seasonal Average Cost Per Acre
1990	24,071	05/05 - 07/26	33,452	48.00
1991	27,111	05/10 - 07/26	27,034	48.00
1992	27,638	04/27 - 09/15	26,722	55.00
1993	28,229	04/26 - 09/10	3,570	23.00
1994	23,650	04/20 - 09/08	5,381	29.00
1995	25,154	05/02 - 09/14	2,047	33.00

DEVELOPMENT OF A NOVEL SPRAYABLE PHEROMONE PRODUCT FOR PINK BOLLWORM CONTROL

Kirk Smith -- biosys
Tom Walgenbach -- biosys
Jeff Springer -- Advanced Polymer Systems

Abstract

The company biosys, was founded in 1983. Its purpose was to develop entomopathogenic nematodes as commercial insect control products. This effort has been achieved with products now being sold world-wide for control of a variety of insect pests in diversified commodities. biosys is now positioning itself to be the world leader in the development and commercial sales of biological control products. A recent merger with Crop Genetics International brought a portfolio of three, EPA registered insect control Baculoviruses. Two virus products, Spod-X® (for beet armyworm control)

period which ended July 26, 1990. Following final treatments, servicing was reduced to once a week through the week ending September 1, 1990.

Based on trap density tests conducted in Maricopa and Pinal counties in 1991-1992, trap density was increased to one trap per ten acres on fiber treated fields. Trap density on rope treated acreage remained at one trap per field. Traps were moved to field perimeters and servicing schedules reduced to two times per week. (Leggett et al. 1994) From 1993-1995, trap inspection was further reduced to once a week with trap density remaining at one trap per ten acres on field perimeters. Boll sampling for larval infestation rates was conducted annually on 45 randomly selected fields according to the protocol established in 1989.

Pheromone Treatment Program

Two primary control regimens were employed in 1990:

1. The Shin Etsu or Mitsubishi PBW Rope was used where possible on the earliest cotton (first planted) and around sensitive sites where aerial application of pesticides was to be avoided. The Rope is a high rate system. Gossyplure is contained in a 8" polyethylene reservoir sealed at both ends. The gossyplure diffuses through the walls of the reservoir to produce its extended release capabilities at approximately a 28g rate per acre. (Flint et al. 1985) Tests have shown the rope to be effective for up to sixty days. Ropes were hand tied using labor crews on 11,826 acres of cotton at or near six leaf stage before pinsquare at a rate of 400 Ropes per acre on approximately a 10 by 10 foot grid. The majority of the Rope acreage was tied between April 28, 1990 and mid-May. No insecticide oversprays or pheromone fiber applications were made on Rope fields.

2. The remaining 13,174 acres in Parker Valley were provided four scheduled treatments on a maximum interval of 14 days utilizing the Ecogen pheromone hollow fiber ("Attract and Kill", U.S. Patent no. 4671010, Staten and Conlee) and azinphos methyl (an organo phosphate insecticide) either alone or in combination beginning at the six leaf stage. Applications were made by air utilizing standard fiber pods. Swath width was 50 feet. The extent of use of this system has prior review (Baker et al. 1990).

The first and fourth applications were dual treatments of both the fiber at 10 gram rate of formulated material (approximately 0.7g AI) in combination with an overspray of azinphos methyl at the rate of 16oz per acre in an ultra low volume formulation. The fiber was premixed with an adhesive (Biotac) at the rate of 3.5oz per acre. Included also in the Biotac was 0.5oz (formulated material) of the pyrethroid permethrin to complete the Attract and Kill mode of operation.

Second and third applications were made on a 12-14 day cycle thereafter on the same acreage. Both were fiber applications at a 15g rate (approximately 1.05g AI) plus Biotac and permethrin.

The fourth and final application was a dual treatment, identical to the first listed above, the insecticide acting as a clean up on existing moth populations to augment the effectiveness of the fiber.

In all of the fiber treatments previously listed, any individual field trap reading averaging one or more moths per trap per night triggered a reduction of the treatment interval below the 12-14 day target time frame. In no instance were treatments with fiber made on less than an eight day schedule. Most treatments averaged 12 day intervals. Fiber treatments began in a limited fashion on May 8, 1990 and were completed on July 26, 1990.

Rope versus fiber acreage ratios remained at 50:50 in 1991. Fiber treatment protocol was reduced to two scheduled applications beginning at the six leaf stage, the first a dual treatment (fiber at a 10 gram formulated rate in combination with chlorpyrifos at the rate of 32 oz per acre ultra low volume formulation) and the second a single fiber application 12-14 days later. Subsequent treatments were based on trapping triggers (threshold moth counts) on a field by field basis.

In 1992, the rope/fiber acreage ratio was reduced to 35:65. Fiber treatments were reduced to one scheduled dual application. All additional treatments were based on trap triggers or the presence of known in-field infestations. Rope and fiber treatments covered the date ranges of May 2 - June 15, 1992 and May 2 - September 15, 1992 respectively.

Rope to fiber ratios dropped to 05:95 in 1993. No rope was utilized in either 1994 or 1995. In addition, automatic dual fiber insecticide treatments were discontinued after 1992. Trapping triggers were employed season long to differentiate the need for single fiber or dual treatments.

Standardization was essential to program success. Trapping was carried out on a strict schedule. Moth counts were analyzed by supervisors daily as reported by field personnel and entered into a computerized database. Pheromone and insecticide treatments were scheduled within a window of less than twenty-four hours post detection.

Two way radio communication assured constant contact between supervisors and all field personnel. Regular monitoring of trapping, boll collection activities and commercial application of pheromones maintained program quality assurance at a high level.

The integration of a solid cultural control regimen represented a key component in program success. Agricultural ordinances enacted by the Colorado River Indian Tribes on tribal leased land mandated well defined planting, chemical termination, stalk destruction and tillage deadlines leading to a one hundred percent compliance record on the part of Parker cotton growers. This in turn has assisted in reducing overwintering pink bollworm populations.

Communication between Arizona Cotton Research & Protection Council program personnel, local growers and pest control advisors has also been an essential element in the full integration of community wide activities. Weekly meetings allowed program managers to update growers on pink bollworm population levels, current control strategies and financial status reports. Secondary pest status discussions with pest control advisors minimized duplication of treatment strategies.

Results and Discussion

Results of the six year suppression program may best be analyzed by comparing statistical summary data with stated program objectives, namely:

1. To demonstrate the efficacy of pheromone technology.

Despite the selective use of chemical insecticides, the Parker Program has primarily utilized pheromones to achieve suppression of the pink bollworm. A progressive season long reduction of pink bollworm male moths trapped has been documented over the duration of the program (Figure 1). A dramatic reduction of larval infestation rates has been achieved over the course of program activities. An overall larval infestation level of 23.35 percent in 1989 has progressively been reduced to a season long average of 0.38 percent infestation in 1995 (Table 1). These data indicate that (particularly since 1992) pink bollworm populations have been reduced, establishing a new general equilibrium position which is sustainable.

2. To reduce the grower's use of conventional insecticides.

Parker Valley has a history of heavy pink bollworm infestation levels. During the late 1980's, growers averaged 12-15 insecticide treatments per season for pink bollworm control. Arizona Department of Agriculture pesticide use documents were reviewed for all program study fields for the period of 1990-1992. All grower treatments labeled for pink bollworm (as either a primary or secondary pest) were then summarized and reported as the average number of treatments applied by area growers for each cotton season. This, coupled with the average number of program insecticide applications per season, documents a significant reduction in the use of conventional pesticides for pink bollworm control; from an average of 6.2 applications in 1990 to 3.5, 2.6, 1.1, 0.7 and 0.3 per season in 1991-1995 respectively. These results represent a dramatic reduction in the use of traditional insecticides.

leafminer, *Liriomyza sativae* Blanchard (2–4) and mites (N. C. Toscano, personal communication). Broad-spectrum insecticides also kill beneficial fauna important in the control of *Spodoptera exigua* (Hübner) and *Heliothis zea* (Boddie). Furthermore, use of pesticides has resulted in above-tolerance residues on fresh market tomatoes (5). Researchers suspect that high levels of insecticide resistance have developed in many TPW populations. Alternatives to the traditional insecticidal approach for TPW control are urgently needed.

II. FORMULATION DESCRIPTION

Development of a system using sex pheromone for control of the tomato pinworm was initiated in 1979 soon after identification of the TPW pheromone as a 96:4 mixture of (*E*)- and (*Z*)-4-tridecenyl acetate (W. L. Roelofs, unpublished) and description of adult sex pheromone biology of the adult (6). The TPW was chosen as a candidate for product development because of previous success with another gelechiid, the pink bollworm, *Pectinophora gossypiella* (Saunders) (PBW) (7). As with PBW, development centered on use of hollow fibers as controlled-release devices. The end-sealed hollow-fiber dispenser emits controlled amounts of active ingredient through its single open end. Emission rates and longevity can be varied by adjusting configuration and density per unit area (8,9). Fibers can be applied singly or in groups, either on adhesive-backed tape or in flowable polybutene stickers. Application may be made with specialized equipment or simply by hand.

III. SYSTEM DEVELOPMENT

A. Release Rates

A prerequisite for evaluating mating disruption was to develop an attractive lure and trap for use in monitoring and determining pest density of TPW adults (10). Early research with hollow-fiber dispensers revealed an inverse relationship between pheromone release rate and numbers of moths captured in traps (11). A single hollow fiber attracted significantly more moths than 5-, 7-, 9-, 10-, 30-, or 100-fiber lures. Additional dose–response testing conducted in 1982 also demonstrated a clear upper threshold of male response to the sex pheromone (Scentry, unpublished data). Dispensers designed with two fibers caught 35% to 52% more moths than six-fiber dispensers and 67% to 88% more moths than 18-fiber dispensers. Since then two-fiber dispensers have been used as a standard lure for monitoring with traps. Active life of this lure is approximately 4 weeks.

In the laboratory, release rates of (*E*)-4-tridecenyl acetate in hollow fibers were measured by meniscus regression. Analyses indicated rates were 2.72 µg/day and 5.93 µg/day at 72°F and 100°F, respectively. The same fiber configuration was loaded with 96:4 (*E*)- and (*Z*)-4-tridecenyl acetate and analyzed for emission rate by gas chromatography. Fibers were mixed with polybutene adhesive used in field application and exposed in the field for 0, 7, 14, 21, and 28 days. Average daily temperature during this period was 70.6 ± 7°F. Residual pheromone was extracted and quantitated by gas chromatography (fused silica Carbowax 20M column). One hundred fibers were analyzed from each age class. Results indicated a mean release rate of 3.7 µg/fiber per day after the initial burst of pheromone on days 1 and 2.

B. Traps

The trap design most effective for capturing TPW was determined by Wyman in 1979 (11). A wing-style trap was six times more effective in capturing TPW moths than either Delta-style or mineral oil traps. Superiority of the wing trap was attributed to ease of access to the sticky surface and emission of pheromone from the trap. The horizontal orientation of the wing-style trap is more efficient for capturing these moths as they alight. Research in California showed a close correlation between trap captures of TPW, larvae in foliage, and percentage of infested fruit in untreated fields (12). Trap-monitoring guidelines and economic thresholds have been established for TPW on pole tomatoes in southern California (13,14).

C. Mating Disruption

The first replicated mating disruption trial of TPW sex pheromone in hollow-fiber formulation was conducted in Florida during 1979. Hollow-fiber dispensers in tape formulation were hand-stapled to tomato stakes in 2.8-ha plots at approximately 1235 dispensers per hectare. Each dispenser contained 50 fibers, producing a release rate of 40 g AI/ha. Adjacent 2.8-ha plots were used as controls. Treatments were replicated three times. Trap captures in treated plots remained low after application (Fig. 1), but larval infestations failed to develop in either treatment. Both the pheromone and control plots were treated equally with conventional insecticides during the period.

In 1980, an unreplicated mating disruption trial was conducted in Mexico's Culiacan valley under supervision of Sanidad Vegetal. Two applications using hollow-fiber dispensers in the tape formulation with 50 fibers per tape were hand-placed on tomato stakes at 312 dispensers per hectare (10 g AI/ha) in the first application and 625 dispensers

BEHAVIOR-MODIFYING CHEMICALS FOR INSECT MANAGEMENT

Applications of Pheromones and Other Attractants

edited by

RICHARD L. RIDGWAY
Agricultural Research Service
U.S. Department of Agriculture
Beltsville, Maryland

ROBERT M. SILVERSTEIN
College of Environmental Science and Forestry
State University of New York
Syracuse, New York

MAY N. INSCOE
Agricultural Research Service
U.S. Department of Agriculture
Beltsville, Maryland

MARCEL DEKKER, INC. New York and Basel

18

Development and Commercial Application of Sex Pheromone for Control of the Tomato Pinworm

JACK W. JENKINS / Scentry Inc., Buckeye, Arizona

CHARLES C. DOANE / Scentry Inc., Buckeye, Arizona

DAVID J. SCHUSTER / Gulf Coast Research and Education Center, University of Florida, Bradenton, Florida

JOHN R. McLAUGHLIN / Agricultural Research Service, U.S. Department of Agriculture, Gainesville, Florida

MANUEL J. JIMENEZ / University of California, Cooperative Extension Service, Community Civic Center, Visalia, California

I. INTRODUCTION

The tomato pinworm, *Keiferia lycopersicella* (Walsingham), is a primary pest of tomatoes in North America. The insect occurs in many tomato-growing areas but causes greatest damage in Mexico, southern California, southern Texas, and Florida. The pest has also been reported in Haiti, Cuba, Central America, and Peru (1). Larvae are leafminers during early instars and leaffollers as they increase in size. Foliar injury is of little economic importance although severe infestations can suppress growth of younger plants. Larger larvae also enter tomatoes below the fruit calyx, predisposing them to decay. Traditional methods of controlling tomato pinworm (TPW) involve repeated applications of broad-spectrum insecticides, often in mixtures aimed at control of other pests as well; however, TPW larvae within the plant tissue are shielded from most insecticides, reducing their effectiveness. Insecticides recommended for TPW control have caused outbreaks of secondary pests such as the vegetable

four times on 0.4-ha plots. Point-source pattern was consistent between treatments—36 fibers per site, 1300 sites per hectare. Trap counts and mating tables were used to measure efficacy. Test plots were monitored twice weekly for 8 weeks before application, and treatments were assigned to plots so preapplication populations did not differ statistically.

The trap captures are summarized in Table 3. Counts did not differ significantly among the three pheromone formulation treatments, but all treatments were significantly lower than catches in the untreated check. There was a 98.7% trap-catch reduction in plots treated with PET lures, followed by Celcon lure plots at 98.3%, and Celcon fiber plots at 96.1%. In no pheromone treatment did average trap count exceed 1.5 moths per trap a week during 7 weeks of post-treatment monitoring.

Mating table data correlated well with trap data in check plots, $r = 0.88$. Peak trap pressure came 5 weeks after treatment, which coincided with peak mating table activity, as seen in Table 4. Mating table data did not differ significantly among the three pheromone formulation treatments, but all treatments had significantly less mating than in the untreated check.

In 1981 a large demonstration was supervised by Sanidad Vegetal in Culiacan, Mexico. Fields comprising 600 ha of staked tomatoes treated with pheromone were compared with 180 ha of fields treated only with conventional insecticides. Applications began during the

TABLE 3 Average Number of Male TPW Males Captured Per Trap Per Week in Pheromone-Treated and Untreated Fields, Florida, 1981

Treatment	Number of weeks after application							Total[a]
	1	2	3	4	5	6	7	
PET tape	0	0	0.2	0	0.7	0.5	0.2	1.7[b]
Celcon tape	0.2	0	0	0.2	1.0	0	0.7	2.2[b]
Celcon fiber	0	0.2	0.2	1.2	1.5	1.0	0.7	5.0[b]
Control	2.0	2.7	6.0	28.2	60.5	18.7	10.0	128.2[a]

[a]Numbers within the column followed by the same letter are not significantly different ($p > 0.05$; Duncan's multiple range test, 1955).

TABLE 4 Percentage of Mated TPW Females after Placement of Virgin Females on Mating Tables in Pheromone-Treated and Control Tomato Fields, Florida, 1981

Treatment	Number of weeks after application						Total[a]
	2	3	4	5	6	7	
PET tape	0	0	11.1	17.0	58.9	25.0	17.3[b]
Celcon tape	3.6	0	62.5	9.7	46.8	33.3	19.1[b]
Celcon fiber	10.3	41.7	33.3	52.8	38.0	68.7	36.8[b]
Control	58.9	72.9	92.8	100.0	83.6	79.2	78.2[a]

[a]Means within the column followed by the same letter are not significantly different ($p > 0.05$; Duncan's multiple range test, 1955).

period of highest TPW pressure in tomatoes in Culiacan. Pheromone in Celcon hollow fibers was mixed in sticker and applied by hand at 10 g AI/ha. The distribution pattern was 36 fibers per point and 1300 points per hectare. A mean of 2.6 pheromone applications were made in tomato fields during the demonstration. Pest populations were monitored in traps before and after treatment. All conventional insecticide applications in both pheromone-treated fields and check fields were recorded. Fewer conventional insecticide applications for all insect pests were required in pheromone-treated plots (Table 5).

TABLE 5 Results from 600 ha of Tomatoes Treated with TPW Pheromone in Hollow Fibers Compared with 180 ha Treated with Conventional Insecticide, Culiacan, Mexico, 1981

Treatment	Average number of applications		Average % infested fruit
	Pheromone	Insecticide	
Pheromone	2.6	5.8	1.2
Control	0	12.3	5.4

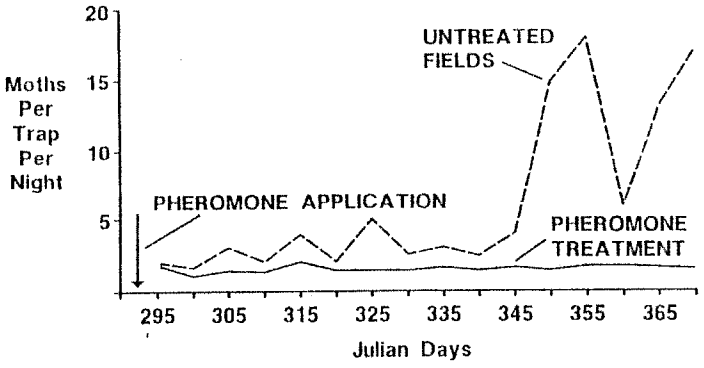


FIGURE 1 Trap captures of male TPW in pheromone-treated and untreated fields, Florida, 1979.

per hectare (20 g AI/ha) 20 days later. The 4-ha treated field was compared with an adjacent field that remained completely untreated. Traps baited with standard two-fiber lures were used to monitor male populations. Trap counts were greatly reduced in the pheromone-treated field (Fig. 2). Samples of 100 tomatoes collected at random on four dates were examined for TPW infestations. The mean TPW infestation in the treated field was 2% compared with 34% in the untreated field (Table 1).

Mating tables were first used in Florida in 1980 to measure disruption. Three sets of paired blocks, approximately 0.1-ha each, were established and half were treated with the hollow-fiber tape dispensers at 10 g AI/ha. All fields received insecticides at the discretion of the pest control advisor. A single mating table was placed in the center of each plot at canopy height. Virgin TPW females, 3 days old, were placed on mating tables on bouquets of excised tomato foliage in vials of water. Depending upon availability, three to seven females were placed on the table each day. Females were collected after the daily mating period and examined for presence of spermatophores. Results from four dates indicated only 4.2% of females were mated in pheromone-treated plots, compared with 52.6% in the check plots (Table 2).

During 1981 in Florida, plots treated with three formulations of hollow fibers applied at 10 g AI/ha were compared with an untreated check. Treatments were polyethylene fibers on sticky tape (PET), Celconese plastic fibers on sticky tape (Celcon), and Celconese fibers applied with polybutene sticker. Treatments were replicated

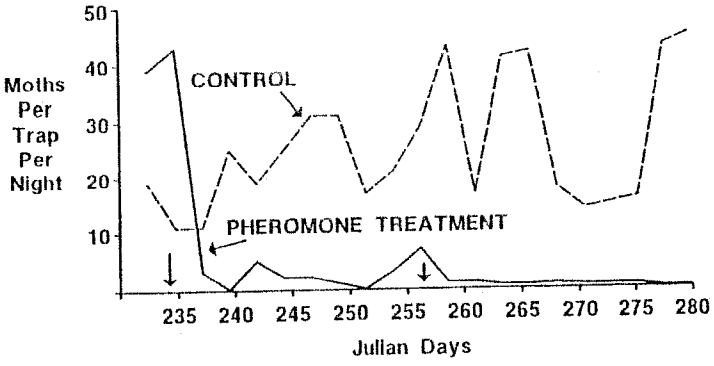


FIGURE 2 Trap captures of male TPW in pheromone-treated and untreated fields. The two vertical arrows indicate dates of pheromone application, Culiacan, Mexico, 1980.

TABLE 1 Average Percentage of Fruit Infested with TPW in Pheromone-Treated and Untreated Plots, Culiacan, Mexico, 1980

	% Infestation			
Treatment	9/24	10/2	10/4	10/6
Pheromone	2	3	1	2
Untreated	40	37	28	33

TABLE 2 Mating Frequency of Virgin TPW Females on Mating Tables in Plots Treated with Pheromone and Control Plots, Florida, 1980

	Number of females		
Treatment	Mated	Unmated	% mated
Pheromone	1	23	4.2
Control	10	9	52.6

The A&K TPW is applied by hand directly to plant foliage or to stakes supporting the tomatoes and does not come into contact with the tomato fruit. No special equipment is needed to apply the mixture. Clusters of fibers and adhesive are applied at canopy level.

VI. INTEGRATED PEST MANAGEMENT

A recent demonstration by University of California researchers showed that TPW pheromone products applied to cherry tomatoes provided effective control (17). Native beneficial insects were conserved and augmented by release of *Trichogramma pretiosum* Riley. Except for a single application of *Bacillus thuringiensis* on one field treated with A&K TPW, no insecticides were needed for any insect pest during the season. In comparison, these same fields had required weekly insecticide applications the previous year. Furthermore, TPW populations in nearby fields in 1987 were not controlled, despite weekly applications of the standard insecticides. In fact, TPW infestations were so high on some insecticide-treated fields that tomatoes were rejected by packers. During 1987, the average percentage of infested fruit in pheromone-treated fields was less than 5%, compared with more than 20% in the same fields in 1986 under traditional insecticide protection.

VII. CONCLUSION

Concern for pesticide residues on tomatoes and difficulty in controlling the TPW economically with available conventional insecticides have created a dilemma for tomato growers. Experiments have proved that controlled-release formulations of TPW sex pheromone provide an effective control of this pest through mating disruption. One system, A&K TPW is registered for control of tomato pinworm, both in the United States and in Mexico. This system can be integrated with other pest management practices, such as narrow-spectrum insecticides and biological and cultural control methods, to further reduce populations of primary and secondary pest species.

REFERENCES

1. Lin, S. Y. H. and Trumble, J. T. Bibliography of the tomato pinworm, *Keiferia lycopersicella* (Walsingham) (Lepidoptera: Gelechiidae). *Bibliogr. Entomol. Soc. Am.* 1:65–74 (1983).

2. Oatman, E. R. and Kennedy, G. C. Methomyl induced outbreak of *Liriomyza sativae* on tomato. *J. Econ. Entomol.* 69: 667–668 (1976).
3. Johnson, M. W., Oatman, E. R., and Wyman, J. A. Effects of insecticides on populations of the vegetable leafminer and associated parasites on fall pole tomatoes. *J. Econ. Entomol.* 73:67–71 (1980).
4. Oatman, E. R., Van Steenwyk, R. A., and Trumble, J. T. Insect pest management of fresh market tomatoes. In *California Fresh Market Tomato Research and Education Programs, 1981–82.* (Pasateri, F. P., ed.), 1981, pp. 95–110.
5. Food and Drug Administration. Results of FDA Surveillance of Mexican Produce for Pesticide Residues. (Compliance program 7305.008. Fiscal year, 1979.) Bureau of Foods and Office of Associate Comm. for Reg. Aff., FDA Administration. April 23, 1979.
6. McLaughlin, J. R., Antonio, A. Q., Poe, S. L., and Minnick, D. R. Sex pheromone biology of the adult tomato pinworm, *Keiferia lycopersicella* (Walsingham). *Fla. Entomol.* 62:34–41 (1979).
7. Doane, C. C. and Brooks, T. W. Research and development of pheromones for insect control with emphasis on the pink bollworm. In *Management of Insect Pests With Semiochemicals.* (Mitchell, E. R., ed.). Plenum Press, New York, 1980, pp. 285–303.
8. Ashare, E., Brooks, T. W., and Swenson, D. W. Controlled release from hollow fibers. In *Controlled Release Polymeric Formulations.* (Paul, D. R. and Harris, F. W., eds.). ACS Symp. Ser. No. 33, American Chemical Society, Washington, D.C., 1976, pp. 273–282.
9. Brooks, T. W., Ashare, E., and Swenson, D. W. Hollow fibers as controlled vapor release devices. In *Textile and Paper Chemistry and Technology.* (Arthur, J. C., ed.). ACS Symp. Ser. No. 49, American Chemical Society, Washington, D.C., 1977, pp. 116–126.
10. Roelofs, W. E., Brooks, T. W., Burkholder, W. E., Cardé, R. T., Chambers, D. L., Shorey, H. H., Wood, D. L., Ludvik, G. F., and Russel, P. L. *Establishing Efficacy of Sex Attractants and Disruptants for Insect Control.* Entomological Society of America, College Park, Md., 1979, 97 pp.
11. Wyman, J. A. Effect of trap design and sex attractant release on tomato pinworm catches. *J. Econ. Entomol.* 72:865–868 (1979).
12. Van Steenwyk, R. A., Oatman, E. R., and Toscano, N. C. Optimal timing of pesticide applications for tomato pinworm through a pheromone monitoring program in commercial fall fresh market tomatoes. *Calif. Dept. Food Agric. Final Rep.*, 1979–1980.
13. University of California. Tomato pinworm, *Keiferia lycopersicella.* In *Integrated Pest Management for Tomatoes.* Univ. Calif. Statewide IPM Project. Div. Agric. Sci. Publ. 3274. 1985, pp. 57–59.
14. Van Steenwyk, R. A., Oatman, E. R., and Wyman, J. A. Density treatment level for tomato pinworm (Lepidoptera: Gelechiidae) based on pheromone trap catches. *J. Econ. Entomol.* 76:440–445 (1983).
15. Van Steenwyk, R. A. and Oatman, E. R. Mating disruption of tomato pinworm (Lepidoptera: Gelechiidae) as measured by pheromone trap, foliage, and fruit infestation. *J. Econ. Entomol.* 76:80–84 (1983).
16. Buckley, R. C., VanSickle, J. J., Bredahl, M. E., Belibasis, E., and Gutierrez, N. Florida and Mexico competition for the winter fresh vegetable market. *USDA ESA Agric. Econ. Rept.* 556. 1986, 101 pp.
17. Jiménez, M. J., Toscano, N. C., Flaherty, D. L., Ilic, P., Zalom, F. G., and Kido, K. Controlling tomato pinworm by mating disruption. *Calif. Agric.* 42:10–12 (1988).

Each pheromone application replaced 2.5 conventional insecticide applications. Throughout the test the highest level of infested fruit in pheromone-treated fields was lower than levels in pesticide-treated check fields.

The relationship between point-source strength and number of point sources was examined for TPW in 1980 by evaluating two distribution patterns and application rates of the hollow-fiber system (15). Pinworm populations were monitored by adult capture in traps and larval infestation in foliage and fruit. Within the test range, distribution patterns did not result in significantly different larval counts; however, all rates and patterns resulted in significant reduction of moth captures compared with the untreated control. The lack of difference in larval infestations between treatments and the control was apparently due to poor isolation and to immigration of mated females from adjacent plots.

Mating disruption was further evaluated in California pole tomatoes in 1981, when the hollow-fiber formulation was applied at an average density of six fibers per site with approximately 5500 sites per hectare for an application rate of 10 g AI/ha (15). Fibers were mixed with sticker and applied by hand to tomato stakes at canopy level. Applications were made once each month during August, September, and October. Three plots, 16-ha each, were treated with pheromone. All plots, including corresponding controls, were treated with mixtures of insecticides for beet armyworm (BAW) control. The TPW populations were monitored by traps and plant inspection.

Moth captures were greatly suppressed in pheromone-treated plots compared with check plots (Table 6). Larval infestations and the percentage of fruit infested were generally reduced in pheromone-treated

TABLE 6 Average Trap Captures and Larval Infestation of TPW in Pheromone-Treated and Control Tomato Fields, California, 1981

Treatment	Moths/trap/night	Mean no./meter of row		Mean % infested fruit
		Mines	Larvae	
Pheromone	0.6[b]	8.0[b]	1.6[b]	0.6[a]
Control	38.6	9.9	2.3	0.8

[a]Means are significantly different (matched pair comparison) from corresponding control: $p < 0.1$.
[b]$p < 0.01$.

plots as well, although not as dramatically as trap captures. The TPW infestations were low because of insecticide applied for BAW control.

IV. ECONOMICS

In his 1981 trial, Van Steenwyk (15) estimated hand application cost about 80 dollars/ha, not including the cost of the pheromone-filled fibers. It was concluded, at that time, that although pheromone treatments suppressed TPW populations, the degree of control did not justify the expense of the system. In normal commercial programs, cost for hand application is far less. A single laborer can treat 1 ha/hr using fibers in sticker. Application cost is less than 5 dollars/ha in the United States and less than 30 cents/ha in Mexico.

High costs of labor, land, and water in southern California have resulted in a gradual shift of fresh market tomato production to Mexico. Tomatoes are Mexico's largest export crop, with revenues in the hundred's of millions dollars annually (16). The Culiacan valley of Mexico produces as much as 50% of all fresh market tomatoes consumed in the United States each winter. Growers are highly motivated to protect this market through economic production of clean tomatoes. In 1979 the USDA-FDA stopped large quantities of tomatoes from importation from Mexico because of pesticide residues above tolerance (5). This action caused considerable economic loss to Mexican growers. Alternative methods of pest control, especially nontoxic alternatives, such as pheromones, are therefore desirable.

V. PRODUCT DESCRIPTION

The TPW mating disruption system development efforts culminated in 1982 with full EPA registration of Attract'n Kill Tomato Pinworm (A&K TPW). This is the only sex pheromone product currently registered for control of TPW. Efforts to develop a similar product are in progress at Shin-Etsu Chemical Company.

The agent A&K TPW is registered in Mexico, Florida, and California. The system is most widely used in the Culiacan valley and San Quintin regions of Mexico. Monthly applications normally begin no later than 2 weeks after transplanting. Pheromone traps and larval sampling are used to determine population levels and product longevity. Typically, trap captures will average fewer than one moth per trap a night for 3 to 4 weeks after an application. Warm temperatures increase the release rate and decrease product longevity. When captures increase to an average of five moths per trap a night, it is recommended that the pheromone be reapplied.

INTRODUCTION

The fresh market tomato industry in Sinaloa, Mexico, worth hundreds of millions of dollars annually (Buckley et al., 1986), was threatened during the 1980s by a complex of lepidopterous pests, including the tomato pinworm, *Keferia lycopersicella* Walsingham, the tomato fruitworms *Heliocoverpa zea* and *Heliothis virescens*, and the beet armyworm, *Spodoptera exigua* (Alvarado-Rodriguez, 1988). Historically, these pests have been controlled with up to 40 applications per crop of multiple, broad-spectrum insecticides such as methomyl, permethrin and fenvalerate (Brewer et al., 1990). Not surprisingly, the extensive use of these insecticides has resulted in development of substantial resistance in the pest populations (Brewer and Trumble, 1991). Other serious problems also have occurred, such as the pesticide-induced appearance of secondary pests like the vegetable leafminers, *Liriomyza trifolii* (Burgess) and *Liriomyza sativae* Blanchard (Oatman and Kennedy, 1976; Johnson et al., 1980 a, b), adverse effects on key biocontrol agents (Oatman et al., 1983; Trumble, 1985), and excessive pesticide residue levels which have prevented export to the US (Anonymous, 1979). Therefore, an obvious need existed for the development and implementation of an IPM program that could offer a more sustainable approach to pest control and minimize the potential for environmental and human health concerns while maintaining or enhancing economic viability.

In spite of a low potential for environmental damage and a very low mammalian toxicity, control programs for conventionally produced tomatoes traditionally have not included the use of microbial pesticides or alternative strategies such as parasite release or mating disruption. Historically, this lack of effort to include *Bacillus thuringiensis* Berliner or related materials in control strategies was because of the perception that high value, short term crops were not as suitable as perennial crops for incorporating use of microbial pesticides (Biever and Hostetter, 1978). Similarly, release of *Trichogramma pretiosum* Riley in tomatoes for fruitworm control, although generally efficacious (Oatman, 1988), were not widely accepted because of high cost, supply problems and ease of use of a prophylactic control program based on broad spectrum insecticides. Mating disruption techniques, while successful in controlling *K. lycopersicella* on cherry tomatoes, have had mixed success in fresh market tomatoes (Van Steenwyk and Oatman, 1983; Jenkins et al., 1990).

One of the best methods for influencing the growing practices for vegetable production is to provide a complete management approach for the pest complex rather than a short-term solution to a single pest problem (Trumble, 1990). This approach has at least two advantages. First, techniques to control resistance development can be incorporated. Second, a complete management system allows the development of low or reduced pesticide input programs which can be analyzed using available economic techniques for com-

Agriculture, Ecosystems and Environment, 00 (1992) 000–000
Elsevier Science Publishers B.V., Amsterdam

Development and economic evaluation of an IPM program for fresh market tomato production in Mexico

John T. Trumble[a] and Benito Alvarado-Rodriguez[b]

[a] *Department of Entomology, University of California, Riverside, CA, USA*
[b] *Agriculture Research Department, Campbell's-Sinaloapasta, Apdo. Postal No. 185, Guasave, Sinaloa, 81000, Mexico*

(Accepted 22 July 1992)

ABSTRACT

Trumble, J.T. and Alvarado-Rodriguez, B., 1992. Development and economic evaluation of an IPM program for fresh market tomato production in Mexico. *Agric. Ecosystem. Environ.*, 00: 000–000.

An integrated pest management (IPM) program based on intensive sampling, parasite release, use of the mating disruption technique, and applications of microbial pesticides and abamectin was developed for the fresh market tomato industry in Sinaloa, Mexico. The IPM program for tomatoes was compared with conventional practices and an unmanaged control in each of three major agricultural valleys in autumn and winter crops, and in two valleys for spring plantings. The amount of marketable fruit production was similar for all treatments in the autumn plantings, but significantly higher in the IPM program during the winter and spring plantings. The densities of *Liriomyza sativae* Blanchard and *Keferia lycopersicella* (Walsingham) eggs, larvae and adults were substantially reduced in the IPM treatment. Percent fruit damage by *Spodoptera exigua* (Hübner), *Heliocoverpa zea* (Boddie) and *Heliothis virescens* (Fabricius) was generally higher in the IPM treatment (4.2–10.9%) as compared with the conventional treatment (0.9–3.7%). However, the percent fruit damage by *K. lycopersicella* was significantly reduced in the IPM treatments (6.49–30.4%) vs. the conventional treatments (4.65–84.2%) in the winter and spring plantings. Net profits (value of fruit at harvest minus the cost of control) were substantially higher in the lower input IPM plots than in conventional treatments. In the autumn, net profits ranged from US$304 to US$579 ha^{-1} higher in the IPM treatment for carton values of US$5–US$11, respectively. In the winter and spring plantings, only the IPM approach was profitable. The IPM program offers substantial, long-term benefits in comparison with the conventional approach. Not only was the cost of the IPM program considerably less, but it: (1) reduced the potential for pesticide resistance development; (2) reduced the possibility of potential mammalian toxicity or non-target effects by using less toxic pesticides that are specific in activity; (3) provided less chance for fruit contamination or environmental damage.

Correspondence to: Professor John T. Trumble, Department of Entomology, University of California, Riverside, CA 92521, USA.

TABLE 2

Control costs (US$) for fresh market tomato production in Guasave

Treatment	No. applications	Cost per application ha	Total cost
Autumn planting			
Conventional	35	35.30	1235.50
IPM			
Avermectin	1	80.40	80.40
Pheromones	3	76.80	230.40
Trichogramma	9	2.30	20.70
B. thuringiensis	1	17.00	17.00
		Total	348.50
Winter Planting			
Conventional	35	35.30	1235.50
IPM			
Avermectin	5	80.40	402.00
Pheromones	4	76.80	306.00
Trichogramma	8	2.30	18.40
B. thuringiensis	9	17.00	153.00
		Total	879.40
Spring Planting			
Conventional	35	35.30	1235.50
IPM			
Avermectin	8	80.40	643.20
Pheromones	4	79.80	306.00
Trichogramma	5	2.30	11.50
B. thuringiensis	5	17.00	85.00
		Total	1045.70

phia, PA) at 0.5 l ha^{-1}. Every attempts was made to follow application programs used by the commercial fresh market tomato industry in each valley, but numbers of applications vary between growers and numbers of compounds included in each tank mix are similarly variable. Growers that spray less frequently may use more chemical in each application. Thus, the conventional treatment represents the best approximation in both efforts and costs of an average control program. Sprays were applied with a backpack sprayer following local commercial practices.

The second treatment was designed as a lower input IPM system. This treatment included mass releases of *T. pretiosum* for fruitworm suppression, which have proved successful on processing tomatoes in the area (Elizondo-Alapisco and Alvarado-Rodriguez, 1988). Five to nine releases of 100 000 *T. pretiosum* per release were made on each plot. The parasites were provided as pupae within *Sitotroga* sp. eggs glued on small cards, and these cards were clipped to foliage systematically throughout the plot *Bacillus thuringiensis*

MATERIALS METHODS

Fresh market tomatoes are grown in the Culiacan, Guasave and Los Mochis Valleys of Sinaloa, Mexico. The region uses three discrete plantings: autumn (October–December), winter (November–February) and spring (January–May). Following a series of pilot studies in the spring of 1988, three, ¼ ha plots were established in 1988–1989 for each of the planting dates within each of the three valleys with the exception of Culiacan, where the spring planting was lost because of a misunderstanding resulting in poor agronomic practices. Each ¼ ha plot was at least 1 km from the others in each site in order to avoid interference between techniques. Selection of a treatment program for each plot was made randomly. Within each planting, each site was considered a replicate.

Three treatments were tested concurrently in each valley. The first was a conventional treatment, consisting of multiple applications (see Tables 1–3) of two broad spectrum pesticides, methamidophos (Tameron®, Bayer, Bayerwerk, Germany) at 1.0 l ha^{-1} and permethrin (Pounce®, FMC, Philadel-

TABLE 1

Control costs (US$) for fresh market tomato production in Culiacan

Treatment	No. applications	Cost per application ha⁻¹	Total cost
Autumn Planting			
Conventional	35	35.30	1235.50
IPM			
Endosulfan	1	19.80	19.80
Avermectin	3	80.40	241.20
Trichogramma	9	2.30	20.70
Pheromones	3	76.80	230.40
B. thuringiensis	2	17.00	34.00
		Total	546.10
Winter Planting			
Conventional	35	35.30	1235.50
IPM			
Endosulfan	3	19.80	59.40
Avermectin	2	80.40	160.80
Pheromones	3	76.80	230.40
Trichogramma	4	2.30	9.20
B. thuringiensis	3	17.00	51.00
		Total	510.80

were grown using local, horticultural practices including trellising. Plants were grown 0.5 m apart in rows with 1.5 m centers.

Insect population assessment

Insect populations were monitored in all treatments and locations weekly. The leafminer population was assessed acording to the methodology of Johnson et al. (1980), and consisted of using ten pupal trays per plot. *Spodoptera exigua* larval populations were assessed by inspecting 60 tomato plants randomly selected on a diagonal transect within each experimental plot. *Keferia lycopersicella* adults were monitored using three wing style pheromone traps (Wyman, 1979) per treatment per valley as described by Toscano et al. (1987). Egg densities were determined by randomly sampling 30 leaves in rows 1, 5 and 10 for each side of the experimental plot as well as an additional sample from the center of the field. The larval population of tomato pinworm in foliage was monitored by inspecting ten whole plants randomly selected from each of rows 1, 5 and 10 from each side of the experimental plot as well as a sample from the center of the field. Data were pooled within plantings for analysis of variance (ANOVA) and, if significant at the $P<0.05$ level, followed by means separation with Fisher's Protected Least Significant Difference (PLSD) test (Fisher, 1949). All analyses were conducted using Super ANOVA (Abacus Concepts, Berkeley, CA).

Marketable fruit production

Plants were harvested from 10 m sections of rows 1, 5 and 10 for each side of the experimental plot as well as an additional sample from 30 m of row from five rows in the center of the field. All orange and red fruit were harvested. Plots were harvested as long as fruit production continued (up to four times per plot). All fruit was counted and weighed. This approach avoided the underestimation of yield problems that occur when only percent fruit damage is assessed (Welter et al., 1989). Percent fruit damage assessment for tomato fruitworm and *S. exigua* consisted of inspecting 100 randomly collected fruit in each experimental plot; fruit losses were evaluated together for these insects because of difficulty in separating feeding damage by species when infestations are high. *Keferia lycopersicella* damage was monitored by inspecting 50 randomly sampled fruit each from rows 1, 5 and 10 for each side of each experimental plot and a sample of 50 fruit from five rows in the center of the field (a total of 650 fruit per plot). For all damage estimates, the number of fruit were weighed by the the damage level on each harvest date to generate a seasonal average for fruit damage by treatment. All percentage data were transformed by an arcsine square root transformation, analyzed by AN-

TABLE 3

Control costs (US$) for fresh market tomato production in Los Mochis

Treatment	No. applications	Cost per application ha	Total cost
Autumn Planting			
Conventional	35	35.30	1235.50
IPM			
Avermectin	2	80.40	160.80
Pheromones	3	76.80	230.40
B. thuringiensis	1	17.00	17.00
Trichogramma	9	2.30	20.70
			Total 428.90
Winter Planting			
Conventional	35	35.30	1235.50
IPM			
Endosulfan	1	19.80	19.80
Avermectin	8	80.40	643.20
Pheromones	4	76.80	306.00
Trichogramma	8	2.30	18.40
B. thuringiensis	8	17.00	136.00
			Total 1123.40
Spring Planting			
Conventional	35	35.30	1235.50
IPM			
Avermectin	8	80.40	643.20
Pheromones	3	76.80	230.40
Trichogramma	5	2.30	11.50
B. thuringiensis	7	17.00	119.00
			Total 1004.10

(Javelin®, Sandoz Chemical, Palo Alto, CA) was applied at 2.4 l ha^{-1} when *S. exigua* populations exceeded a threshold of one larvae per four plants. The mating disruption technique (pheromones from Scentry, Buckeye, AZ.) consisted of monthly applications of pheromones in hollow fibers mixed in a flowable polybutene adhesive. Fiber/adhesive mixtures were applied at approximately 2 m intervals along every other row with a wooden stick to transfer a small amount of mixture. The application rate was 13 g a.i. ha^{-1}. Abamectin application (Agrimec®, Merck and Co., Rahway, NJ) at 0.0135 kg a.i. ha^{-1} was made when *K. lycopersicella* larvae exceeded the threshold of three to four larvae per 3 m of row (0.25 per plant) (modified from Wiesenborn et al., 1990). In addition, up to three applications of endosulfan (Hoechst AG, Frankfurt, Germany, 35%, 1.5 l ha^{-1}) were needed (Tables 1–3) in some plots to help control outbreaks of the potato aphid, *Macrosiphon euphorbiae* (Thomas).

The third treatment was an untreated control. As with all plots, the controls

TABLE 4

Populations of immatures of leafminers and eggs of *K. lycopersicella* and percent fruit damage by lepidopterous pests for commercial, IPM and control programs

Treatment and season	*Liriomyza* spp. per tray day[a]	*K. lycopersicella* eggs per leaf	Mean % fruit damage[a]	
			S. exigua–fruitworms[b]	*K. lycopersicella*
Autumn planting				
Control	66.0a	0.030a	13.0b	17.56a
Conventional	73.3a	0.007a	1.4b	4.65a
IPM	57.8a	0.008a	10.9b	6.49a
Winter planting				
Control	31.4a	0.12a	17.3a	50.7ab
Conventional	152.9b	0.20a	3.7a	68.5b
IPM	44.6a	0.07a	9.9a	30.4a
Spring Planting[c]				
Control	10.5b	1.95ab	11.1b	69.7b
Conventional	185.1a	2.92a	0.9a	84.2a
IPM	4.1b	0.77b	4.2a	30.2c

[a] Arcsine transformation followed by ANOVA and means separation by Fisher's Protected LSD; data back transformed for presentation; means followed by the same letter are not significantly different at the 0.05 level.
[b] Fruitworms include *Heliocoverpa zea* and *Heliothis virescens*.
[c] Based on plots in Guasave and Los Mochis Valleys only.

parasites (Oatman and Kennedy, 1976). Similar results have been reported for pesticide use in other crops (Trumble, 1990). The low leafminer populations in the IPM treatment probably arose from a combination of the control provided by avermectin and the impact of the parasite populations. Although the avermectin was intended as a control agent for *K. lycopersicella*, this compound effectively controls *Liriomyza* spp leafminers with little or no impact on their associated parasites (Trumble, 1985). The lack of differences in leafminer densities in the autumn planting is difficult to explain. It is possible that the parasite population required much of this period to reach levels needed to provide adequate leafminer suppression.

Spodoptera exigua larval populations in the autumn crop followed similar trends in all treatments, and were not significantly different (mean range, 0.003–0.054 larvae per plant, $F=0.631$, d.f. $=2,6$, $P=0.564$). Similar trends were documented in the winter (mean range, 0.08–1.36 larvae per plant, $F=0.889$, d.f. $=2,6$, $P=0.459$) and spring plantings (mean range, 0.18–0.56 larvae per plant, $F=0.508$, d.f. $=2,3$, $P=0.646$). Difficulty in locating the negatively phototactic late instar larvae (Griswold and Trumble, 1985) probably accounted for the lack of statistical separation between treatments by increasing the variance in the samples.

OVA and, if F values were significant at the $P<0.05$ level, followed by means separation with Fisher's PLSD test.

Economic analyses

Production costs associated with pesticide application, and pesticide purchase were supplied by growers, agricultural chemical companies and related chemical supply warehouses. Discussions with growers indicated that the range of applications was from 20 to 45 + per crop, usually with multiple pesticides per application. The numbers of conventional applications therefore were standardized for comparative purposes in the economic analyses at 35 per crop of two pesticides. Prices for pheromones, traps and adhesive were provided by a commercial pheromone production company (Scentry, Inc.) and several growers. Prices for all products tended to vary with the amount of purchase, so for purposes of comparison, all costs were standardized for an average-sized farm (5000–7500 ha) at the levels shown in Tables 1–3. A partial budget analysis was used. Gross profits were calculated by determining the weight of marketable fruit produced per hectare using average fruit weight per 10 m of row for each planting and location (see previous section for sampling plan), dividing by 11.35 kg (the weight of fruit in a standard carton), and then multiplying the number of cartons by the dollar value per carton. The net profit is defined as the value of fruit at harvest minus the cost of control; this is not a true net profit as the horticultural costs (planting, labor, harvest, etc.) were not included. However, because each grower permanently employs a large field crew, such costs are static and vary little with insect control strategy.

RESULTS

Insect population assessment

Liriomyza sativae populations were substantially different among treatments in the winter and spring crops (Table 4). The conventional treatment consistently averaged higher numbers of pupae per tray day^{-1} than the IPM and control treatments, where *Liriomyza* populations remained steadily low throughout the growing season. Although the tomato plant can withstand considerable foliar damage without yield loss (Pastemak et al., 1979), levels observed in the conventional plots probably caused significant effects on fruit yield and size. J. Trumble and E.R. Oatman (unpublished data) found significant fruit size reductions in California when populations reached 20 pupae per tray day^{-1}.

The exceptionally high leafminer populations in the conventional treatment were probably a result of the adverse pesticidal effects on the leafminer

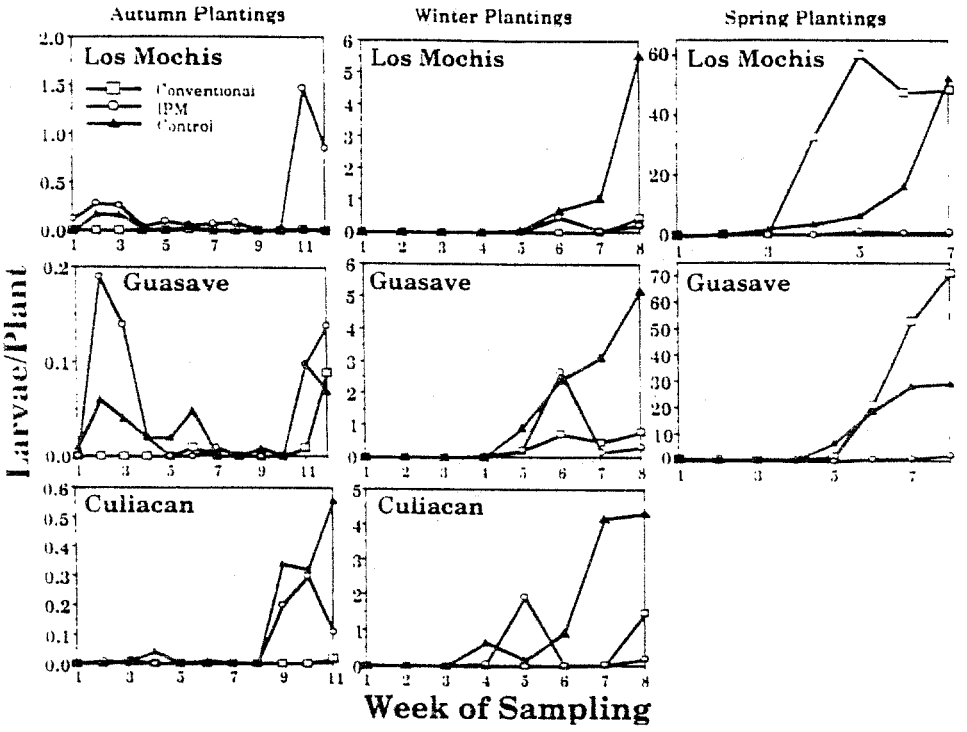


Fig. 2. Mean *K. lycopersicella* larvae per plant in commercial, IPM and control treatments for autumn, winter and spring tomato crops in Sinaloa. Sample sizes and replicate locations are listed in the methods section.

treatments were dramatically less in the spring plantings in Los Mochis and Guasave, where the conventional and control treatments exceeded 30 larvae per plant during the harvest period (Fig. 2). Analysis of data on percent parasitism of *K. Lycopersicella* eggs and larvae suggested that the generally higher levels of *K. lycopersicella* larvae in the conventional as compared with the control treatment were probably owing to undesirable pesticidal effects on biological control agents (B. Alvarado-Rodriguez and J.T. Trumble, unpublished data).

Marketable fruit production

Marketable fruit production varied between plantings and treatments (Fig. 3a). The most marketable fruit was produced in the autumn, and the least in the spring. The growers in the region suggested that this represented a typical pattern of production, which they ascribed in part to a physiological effect of the cooler temperatures occurring in winter and spring in Sinaloa. Treatment differences were significant in the winter and spring plantings, when the IPM

Keferia lycopersicella adult catches in pheromone traps were considerably reduced in the IPM treatment (Fig. 1). The economic threshold level of five moths per trap per night was only occasionally exceeded. In contrast, pheromone traps in the conventional and control treatments routinely surpassed the threshold, reaching a high of 67 moths per trap per night in the spring planting in Los Mochis and 143 moths per trap per night in the winter planting in Guasave, respectively.

Populations of *K. lycopersicella* immatures were lowest in the autumn and highest in the spring planting. Significant differences between treatments in eggs per leaf could be detected only for the spring planting, where the IPM treatment exhibited the lowest egg density ($F=12.361$, d.f. $=2,3$, $P=0.036$) (Table 4). Larval populations increased from a maximum of 1.5 per plant in the autumn to 5.6 per plant in the winter crop and over 70 per plant in the spring (Fig. 2). In the winter planting, when *K. lycopersicella* larval densities became economically important, the IPM program provided control generally equivalent to the conventional practices. Both of these approaches resulted in fewer larvae per plant than the control. Larval densities in the IPM

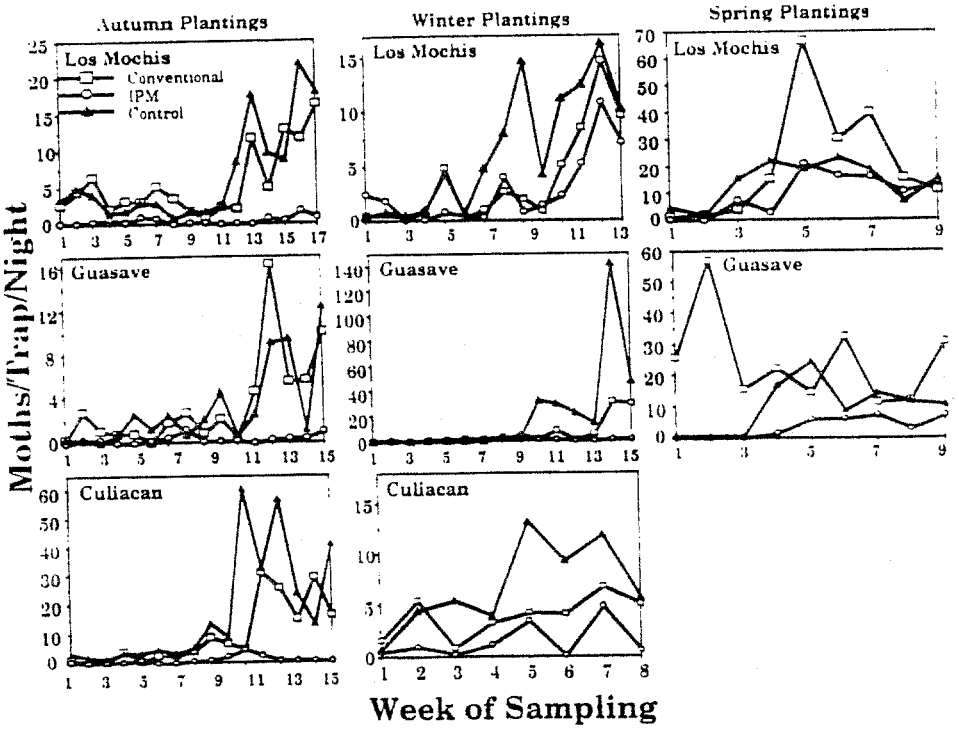


Fig. 1. Mean *K. lycopersicella* moths per trap per night collected in commercial, IPM and control treatments for autumn, winter and spring tomato crops in Sinaloa. Sample sizes and replicate locations are listed in the methods sections.

repeated applications of the two pesticides utilized in the conventional treatment did not provide significant control of this pest in comparison with the control.

The autumn planting produced the largest average fruit weight (Fig. 3b). Within each treatment, there were significant differences in average fruit weight between the autumn planting and either the winter or spring plantings (conventional treatment, $F=24.008$, d.f. $=2,5$, $P=0.003$; IPM treatment, $F=12.248$, d.f. $=2,5$, $P=0.012$; control treatment, $F=11.140$, d.f. $=2,5$, $P=0.014$); winter and spring plantings did not differ. In addition, the mean weight per fruit was not significantly different among treatments (Fig. 3b). Although the average fruit weights were not significantly different, the proportion of the fruit which could be packed into a larger size class was not determined. A weight increase averaging 12 g per fruit in the IPM treatment as compared with the conventional treatment for the spring planting may have increased the proportion of larger fruit, which usually wholesale for US$1–2US$2 more per carton. Because we did not collect size class data, potentially increased revenues for the IPM treatments from sale of a larger sized fruit were not included in the economic analyses.

Fruit damage from *S. exigua*, *Heliocoverpa zea* and *Heliothis virescens* was significantly greater in the IPM treatments compared with the conventional treatments in the autumn and spring plantings (autumn, $F=14.588$, d.f. $=2,6$, $P=0.005$; Spring, $F=26590$, d.f. $=2,3$, $P=0012$) (Table 4). Results from applications of *B. thuringiensis* were not significantly different from damage levels in the control treatments in autumn and winter plantings, but damage was significantly reduced in the spring crop (Table 4).

Economic analyses

The lower input IPM program was substantially more cost effective than the conventional approach. The cost of the IPM program was equivalent to less than 20 applications (two pesticides per application). Costs for control in the IPM treatments were generally lowest in the autumn planting, when insect population pressures were less, and highest in the winter and spring crops when *K. lycopersicella* populations reached maximum levels (Tables 1–3). Despite numerically lower amounts of marketable fruit in the IPM vs. the conventional treatment in the autumn crop, net profits (value of fruit at harvest minus the cost of control) ranged from US$304 to US$579 ha^{-1} higher in the IPM treatment than in conventional treatment for typical market values of US$5–US$11 per carton (Fig. 4). This difference resulted from the substantially reduced control costs in the IPM treatments. Although the analyses were standardized at 35 applications for each crop, growers may use fewer applications during the autumn planting than in the winter and spring (B. Alvarado-Rodriguez, personal communication). This practice serves to re-

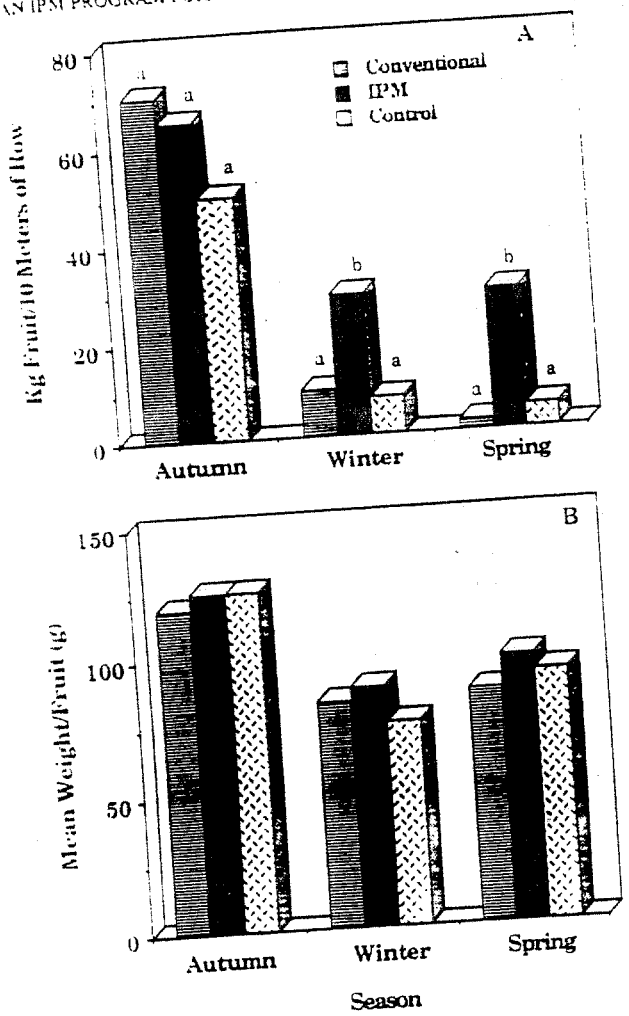


Fig. 3. Mean marketable fruit per 10 m of row (A) and mean fruit weight (B) produced in commercial, IPM and control treatments for autumn, winter and spring tomato crops in Sinaloa. Sample sizes and replicate locations are listed in the methods section. Letters above bars in A refer to means separation by Fisher's PLSD test.

treatments produced significantly more fruit than the conventional or control treatments (winter, $F=5.372$, d.f. $=2,6$, $P=0.046$; spring, $F=9.590$, d.f. $=2,3$, $P=0.049$). Most of this improvement came from the improved control of the tomato pinworm in the IPM system (Table 4). Damage levels were over twice as high in the control and conventional treatments as in the IPM treatments. The combination of avermectin and pheromones in the IPM treatment did not provide complete control and improvements are needed, but the results were far superior to the standard chemical control procedures. Clearly, even

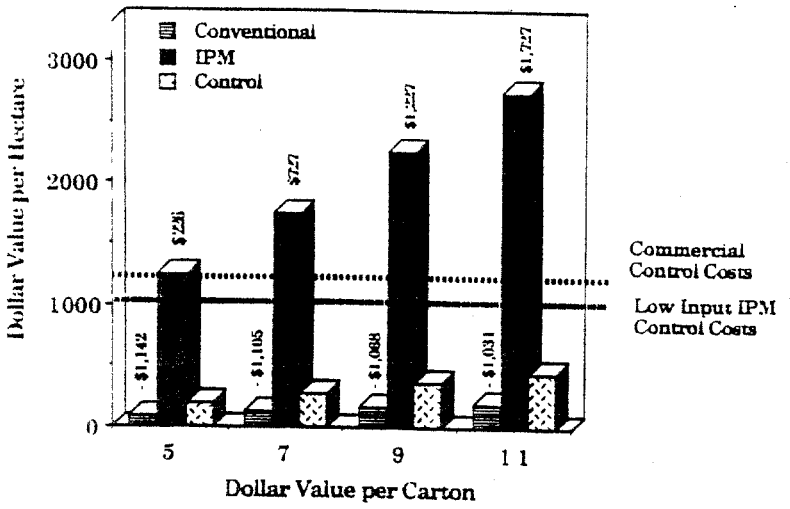


Fig. 6. Net profit analysis from the spring planting in Sinaloa. The height of the bars indicates the value of the crop as the market value (dollar value of the cartons) changes. The dotted lines show the cost of the commercial and the IPM control strategies. The net profits (value of the crop minus the cost of pest control) are shown directly above the commercial and IPM bars.

profits were greater. In fact, in the experimental plots, only the IPM appraoch was profitable. The combination of reduced pest control costs and increased fruit production resulted in a net profit differential between the conventional and IPM treatments ranging from US$1233 to US$2243 in the winter planting and US$1368 to US$2758 in the spring crop for US$5–US$11 per carton markets.

DISCUSSION

The IPM program documented in this study is probably not in its most efficient form because it was designed conservatively. This approach was necessary because it has been the authors' experience that persuading growers to risk profits on a program which has previously failed is a difficult proposition: growers of relatively short term, high value crops such as vegetables tend to be conservative when considering pest control. Additionally, because the low cost of field labor allowed substantial efforts for sampling purposes, sampling plans used to assess insect populations in the IPM program were designed to provide information on insect dispersion as well as simple population intensity. Therefore, the use of more efficient stratified or sequential sampling plans for some of the insects (Zehnder and Trumble, 1985; Shelton and Trumble, 1990) could increase the efficiency and economic viability of the IPM program.

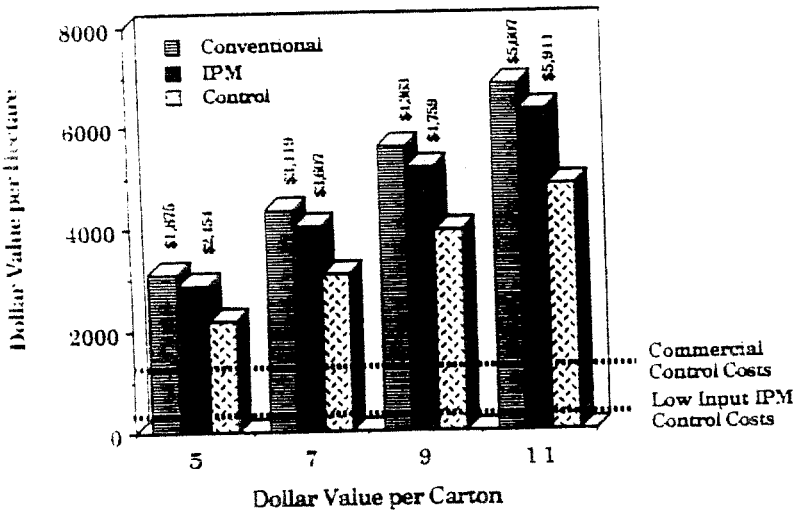


Fig. 4. Net profit analysis from the autumn planting in Sinaloa. The height of the bars indicates the value of the crop as the market value (dollar value of the cartons) changes. The dotted lines show the cost of the commercial and the IPM control strategies. The net profits (value of the crop minus the cost of pest control) are shown directly above the commercial and IPM bars.

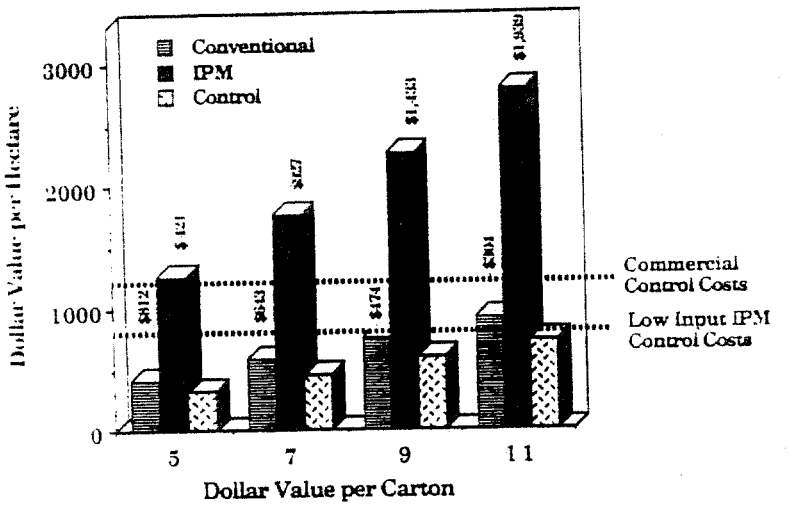


Fig. 5. Net profit analysis from the winter planting in Sinaloa. The height of the bars indicates the value of the crop as the market value (dollar value of the cartons) changes. The dotted lines show the cost of the commercial and the IPM control strategies. The net profits (value of the crop minus the cost of pest control) are shown directly above the commercial and IPM bars.

duce the net profit differential between the IPM and conventional treatments, assuming damage in the conventional treatments was not increased.

In the winter and spring plantings (Figs. 5 and 6), the differences in net

in processing tomatoes for the past two growing seasons, did provide information which suggests the IPM approach has been effective. Data from the Sinalopasta company farm showed damage levels in fruit declining from 8.5% (2300 ha on standard conventional practices) in 1988–1989 to 2.2% (3000 ha on the IPM program) and 2.7% (1800 ha on the IPM program) in 1989–1990 and 1990–1991, respectively. This drop in percent damage occurred in spite of a 60% reduction in pesticide use from 2.45 kg a.i. ha^{-1} in 1988–1989 to 0.99 kg a.i. ha^{-1} in 1989–1990, and 0.84 kg a.i. ha^{-1} in 1990–1991. Recognizing that population variation between years could account for the differences in percent damage and pesticide use, an untreated 10 ha plot was established in 1990–1991 to document *K. lycopersicella* populations. Fruit from this untreated plot showed 71.2% damage. Thus, the available data suggest that the IPM program will function economically in large scale commercial plantings.

The IPM program offers several additional benefits beyond the short-term economic gains. First, use of this program minimizes the potential for resistance development by reducing the amount of pesticide applied and by maximizing the impacts of the beneficial arthropods. Second, the program dramatically reduces the use of pesticides with high mammalian toxicity: methamidophos has an oral LD_{50} of approximately 13 mg kg^{-1} vs. abamectin, which has a contact toxicity in excess of 5000 mg kg^{-1}. Third, the reduced pesticide pressure provides less change for fruit contamination. Fourth, the overall reduction in pesticide application serves to minimize the possibility of subvisual phytotoxic effects of pesticides on tomatoes (Welter et al., 1989).

ACKNOWLEDGMENTS

The authors appreciate the advice and support from the Confederacion de Asociaciones Agricolas del Estado de Sinaloa and Dr. Jose Rodriguez, the permission of F.J. Navarro and Dr. J.M. Ramirez Diaz for use of Campo Experimental land, and the gifts of control materials from Scentry, Inc., Sandoz Crop Protection, and Merck & Co. The Centro Reproductivo de Insectos Beneficos-GUASAVE kindly provided the *T. pretiosum*. The data and support of the Campbell Institute for Research and Technology (CIRT) and the Campbell's–Sinalopasta Co. are appreciated. The authors also appreciate the continuous support and encouragement of former CIRT vice President Dr. Allen M. Stevens, as well as the personal support and inspiration of Dr. Edwardo Alvarez Luna. Appreciation also is expressed to Drs. D. Schuster, M. Blua, T. Paine and M. Diawara for improvements to earlier drafts of the manuscript.

Documenting the cost effectiveness of each of the components of the IPM program was not possible given the data collection methods. However, conclusions can be drawn regarding the usefulness of the mating disruption technique and the application of *B. thuringiensis*, two practice which have historically generated variable results (Biever and Hostetter, 1978; Baker et al., 1990; Silverstein, 1990). The mating disruption technique used in the IPM treatments consistently resulted in the fewest captures of *K. lycopersicella* adults of all the treatments (Fig. 1). Unfortunately, such reductions in adult collections do not ensure reduced fruit damage (Van Steenwyk and Oatman, 1983). However, both egg densities (Table 4) and larval populations (Fig. 2) in the IPM treatments were reduced in comparison with the conventional program, particularly during the spring planting when *K.lycopersicella* densities were at maximum. Although some of the larval mortality was almost certainly because of the effects of abamectin application, this compound is not ovicidal and has not been implicated as an oviposition deterrent (R. Brown, personal communication, 1991). Thus, given that the research plantings were relatively small (2 ha per valley) and subject to exposure to immigration of previously mated female moths, the apparent success of this approach on a limited scale suggests that the technique would likely be more effective as the treated acreage increases (Rothschild, 1981).

Bacillus thuringiensis was useful in the IPM program because it does not kill the key parasite species which control *Liriomyza* species, it is non-toxic to humans and environmentally safe (Trumble, 1990), and helps reduce the fruit damage by lepidopterous pests. A range of *B. thuringiensis* isolates and formulations have recently shown improved efficacy for *S. exigua* suppression (Moar and Trumble, 1990). As such new products achieve registration, their incorporation into the IPM program will improve the economic viability of this approach.

The effective implementation of the IPM program in Sinaloa faced some major constraints. Because tomatoes represent an important balance-of-trade item with the US (Buckley et al., 1986; Ramirez Diaz, 1988), and because the fresh market tomato industry is a major employer in the region, the industry is often subsidized by the Mexican government. The IPM program therefore had to produce at least as much fruit as current practices and employ as many people. By reassigning some of the permanent field crew from physical removal of infested leaves and pesticide application to monitoring, most of these jobs could be maintained.

Unfortunately, getting proprietary information from tomato packing operations to determine if the IPM program has been a commercial success has proven difficult. Although some growers have verbally stated that the IPM program has been successfully implemented, the data necessary to document the claims have not been forthcoming. However, the Campbell's–Sinalopasta Corporation in the state of Sinaloa, which has been using the IPM program

H.W., 1983. Parasitization of lepidopterous pests on fresh market tomatoes in southern California. J. Econ. Entomol., 76: 452–455.

Pastemak, D.M., Twersky, M. and DeMalach, Y., 1979. Salt resistance in agricultural crops. In: Mussell and Staples (Editors). Stress Physiology in Crop Plants. Wiley, New York, pp. 127–142.

Ramirez Diaz, J.M., 1988. Investigacion sobre plagas de tomate en Sinaloa. In: Taller Sobre el Manejo Integrado de Plagas en Tomate. CAADES, Culiacan, Sinaloa, Mexico, 1: 178–182.

Rothschild, G.H.L., 1981. Mating disruption of lepidopterous pests: current status and future prospects. In: E.R. Mitchell (Editor), Management of Insect Pests with Semiochemicals. Plenum Press, NY, pp. 207–228.

Shelton, A.M. and Trumble, J.T., 1990. Monitoring insect populations. In: D. Pimintel (Editor), Handbook of Pest Management in Agriculture, 2nd Edn., Vol. 2. CRC Press, Boca Raton, FL, pp. 45–62.

Silverstein, R.M., 1990. Practical use of pheromones and other behavior-modifying compounds: overview. In: R.L. Ridgway, R.M. Silverstein and M.N. Inscoe (Editors), Behavior-Modifying Chemicals for Insect Management. Marcel Dekker, New York, pp. 1–8.

Toscano, N.C., Youngman, R.R., Oatman, E.R., Phillips, P.A., Jimenez, M. and Munoz, F., 1987. Implementation of an integrated pest management program for fresh market tomatoes. Appl. Agric. Res., 1: 315–324.

Trumble, J.T., 1985. Integrated pest management of *Liriomyza trifolii*: Influence of avermectin, cyromazine, and methomyl on leafminer ecology in celery. Agric. Ecosystems Environ., 12: 181–188.

Trumble, J.T., 1990. Vegetable insect control with minimal use of insecticides. J. HortScience, 25: 159–164.

Van Steenwyk, R.A. and Oatman, E.R., 1983. Mating disruption of tomato pinworm (Lepidoptera: Gelichiidae) as measured by pheromone trap, foliage, and fruit infestation. J. Econ. Entomol., 76: 80–84.

Welter, S.C., Johnson, M.W., Toscano, N.C., Perring, T.M. and Varela, N., 1989. Herbivore effects on fresh and processing tomato productivity before harvest. J. Econ. Entomol., 82: 935–941.

Wiesenborn, W.D., Trumble, J.T. and Oatman, E.R., 1990. Economic comparison of insecticide-treatment programs for managing tomato pinworm (Lepidoptera: Gelichiidae) on fall tomatoes. J. Econ. Entomol., 83: 212–216.

Wyman, J.A., 1979. Effect of trap design and sex attractant release on tomato pinworm catches. J. Econ. Entomol., 72: 865–868.

Zehnder, G.W. and Trumble, J.T., 1985. Sequential sampling plants with fixed levels of precision for *Liriomyza* species (Diptera: Agromyzidae) in fresh market tomatoes. J. Econ. Entomol., 78: 138–142.

REFERENCES

Alvarado-Rodriguez, B., 1988. Manejo integrado de plagas en tomate industrial en Sinaloa. In: Taller Sobre el Manejo Integrado de Plagas en Tomate. CAADES, Culiacan, Sinaloa, Mexico, 1: 33–41.

Anonymous, 1979. Results of FDA surveillance of Mexican produce for pesticide residues (compliance program 7305.008). Bureau of Foods and Office of Assoc. Comm. for Reg. Affairs, FDA Admin., April 29, Washington, DC.

Baker, T.C., Staten, R.T. and Flint, H.M., 1990. Use of pink bollworm pheromone in the southwestern United States. In: R.L. Ridgway, R.M. Silverstein and M.N. Inscoe (Editors), Behavior-Modifying Chemicals for Insect Management. Marcel Dekker, New York, pp. 417–436.

Biever, K.D. and Hostetter, D.L., 1973. Use of entomopathogens in pest management systems for vegetables. In: G.E. Allen, C.M. Ignoffo and R.P. Jaques, (Editors), Microbial Control of Insect Pests: Future Strategies in Management Systems. Natl. Sci. Foundation, USDA Workshop, Univ. Florida, Gainesville, pp. 186–194.

Brewer, M.J. and Trumble, J.T., 1991. Classifying resistance severity in field populations: Sampling inspection plans for an insecticide resistance monitoring program. J. Econ. Entomol., 84: 379–389.

Brewer, M.J., Trumble, J.T., Alvarado-Rodriquez, B. and Chaney, W., 1990. Beet armyworm, (Lepidoptera: Noctuidae) adult and larval susceptibility to fenvalerate, permethrin, and methomyl in managed habitats and selection experiments. J. Econ. Entomol., 83: 2136–2146.

Buckley, K.C., van Sickle, J.J., Bredahl, M.E., Belibasis, E. and Gutierrez, N., 1986. Florida and Mexico competition for the winter fresh market. USDA Agric. Econ. Rep. No. 556.

Elizondo-Alapisco, D. and Alvarado-Rodriguez, B., 1988. Uso de avispitas *Trichogramma pretiosum* en tomate industrial en Sinaloa. In: Taller Sobre el Manejo Integrado de Plagas en Tomate. CAADES, Culiacan, Sinaloa, Mexico, 1: 100–106.

Fisher, R.A., 1949. The Design of Experiments. Oliver and Boyd, Edinburgh.

Griswold, M.J. and Trumble, J.T., 1985. Responses of *Spodoptera exigua* (Lepidoptera: Noctuidae) larvae to light. Environ. Entomol., 14: 650–653.

Jenkins, J.W., Doane, J.C., Schuster, D.J., McLaughlin, J.R. and Jimenez, M.J., 1990. Development and commercial application of sex pheromone for control of the tomato pinworm. In: R.L. Ridgway, R.M. Silverstein and M.N. Inscoe (Editors), Behavior-Modifying Chemicals for Insect Management. Marcel Dekker, New York, pp. 269–280.

Johnson, M.W., Oatman, E.R. and Wyman, J.A., 1980a. Effects of insecticides on populations of the vegetable leafminer and associated parasites on summer pole tomatoes. J. Econ. Entomol., 73: 61–66.

Johnson, M.W., Oatman, E.R. and Wyman, J.A., 1980b. Effects of pesticides on populations of the vegetable leafminer and associated parasites on fall pole tomatoes. J. Econ. Entomol., 73: 67–71.

Johnson, M.W., Oatman, E.R., Wyman, J.A. and van Steenwyk, R.A., 1980. A technique for monitoring *Liriomyza sativae* in fresh market tomatoes. J. Econ. Entomol., 73: 552–555.

Moar, W.J. and Trumble, J.T., 1990. Comparative toxicity of the *Bacillus thuringiensis* strains and formulations against *Spodoptera exigua* (Lepidoptera: Noctuidae). Fl. Entomol., 73: 195–197.

Oatman, E.R., 1988. Biological and integrated control of lepidopterous pests on tomato in California. In: Taller Sobre el Manejo Integrado de Plagas en Tomate. CAADES, Culiacan, Sinaloa, Mexico, 1: 96–97.

Oatman, E.R. and Kennedy, G.C., 1976. Methomyl-induced outbreak of *Liriomyza sativae* on tomato. J. Econ. Entomol., 69: 667–668.

Oatman, E.R., Platner, G.R., Wyman, J.A., van Steenwyk, R.A., Johnson, M.W. and Browning,

2. *The Pear Pest Management System Continuum in California: A Case Study*

Pear and apple producers in California's Sacramento and San Joaquin Valley have struggled for years with a dominant insect pest—the codling moth. In the 1980s, most farmers settled on the organophosphate Guthion (azinphos-methyl) as the insecticide of choice. But because of repeated use of this pesticide over a wide area, resistance began to emerge in the late 1950s. Pest management professionals like Pat Weddle, an independent IPM consultant, knew that steps had to be taken quickly to keep his clients in business. Returning to calendar spraying of broad-spectrum insecticides was not an option in this environmentally sensitive area (Weddle, 1994).

A search ensued for new technologies that would lessen reliance on Guthion as a way to slow, or even reverse Guthion resistance (see the box "Resistance Drives Search for Alternatives" and also Chapter 1). Codling moth mating disruption (CMMD) emerged as the most promising new approach, and since the late 1980s has made possible major strides toward biointensive IPM in pear and apple production regions from California, to the Hood River region in Oregon, Washington State, the Kelowna area of British Columbia, South Africa and Italy's Poa River Valley. (For general overviews of pheromone-based management systems, see Barnes et al., 1992; Howell et al., 1992; Kirsch, 1988).

To gain a perspective on the IPM adoption process, we commissioned Pat Weddle to describe the pear IPM systems now in place in the Sacramento and San Joaquin Valleys. The tables and figures that follow cover pear pest management and draw upon the records of Weddle, Hansen, and Associates, as well as other crop consultants in this region who together cover a significant percentage of the area's pear acreage.

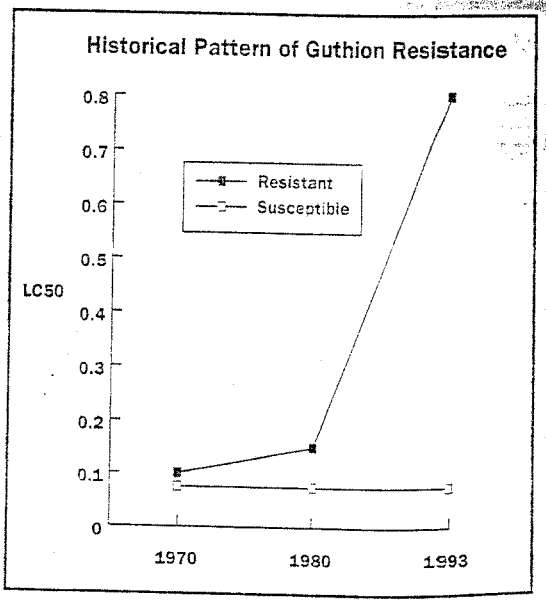
Pat Weddle estimates that in 1996 about 15 to 20 percent of pear acreage in the Delta and Valley region was managed using conventional, chemical-intensive systems; between 50 and 60 percent under "Low" level IPM; 20 to 30 percent under "Medium" level, or transitional IPM systems; and, from 5 to 10 percent under biointensive IPM. Accordingly, based on Pat Weddle's assessment of

Resistance Drives Search for Alternatives

In the early 1990s, 93 percent of California's pear acreage was sprayed three to five times with Guthion (azinphos-methyl). On average, a total quantity of 3.77 pounds of a.i. per acre was applied (Gianessi and Anderson, 1995). Guthion poses significant risks to farm workers and non-target organisms and is one of the most frequently detected pesticide residues in pears.

Resistance is monitored by tracking changes in the "Lethal Concentration" (LC) of pesticides like azinphos-methyl. The LC-50 is the concentration needed to kill 50 percent of codling moths in a laboratory assay. Traps are used every season in commercial orchards to monitor moth population levels. Some insects are captured and taken to the laboratory where they are tested for susceptibility to Guthion and other commonly used insecticides.

LC-50 values have risen more than eight-fold since the late 1980s, as shown in the graph (Weddle, 1994). Codling moth trap counts (an indicator of pest population size) started going up in 1988 despite heavier and more frequent applications of Guthion in California's Sacramento River Delta region. Monitoring and field research were intensified, and a regional project was started in 1994 by growers, pest management consultants and University of California researchers that became known as the Randall Island Project (see Chapter 1).



Source: Weddle, 1994.

Pest Management at the Crossroads

by

Charles M. Benbrook, Ph.D.

with

Edward Groth III, Ph.D.

Jean M. Halloran

Michael K. Hansen, Ph.D.

Sandra Marquardt

Consumers Union

Yonkers, New York

1996

Table 7.1 California Pear IPM Scouting and Pesticide Application Criteria

	No IPM	Transitional Systems		High or Biointensive IPM
		Low	Medium	
Scouting for Major Pests	Limited visual inspections for major pests; no or minimal effort in trapping codling moths and monitoring population levels	Moth traps, plus non-systematic visual spot checking for mite, psylla and other pests; 1 to 2 traps per 40 to 80 acres	Moth traps, systematic monitoring of multiple pests and beneficials (phenology, population histories, statistical analysis of trends); 1 to 2 traps per 20 to 40 acres	Intensive trapping and systematic monitoring of pests and beneficials, plus statistical analyses of factors affecting pest-beneficial interaction; 1 to 2 traps per 10 to 20 acres
Pesticide Application Criteria	CRITERION A: Pesticides applied routinely, often on a calendar schedule	CRITERION B: Pesticide applications timed for optimal efficiency and based on scouting data and presence of target pests	CRITERION C: Criterion B, plus increased use of biopesticides and selection of pesticides/timing of applications to minimize adverse impacts on beneficials; aggressive resistance management	Criterion C, plus mating disruption and use of biopesticides when needed; avoidance of broad-spectrum chemicals; release of natural enemies and other steps to augment populations of beneficials

Source: Pat Weddle, pear pest management case study for Consumers Union.

also treated with CMMD—on average nearly an 85 percent reduction from pre-CMMD levels. Part of the remaining 10 percent of acreage was not sprayed at all, and part was sprayed more often, typically because the blocks, or portions of blocks, are small or isolated from larger areas treated with mating disruption (CMMD works best in large, contiguously treated areas).

Reliance on and dose equivalents of broad-spectrum toxic pesticides in biointensive pear pest management systems in California are well less than one-quarter of the levels typical of pest management systems at the "No" and "Low" end of the pear IPM continuum.

Table 7.2 presents data showing the change in reliance on pesticides as progress is made along the continuum. "Dose Equivalents" are a measure of the number of times the full label rate of a pesticide was sprayed in the orchard. It equals total pounds applied over all applications divided by the maximum allowable one-time rate of application. Synthetic pesticides used by pear growers in the region include herbicides; the insecticides Guthion, Lorsban (chlorpyrifos) and various pyrethroids; and when needed (as in 1996), abamectin for fire blight control. Biopesticides include oils, *Bt*, and the codling moth pheromone. Table 7.2 reports both dose equivalents and the number of applications, because application costs are a significant share of total costs, as shown in the bottom line in of the table.

IPM adoption in San Joaquin Valley pear production, growers already have achieved the USDA's goal of 75 percent adoption of IPM, which encompasses all three levels of IPM adoption.

As argued in the next chapter, we think "Low" level IPM is essentially just using pesticides cost-effectively and typically includes too few preventive practices to lessen reliance on pesticides. For this reason, in Chapter 9 we call for reconsideration of both the IPM adoption goal in agriculture and how its attainment will be monitored.

Early adopters in the Valley improved the cost-effectiveness of CMMD, learning from mistakes and each season's unique combination of circumstances. According to Weddle, "Every season is an adventure but every year we learn more..." that will make the transition easier for others in the future.

Based on his clients' experience, Weddle estimates that the transition from chemical control to "Low" level IPM takes about one year; another one to two years is needed to progress into the "Medium" zone. He projects that it commonly takes another two to three years, at a minimum, to make the final transition to biointensive IPM. Few growers in the Valley have progressed from "No" IPM to biointensive IPM in less than five years, but he feels it can be done if growers are committed to making the change and are able to get the assistance needed. The transition is likely to be smoother in the future compared to five to seven years ago, when mating disruption was first introduced to the Valley. Early innovators have worked many kinks out of CMMD technology, learning from mistakes and each season's unique combination of circumstances. According to Weddle, "Every season is an adventure but every year we learn more."

Yields on pear farms in the "Low," "Medium" and biointensive zones generally fall in the 12 to 25 tons per acre range (the difference is largely a function of tree density and health). Orchards at the chemical-intensive end of the continuum generally harvest 10 to 15 tons per acre. Table 7.1 summarizes the basic scouting and pesticide application criteria used by pear pest managers at various stages along the IPM continuum.

System Performance

After three years of CMMD implementation, pear growers in the Randall Island Project, all of whom are in the "Medium" or biointensive zones along the continuum, have achieved impressive results. The 1995 pear harvest moved into packing sheds with an average of less than one percent worm damage. While some blocks had higher damage, few exceeded 1.5 percent.

The efficacy achieved with CMMD is at least comparable to, and in many instances better than that achieved in orchards still largely reliant on Guthion, in which three to five applications were made, totaling 4 to 7.5 pounds a.i. per acre per season. Randall Island Project participants applied only about 0.5 to 0.7 pound of a.i. per acre over approximately 90 percent of the acreage

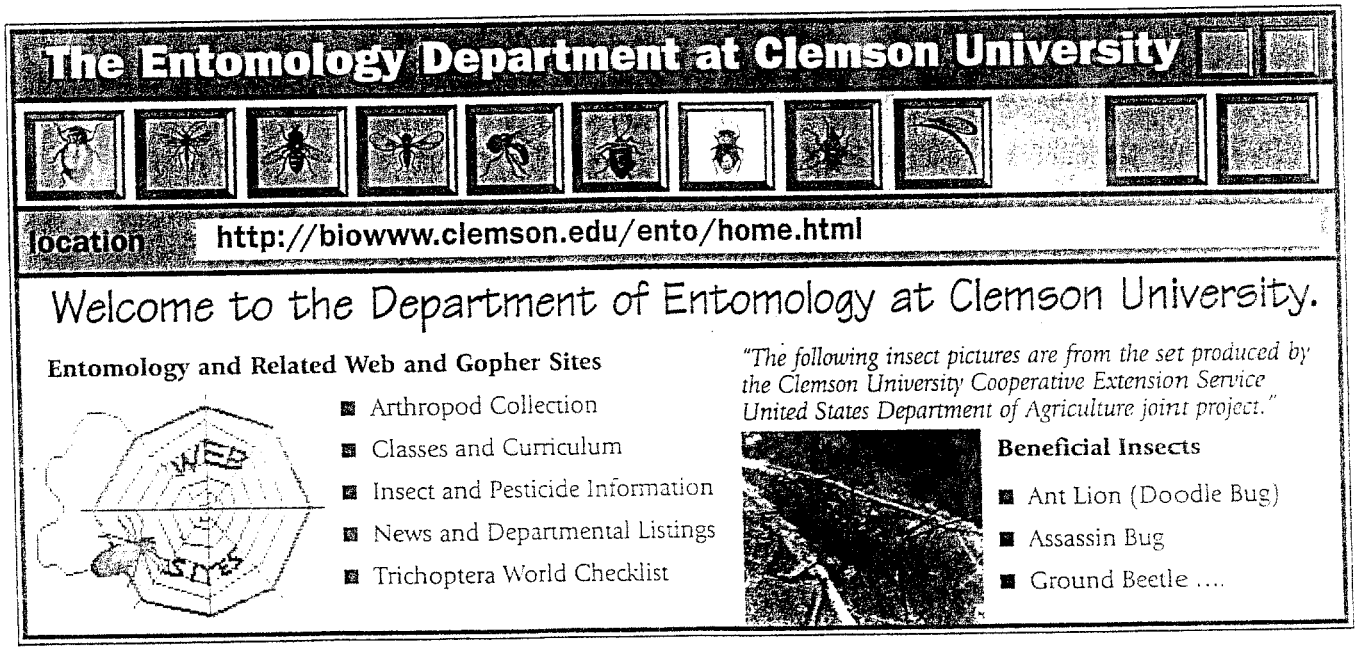
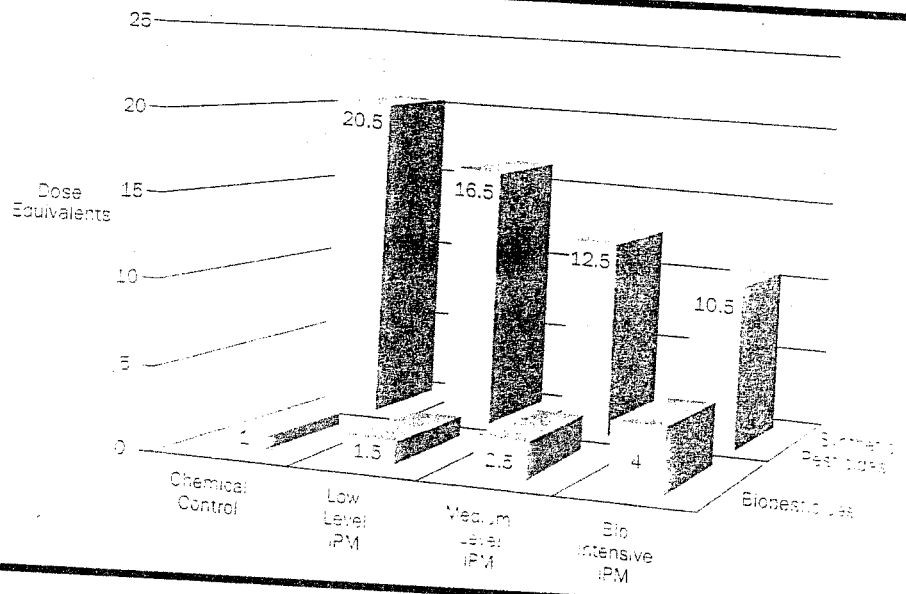
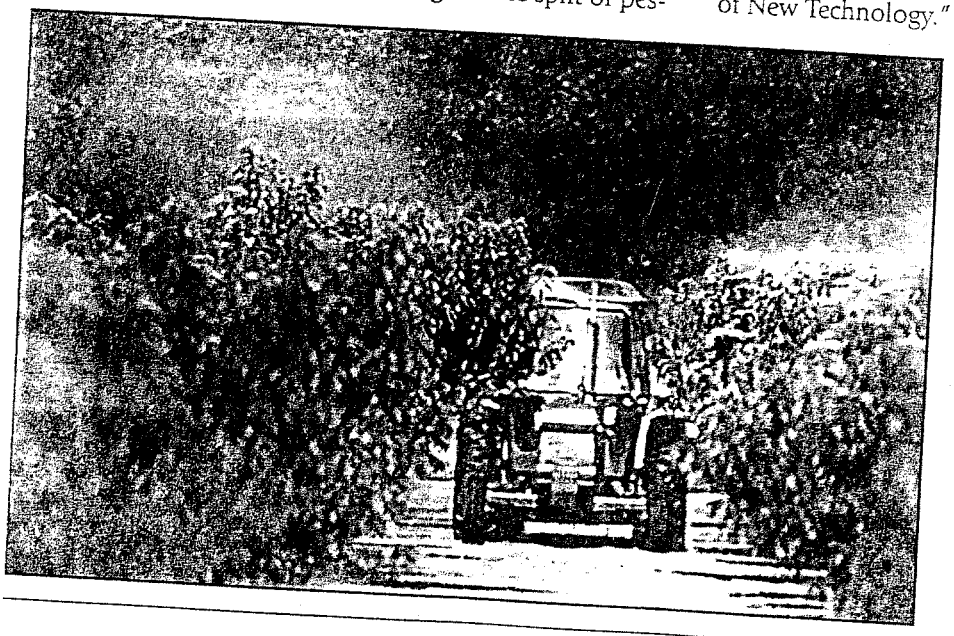


Figure 7.2 Comparison of Synthetic Pesticide and Biopesticide Dose Equivalents* Along the IPM Continuum: California Pear Producers (Number of Dose Equivalents Per Acre)



*In this example, one dose equivalent for a pesticide active ingredient is application of the full label rate on one acre, possibly over more than one applications. (For example, two applications at one-half the label rate equals one dose equivalent).
Source: Pat Weddle.

pest management expenditures but it does shift the distribution of expenditures. By far the biggest change behind the numbers in Table 7.2 is the shift from reliance on Guthion to use of mating disruption. This shift also accounts for a large change in the toxicity and environmental impacts of pesticides applied in the orchard, as well as significant change in the split of pesticide expenditures between synthetic and biopesticide products. As in all biointensive IPM systems, the effort to discover and apply new pest management technologies must be ongoing to improve effectiveness and lower costs. Some of the more promising CMMD technical innovations are reviewed in the box "Lowering the Cost of New Technology."



Peach and pear growers have reduced the pounds of pesticide applied by adopting "alternate row spraying." A high-pressure airblast sprayer is used to gain good coverage of trees in two rows. Then the sprayer moves over two full rows instead of one for its next pass. This method of spraying cuts the amount of pesticide needed per acre by about 20 percent to 40 percent. Credit: Keith Weller, ARS

Table 7.2 California Pear Pesticide Use and Pest Management Cost Summary Along the IPM Continuum

	No IPM	Low IPM	Medium IPM	High IPM
Dose Equivalents (Number)				
Synthetics	14-27	13-20	10-15*	9-12*
Biopesticides	1	1-2	1-4	2-6
Application Equivalents (Number)				
Synthetics	10-15	10-15	10-15*	10-15*
Biopesticides	0**	0**	1	1-2
Synthetics Range lbs a.i./acre	25-40	20-40	10-25	6-20
Best Estimate a.i./acre	35	30	20	13
Cost Ranges ($/Acre)				
Materials	$320-$532	$250-$385	$193-$473	$224-$500
Average $/Acre	$425	$320	$330	$360
Applications	$150-$225	$150-$225	$150-$245	$150-$265
Average $/Acre	$190	$190	$198	$207
Scouting	0	$10-20	$20-40	$40-50
Average $/Acre	0	$15	$30	$45
Total Cost Range	$470-$757	$410-$630	$363-$738	$414-$815
Average $/Acre	$615	$525	$558	$612

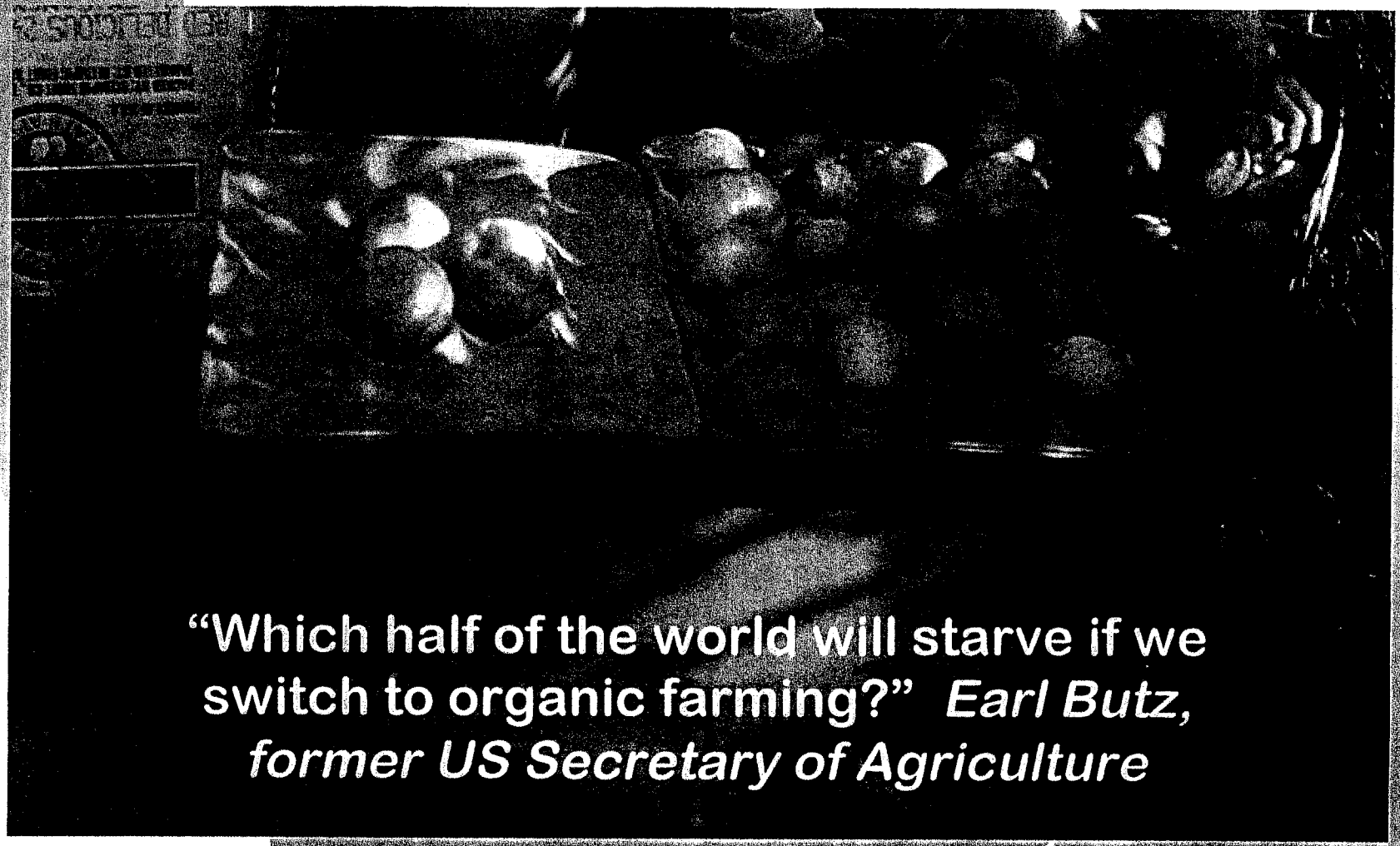
* Heavy spray oil equivalent for mite control.
** Biopesticides applied with synthetic products in tank mixes.
Source: Pat Weddle, case study.

As progress is made along the IPM continuum, the intensity and scope of scouting increases, as do scouting costs. The table shows scouting expenditures per acre rising from nothing under "No" IPM (chemical company representatives, or Pest Control Advisors [PCAs] working for businesses selling pesticides typically scout at no extra charge), to $45.00 per acre under biointensive IPM. This increase in scouting costs, though, is more than offset by the reduction in expenditures on pesticides.

In the world of pest management, the advances envisioned for CMMD could make this approach to codling moth management an example of precision farming at its best.

Figure 7.2 highlights the shift in reliance—measured as dose equivalents—from synthetic pesticides to biopesticides as progress is made along the pear pest management continuum. Under chemical control, over 20 dose equivalents are needed on the average acre, and almost no use is made of biopesticides; under biointensive IPM, the average number of synthetic dose equivalents has dropped by almost half, from 20.5 to 10.5 (including several dose equivalents of non-toxic spray oils for mite control), and the average four dose equivalents of biopesticides is almost a third of the total number of pesticide dose equivalents. Reliance on and dose equivalents of broad-spectrum toxic pesticides in the biointensive system are well below one-quarter of the levels typical of pest management systems at the "No" and "Low" end of the pear IPM continuum.

In working with clients, Weddle stresses a key point that is clear in Table 7.2 – the transition along the IPM continuum generally does not significantly change total



"Which half of the world will starve if we switch to organic farming?" *Earl Butz, former US Secretary of Agriculture*

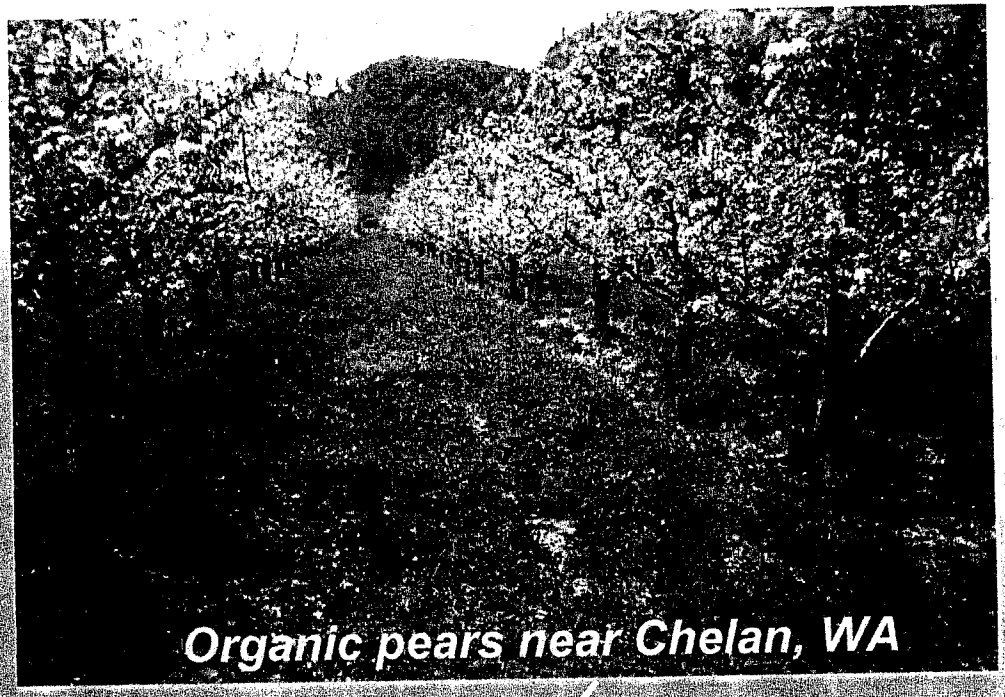
COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY

World Class. Face to Face.

COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY

World Class. Face to Face.

Recent Trends in Organic Tree Fruit Production: 2001



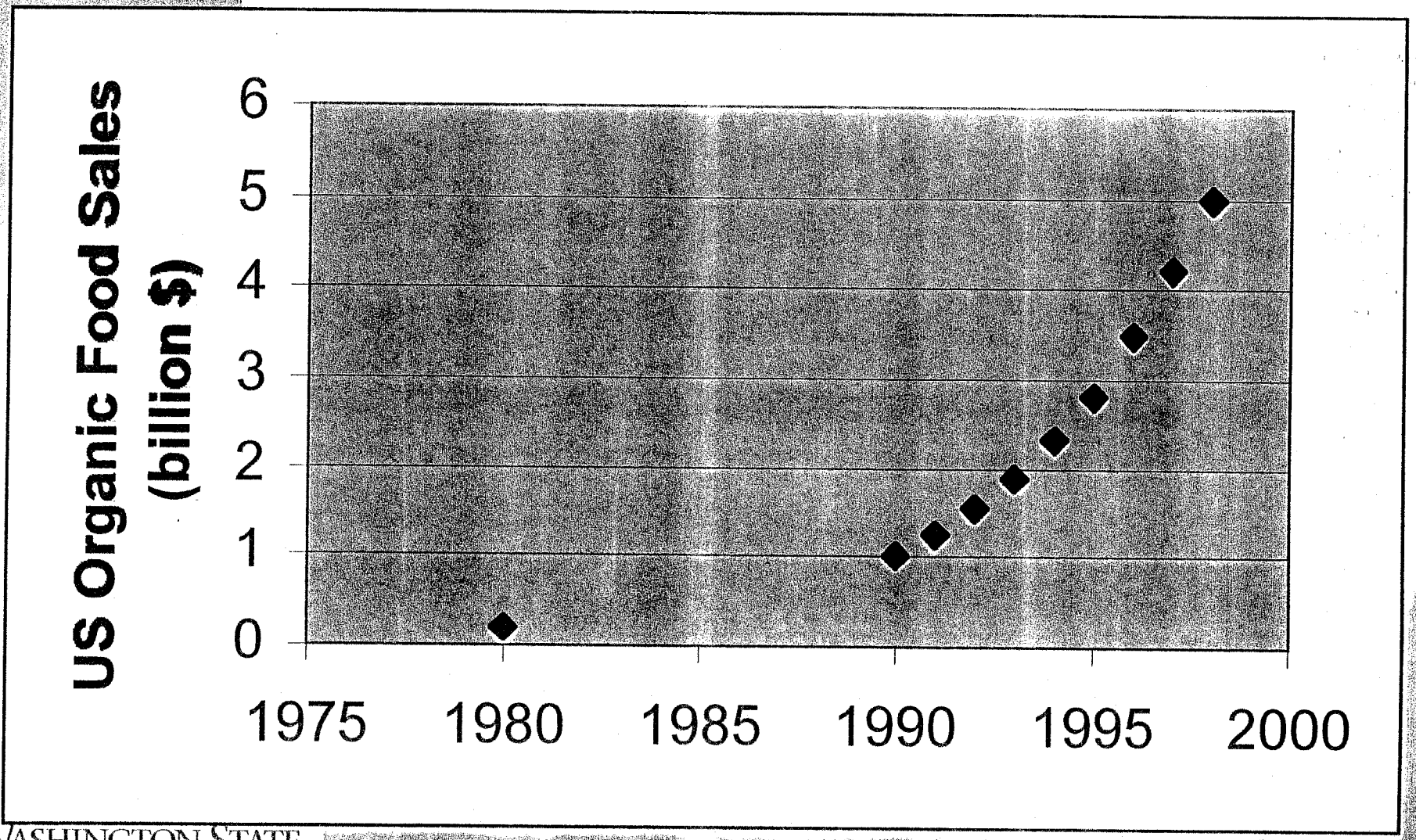
Organic pears near Chelan, WA

David Granatstein

Center for Sustaining Agriculture and Natural Resources, Wenatchee, WA

www.tfrec.wsu.edu "Organic & Integrated"

Organic food sales in the US



WASHINGTON STATE UNIVERSITY

World Class. Face to Face.

Source: Organic Trade Association

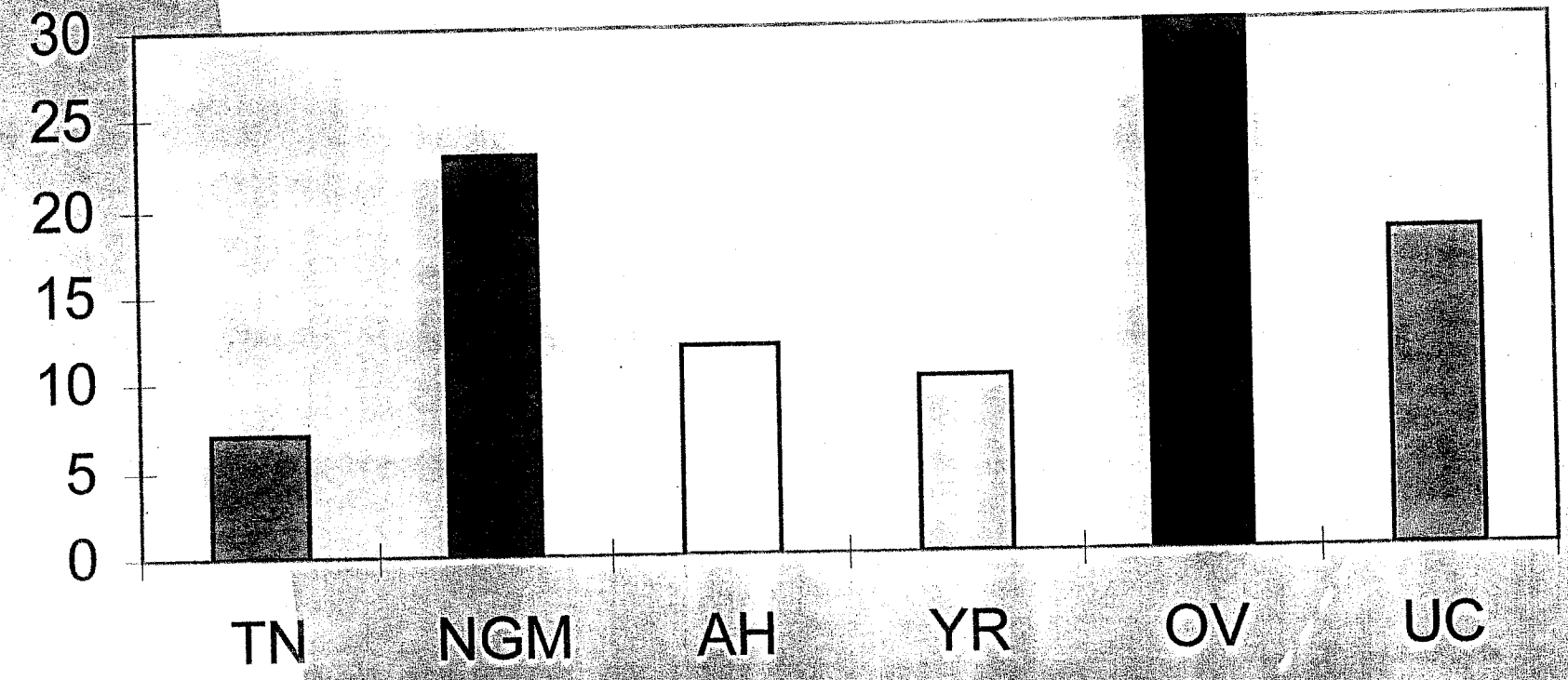
Encouraging Trends

- Consumer trends around "wellness"
- Increased organic food sales and availability
- More public interest in food system, ecosystem services, role of ag in society
- More public and private support for organic farming
- More regulatory certainty (USDA National Organic Standard)
- Organic = "GMO free"

Concerns

- More tree fruit growers and acres, domestic and foreign - More supply than demand right now ?
- "Industrial" organic
- More scrutiny and criticism of organic
- Convergence of conventional and organic on the farm

Segments of total population by environmental attitude (%)



TN = True Naturals
OV = Overwhelmed
NGM = New Green Mainstream
AH = Alternative Healers
UC = Unconcerned
YR = Young Recyclers

(Hartman, 1996)

COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY
World Class. Face to Face.

U.S. Organic Tree Fruit Acreage - 2001

	Apple	Pear	Cherry	All fruit
WA	6540	1308	303	8436
CA	4529	842	179	8662
AZ	2800	--	30	2830
CO	1535	100	133	1923
ID	503	--	--	506
OR	350	500	25	1180
Others	1015	48	57	1198
Total US	17,272	2798	727	23,835
WA trans.	3411	642	280	4408

COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY
World Class. Face to Face.

Estimated World Organic Apple and Pear Acreage - 2001

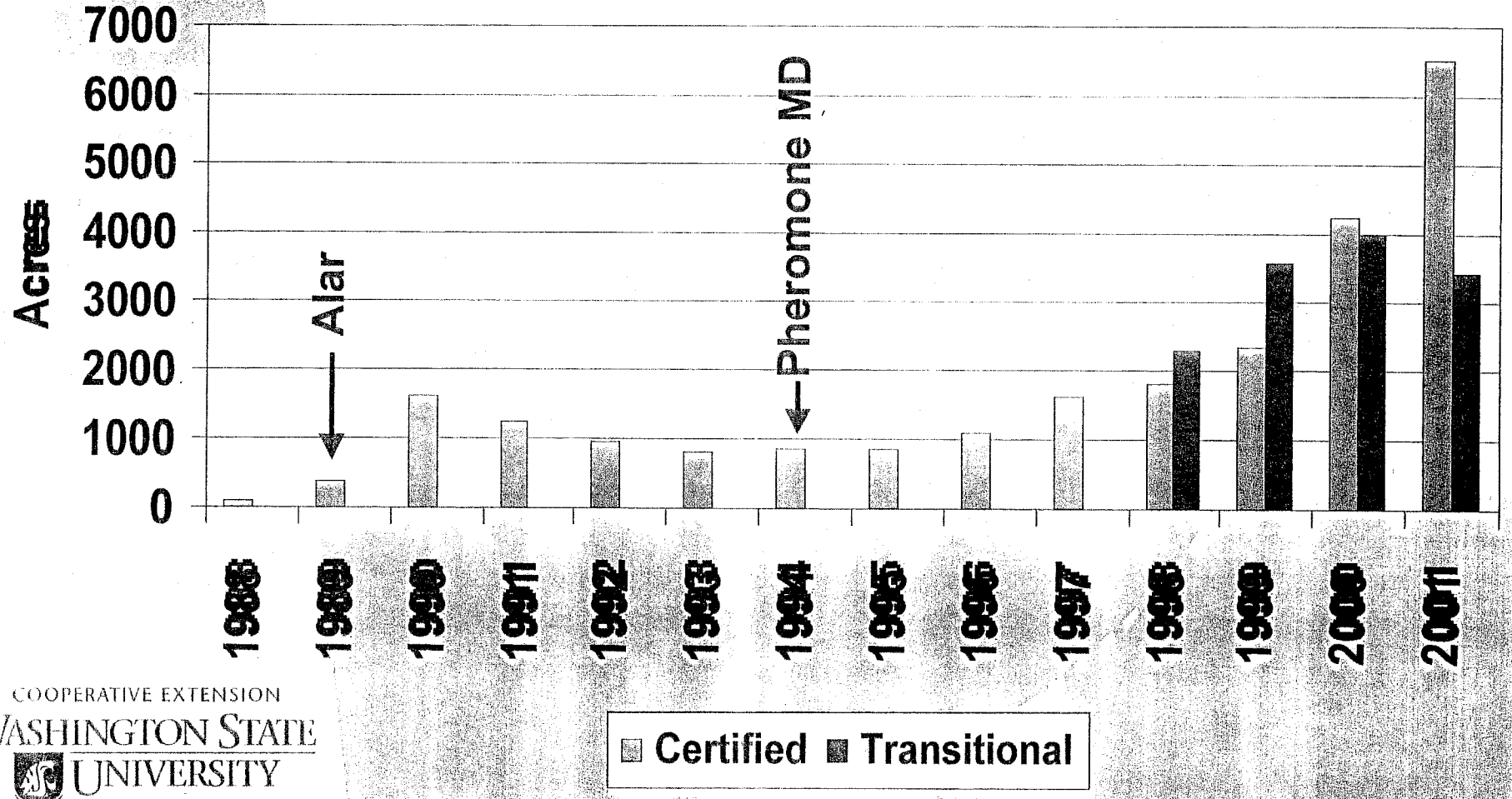
	Certified Acres	
	Apple	Pear
U.S.	17,572	2,798
Canada	800	60
Europe*	8,675	3,665
South America	1,385	932
New Zealand	2,873	163
Total	31,005	7,618

China ??

*Europe data from 2000

COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY
World Class. Face to Face.

Organic Apple Acreage in Washington State



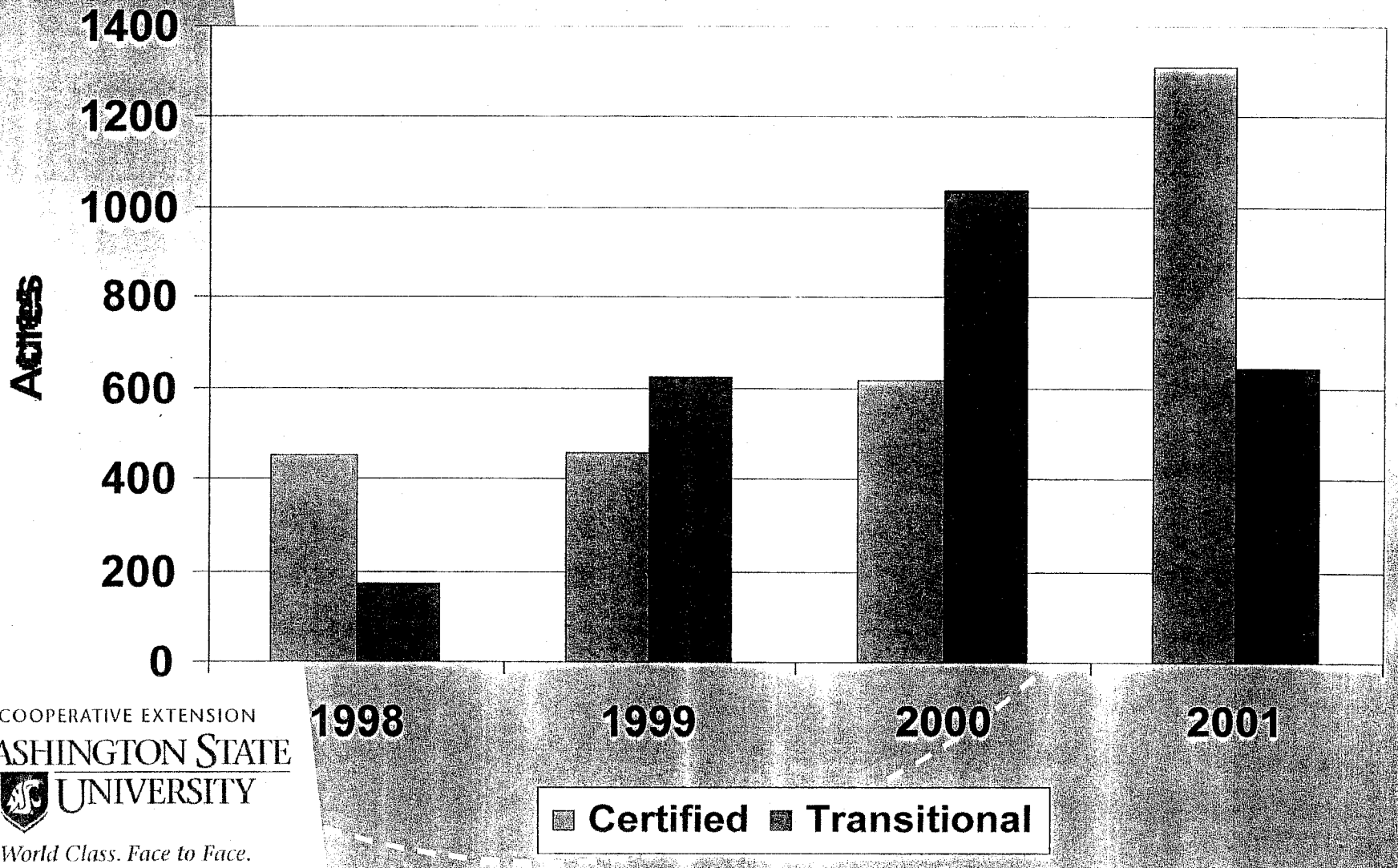
COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY
World Class. Face to Face.

Organic Tree Fruit Acreage in WA - 2001

	Cert.	Trans.	Total
Apples	6540	3411	9951
Pears	1308	642	1950
Cherries	303	280	583
Apricots	49	4	53
Peaches	126	31	157
Nectarines	57	26	84
Plums	54	14	68
Total	8436	4408	12844

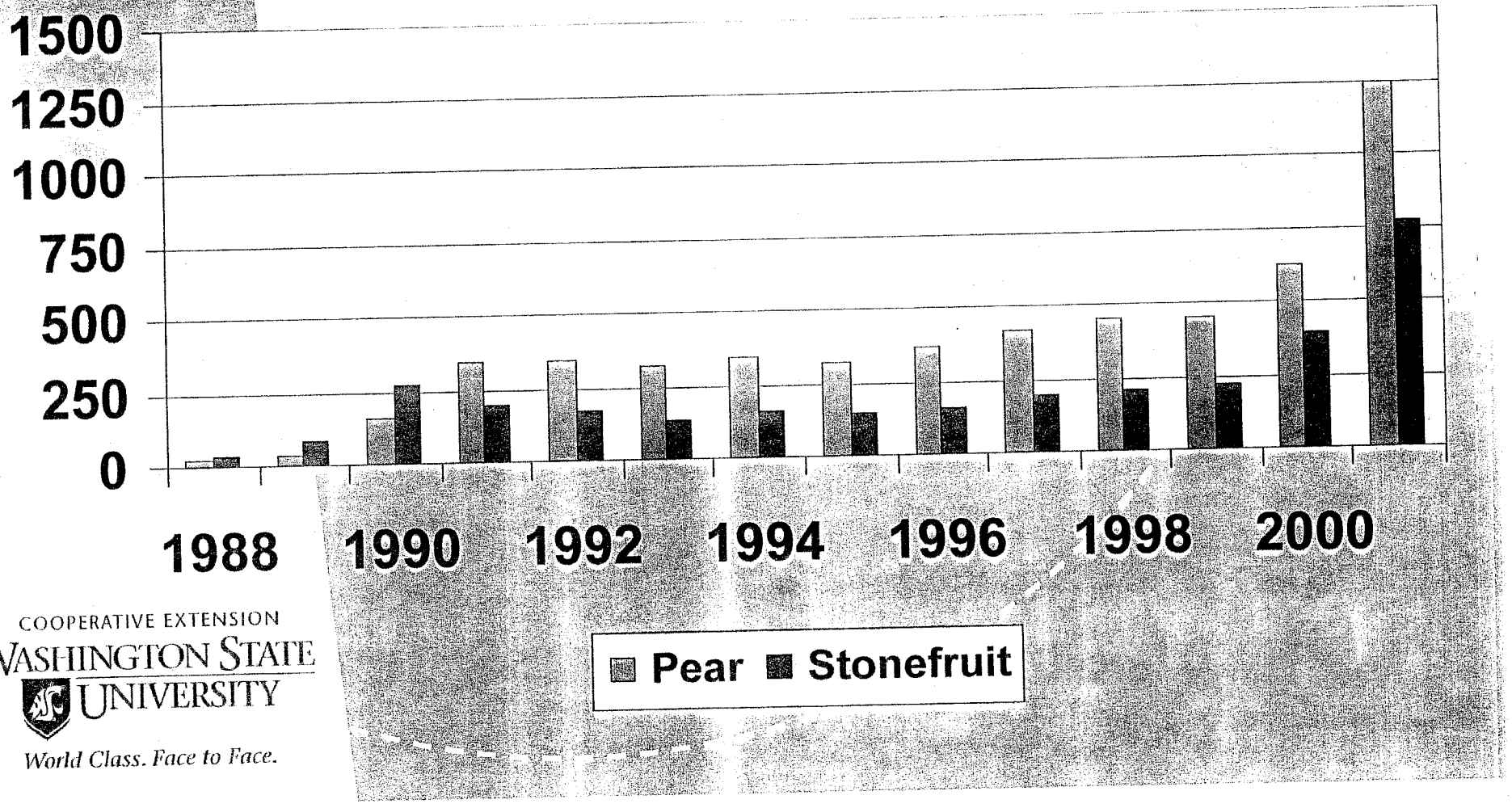
COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY
World Class. Face to Face.

Organic Pear Acreage in Washington State



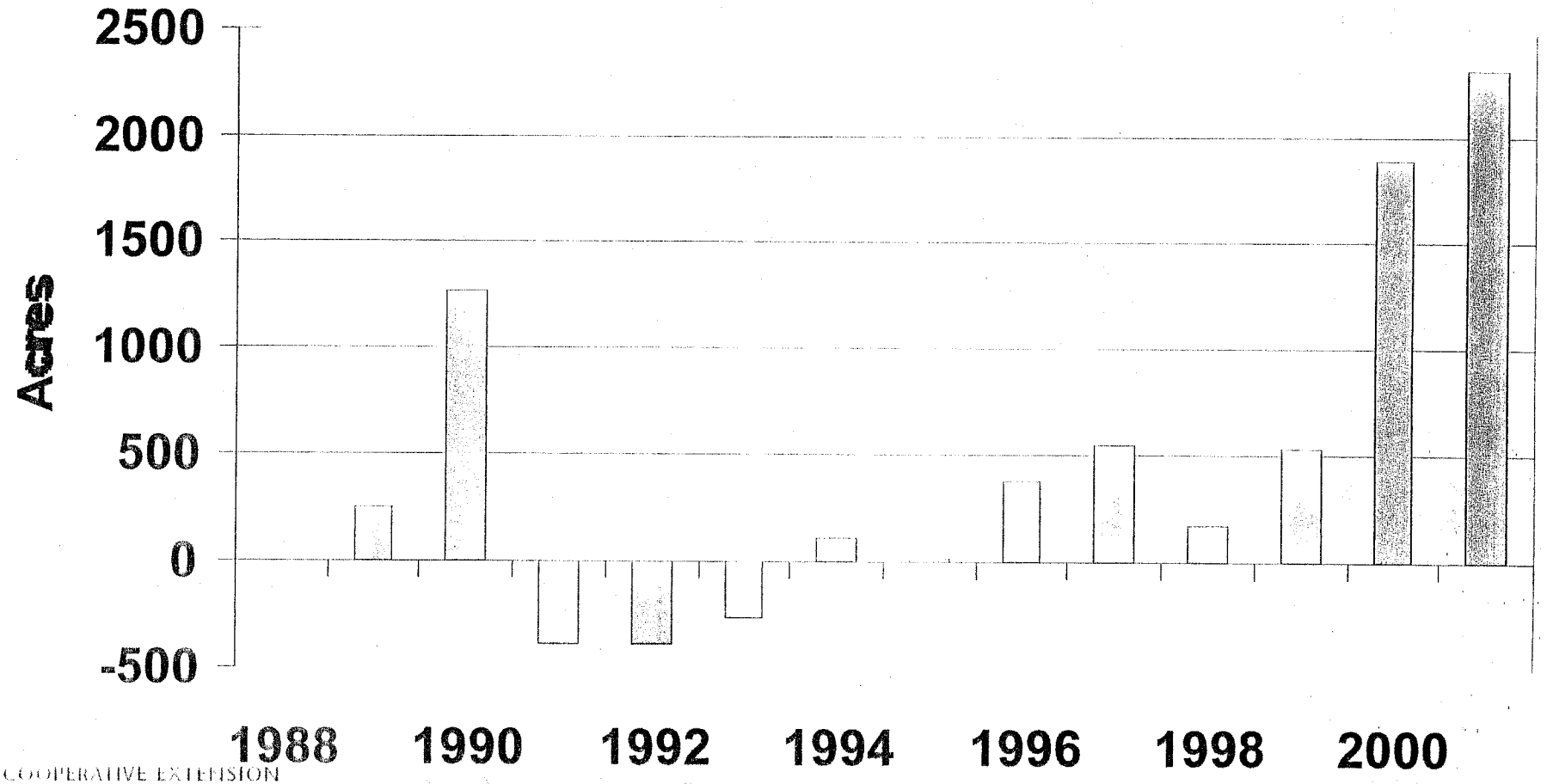
COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY
World Class. Face to Face.

Organic Pear and Stone Fruit Acreage in Washington State



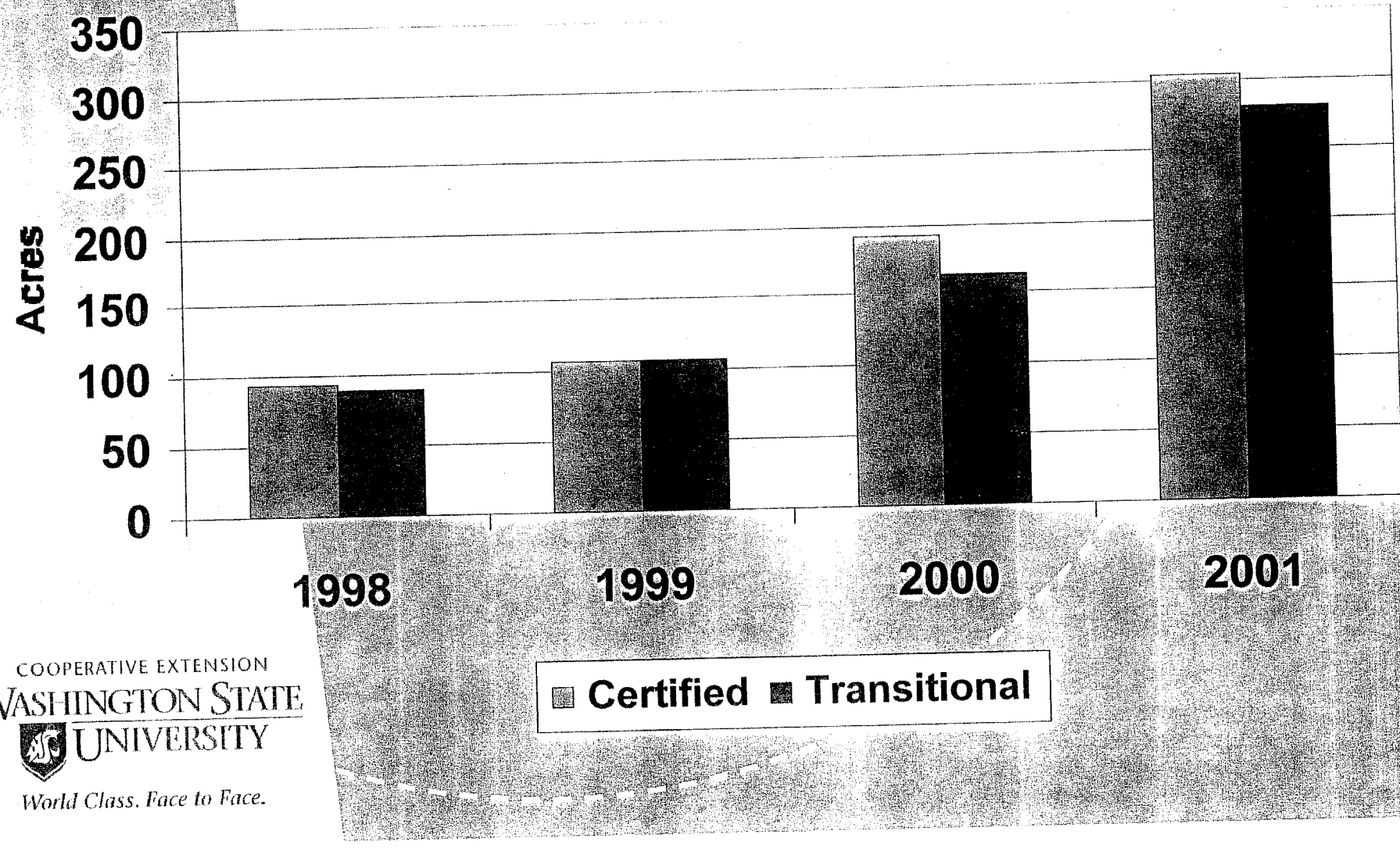
COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY
World Class. Face to Face.

Annual Changes in Organic Apple Acreage – Washington State



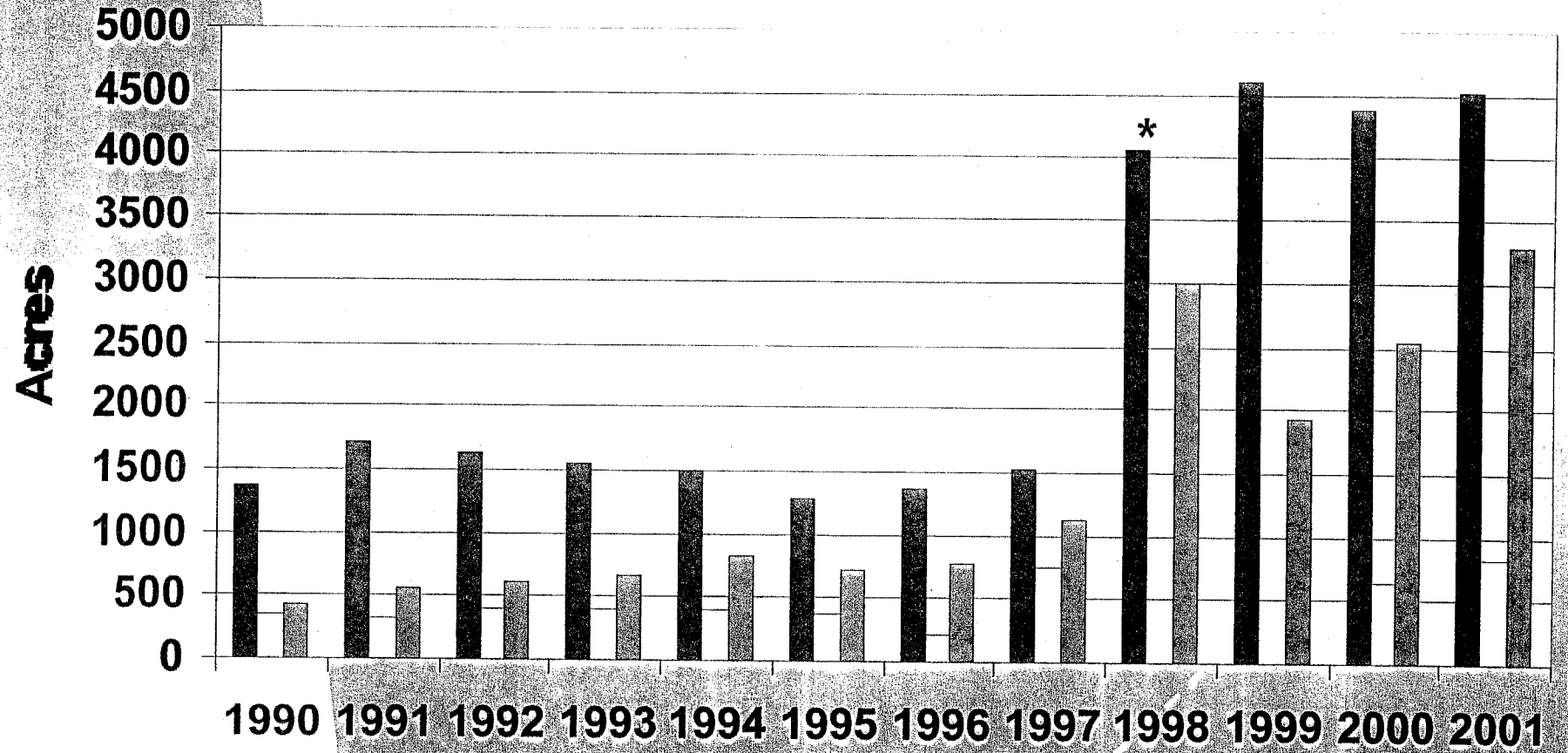
COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY
World Class. Face to Face.

Organic Cherry Acreage in Washington State



COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY
World Class. Face to Face.

California Organic Tree Fruit Trends



* Data source changed to CDFA

COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY
World Class. Face to Face.

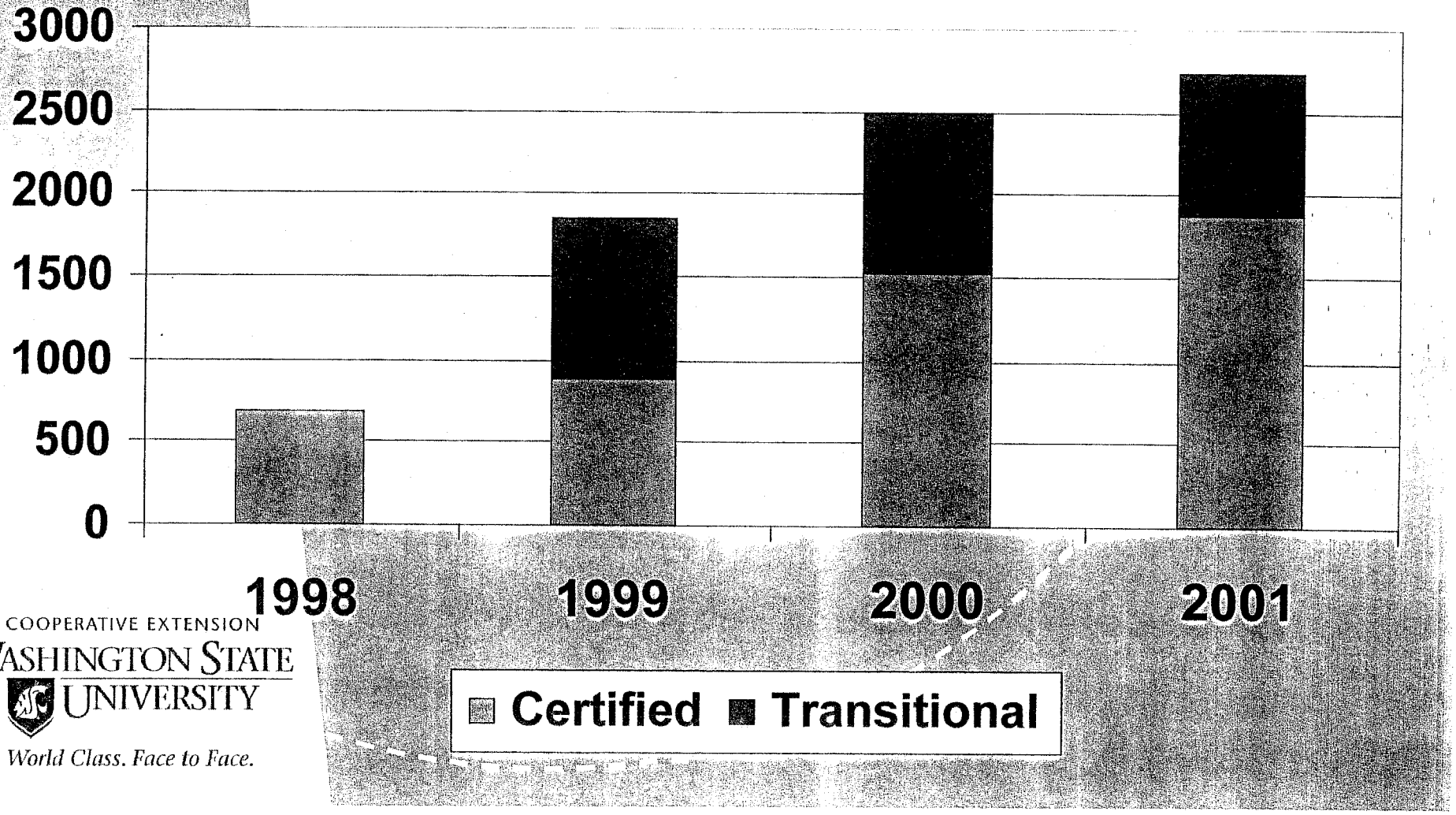
Organic Orchard Acreage as a Percent of Total Washington Orchards

	Apple	Pear
1996	0.68	1.49
1997	0.96	1.68
1998	1.05	1.84
1999	1.36	1.87
2000	2.48	2.54
2001	3.90	5.27
2001 (C+T)	5.92	7.86

Based on USDA-National Agricultural Statistics for bearing acreage

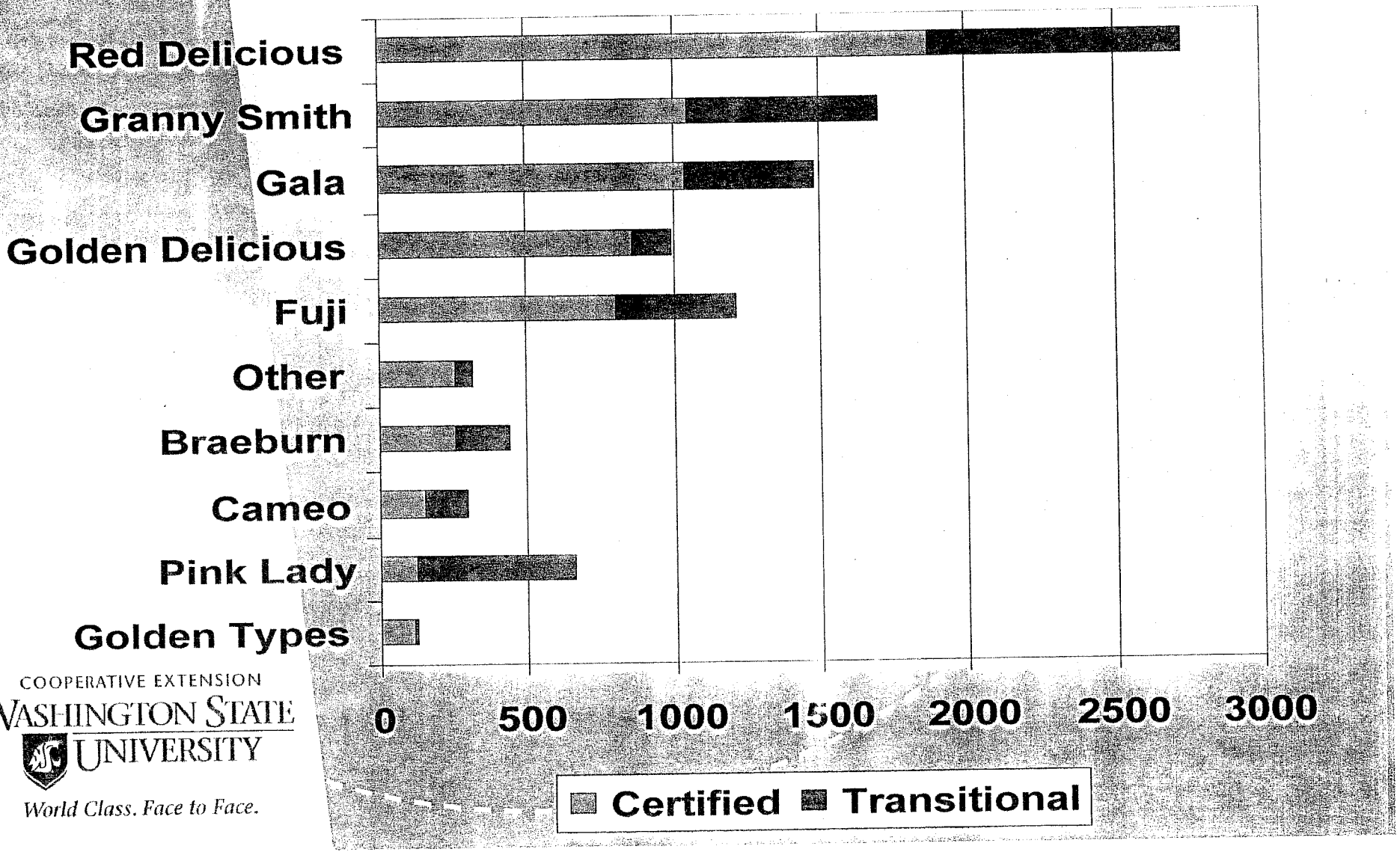
COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY
World Class. Face to Face.

WA Organic Red Delicious Acreage



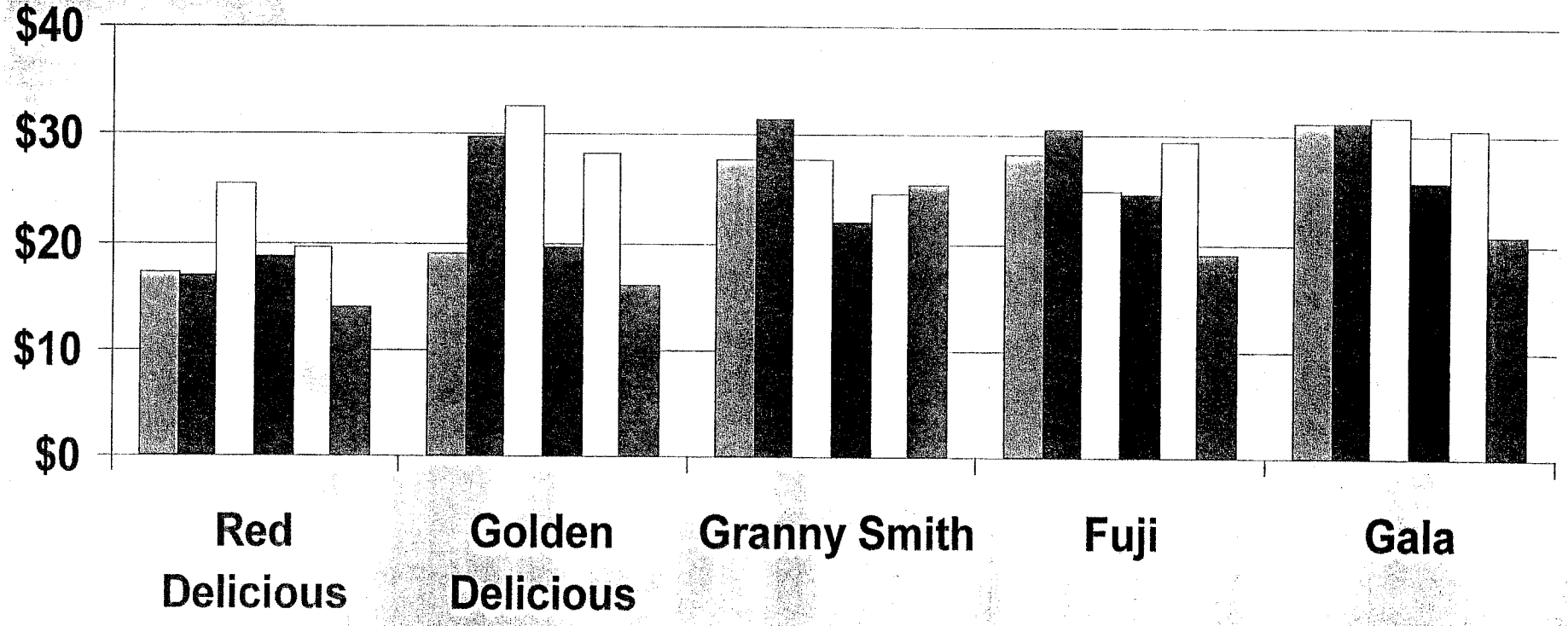
COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY
World Class. Face to Face.

WA Organic Apple Acreage by Variety 2001



COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY
World Class. Face to Face.

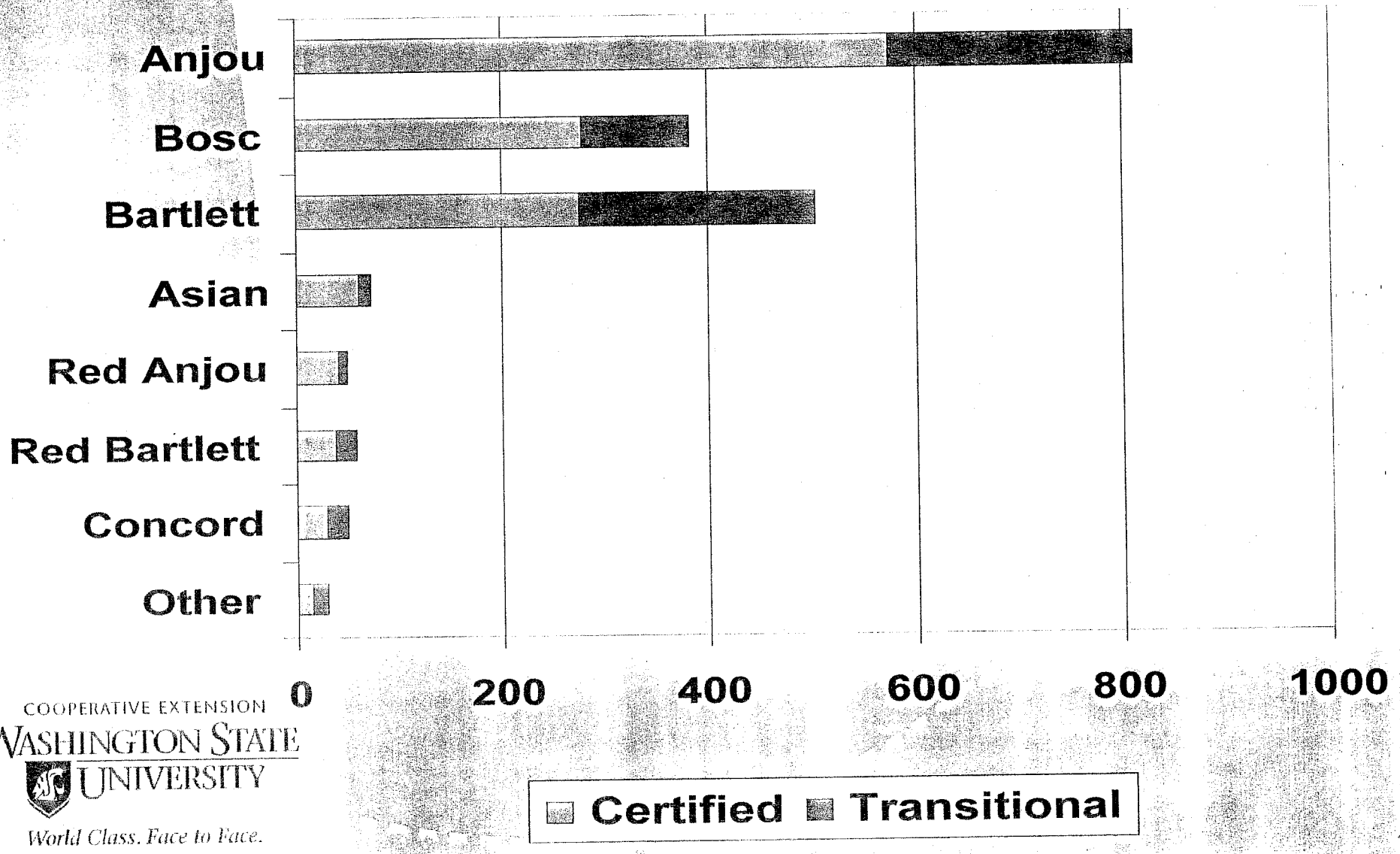
WA Organic Apple Prices
($ per box FOB)



COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY
World Class. Face to Face.

Source: Washington Growers Clearinghouse

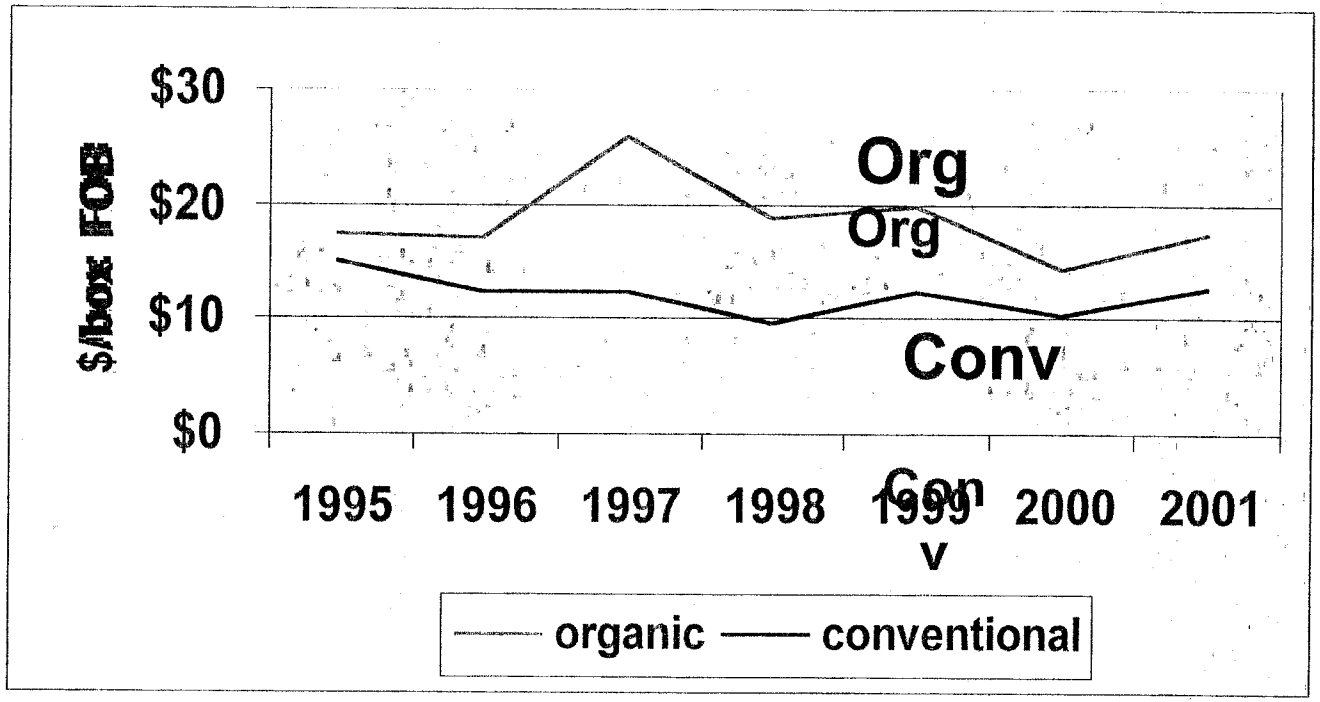
WA Organic Pear Acreage by Variety 2001



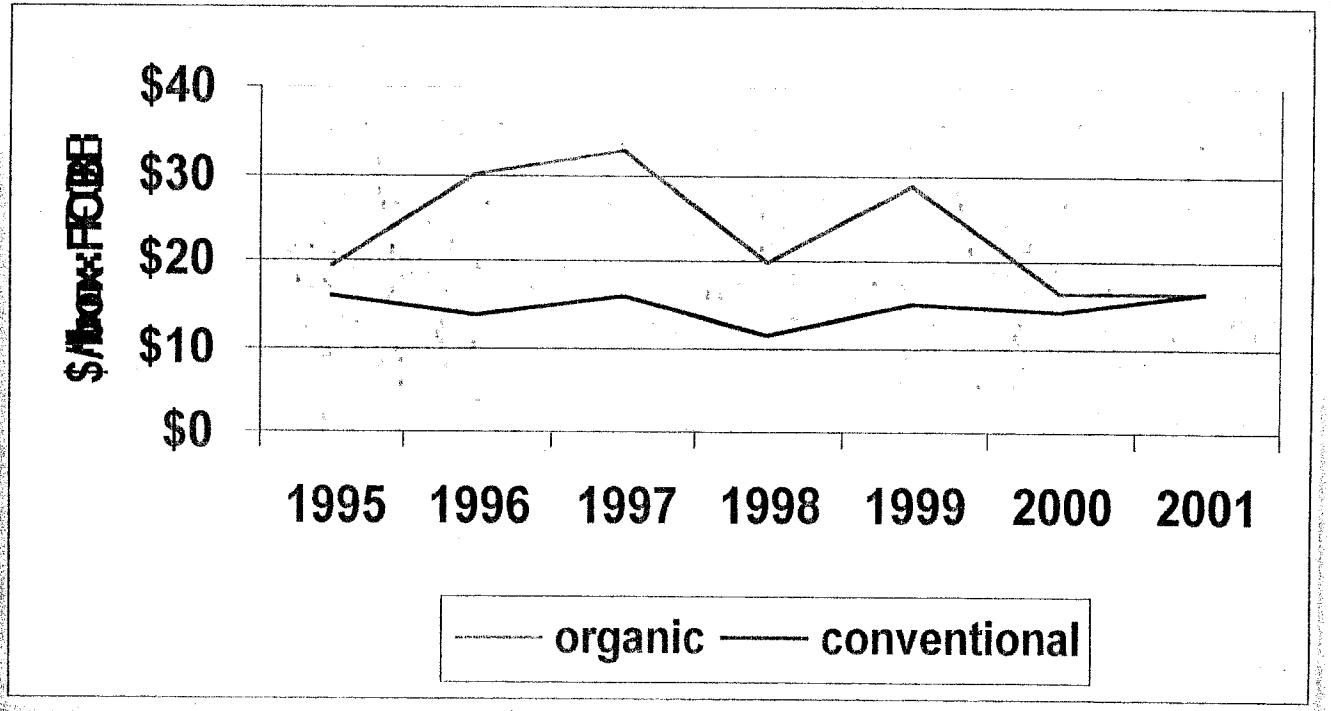
COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY
World Class. Face to Face.

Price Trends

Red Delicious

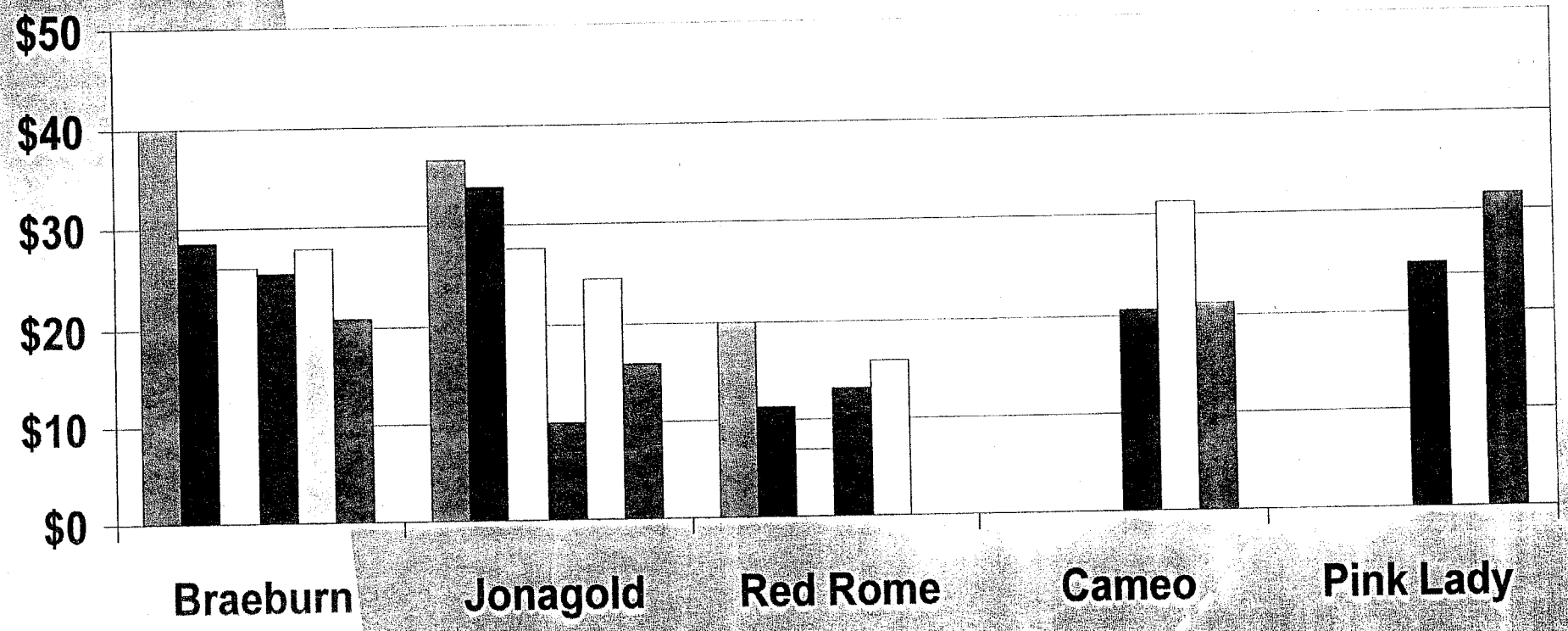


Golden Delicious



COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY
World Class. Face to Face.

WA Organic Apple Prices
($ per box FOB)

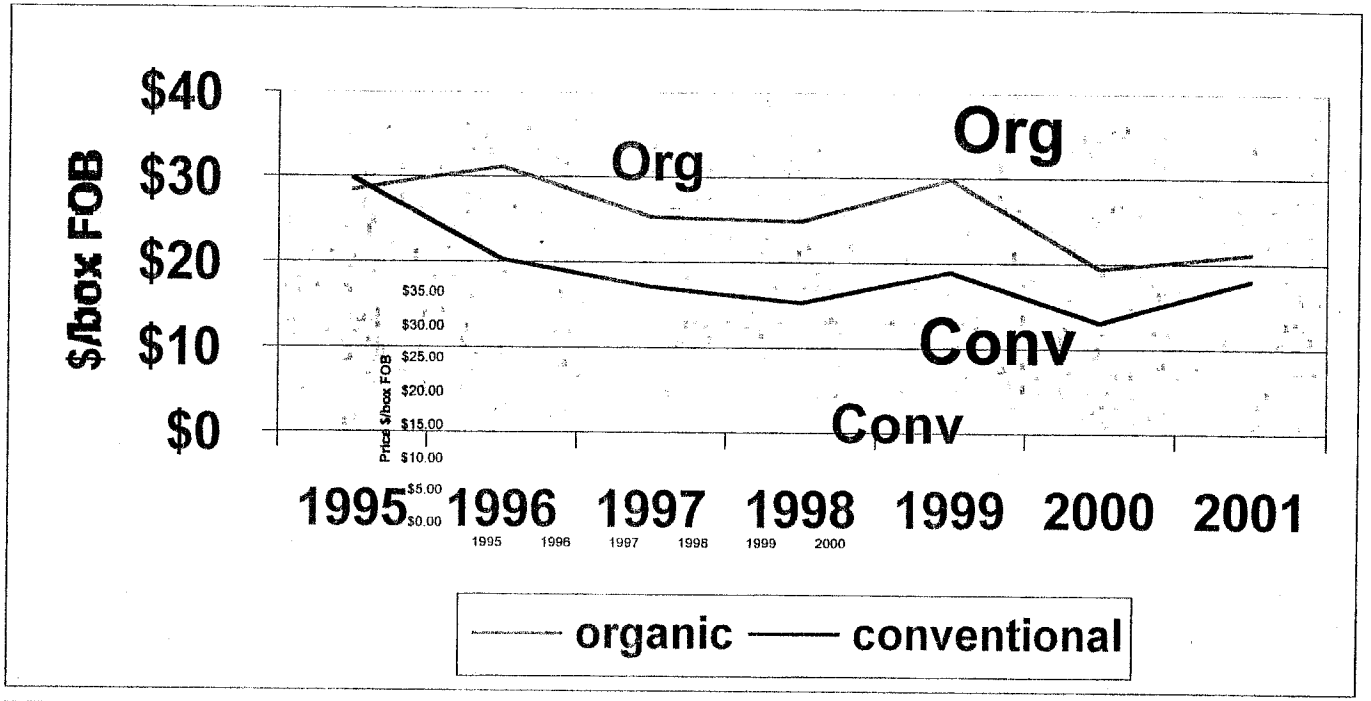


Source: Washington Growers Clearinghouse

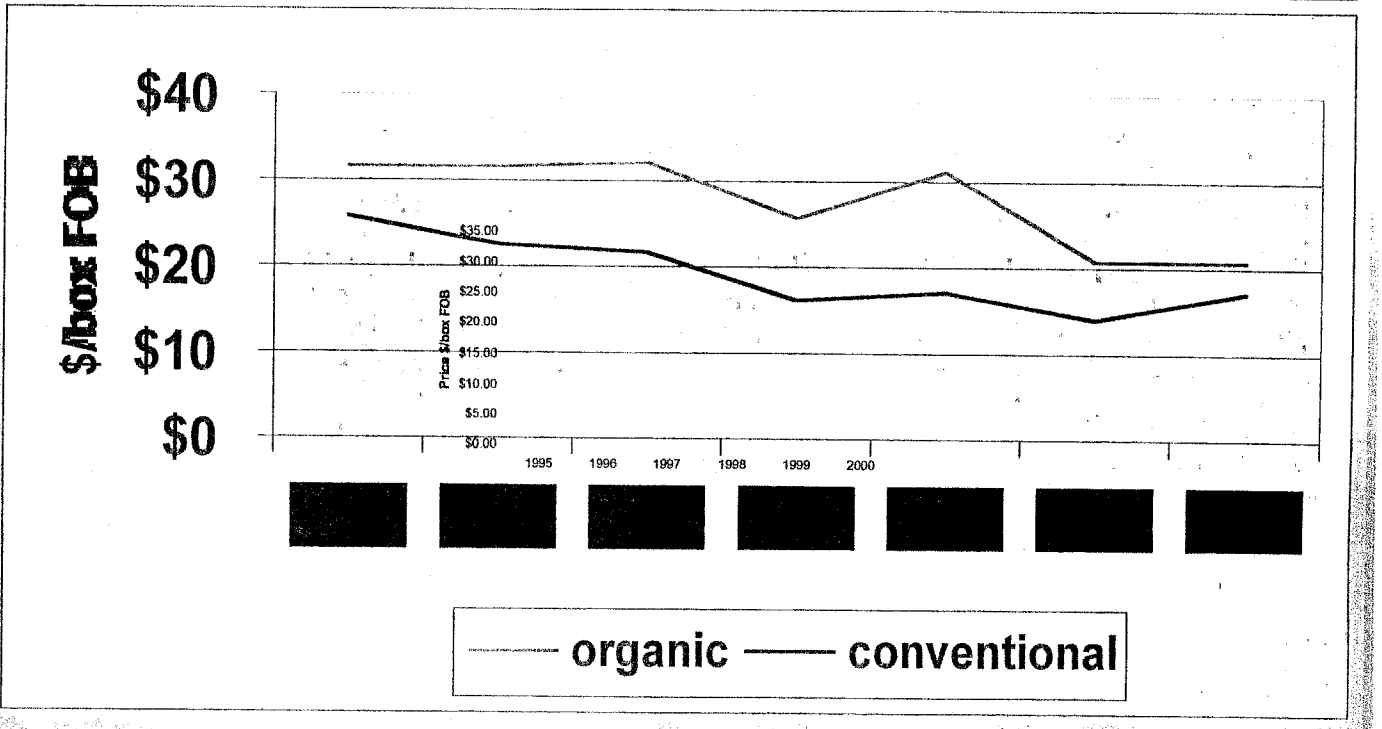
COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY
World Class. Face to Face.

Price Trends

Fuji



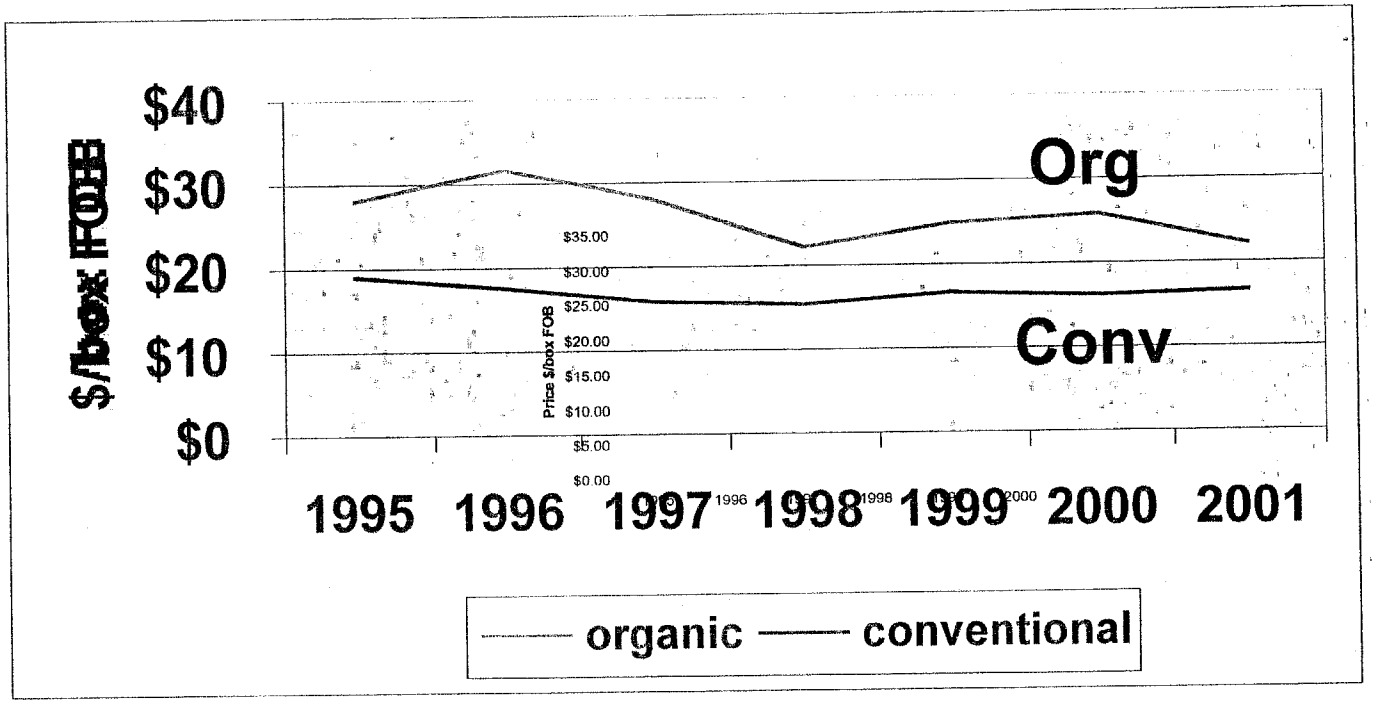
Gala



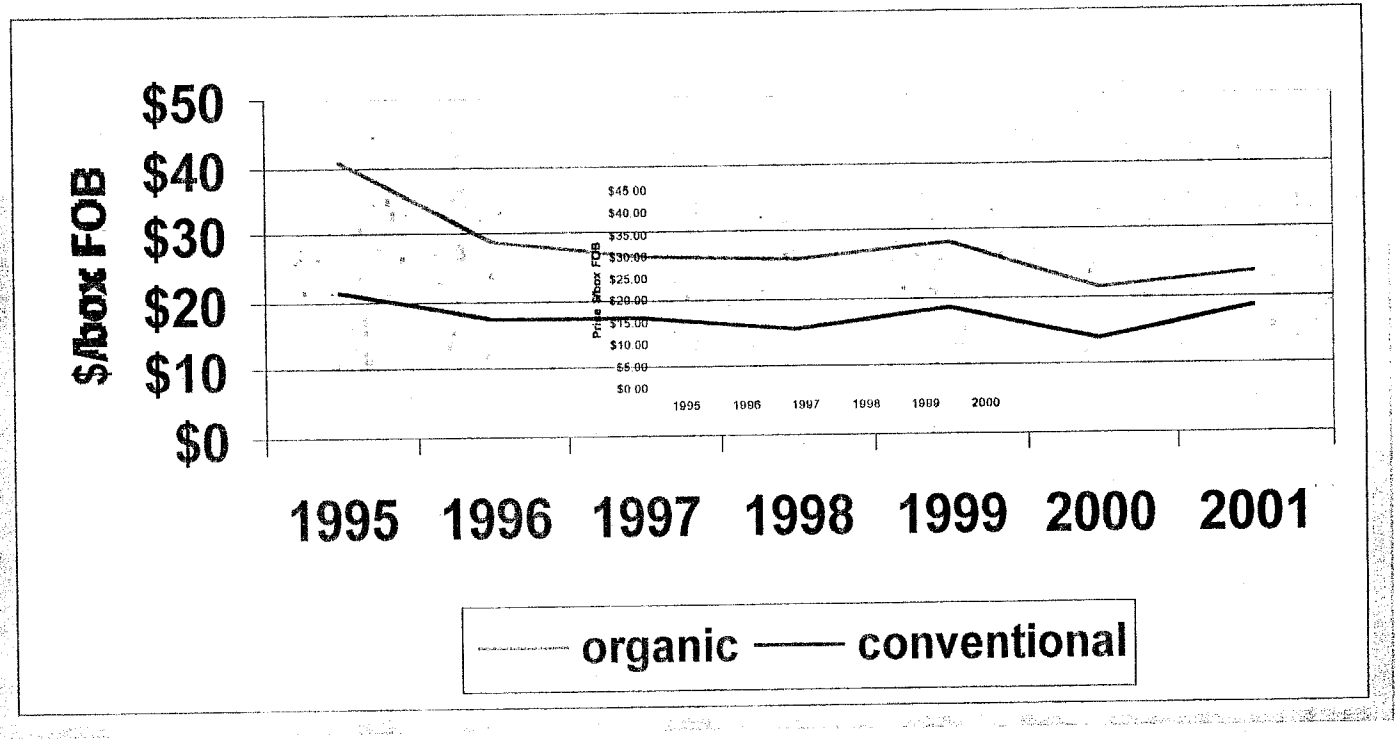
COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY
World Class. Face to Face.

Price Trends

Granny Smith



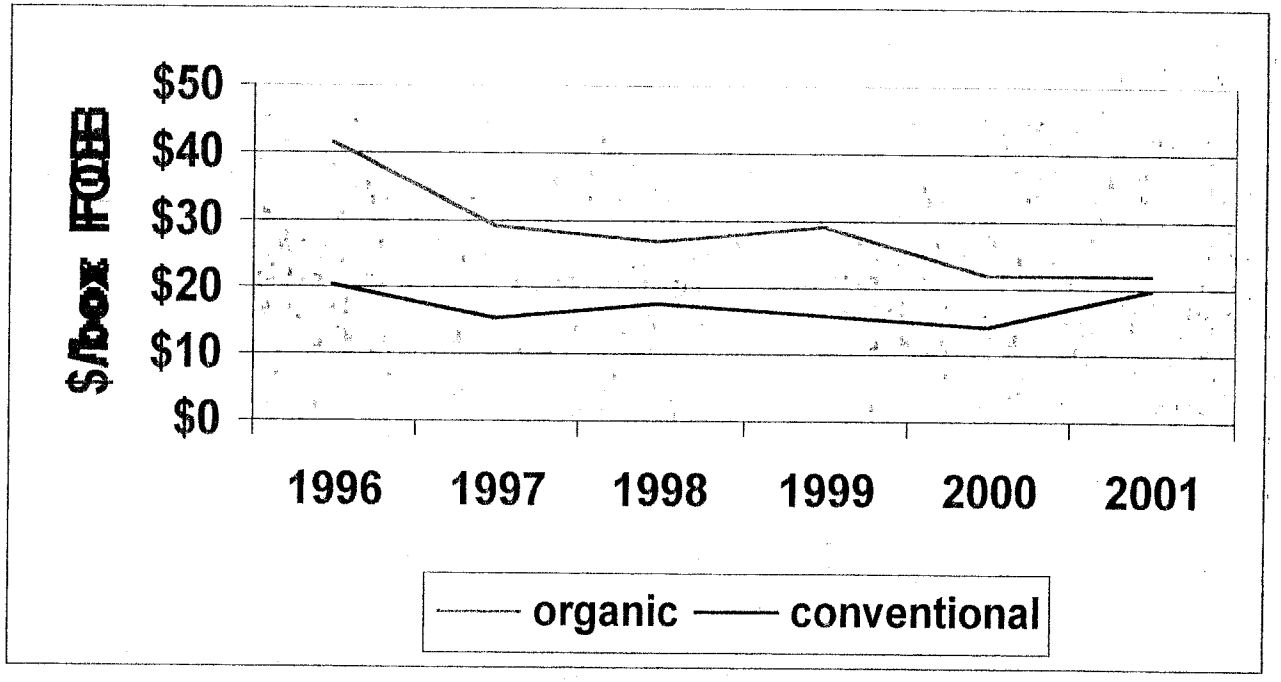
Braeburn



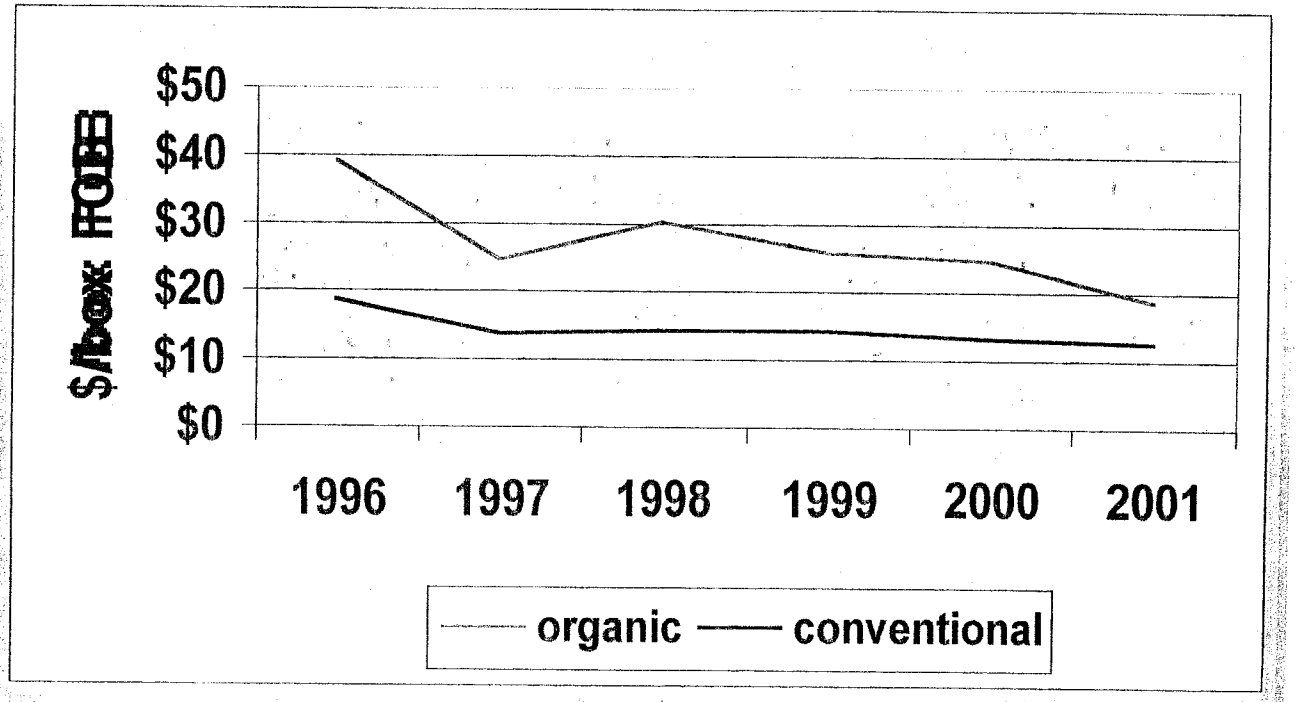
COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY
World Class. Face to Face.

Price Trends

Bartlett

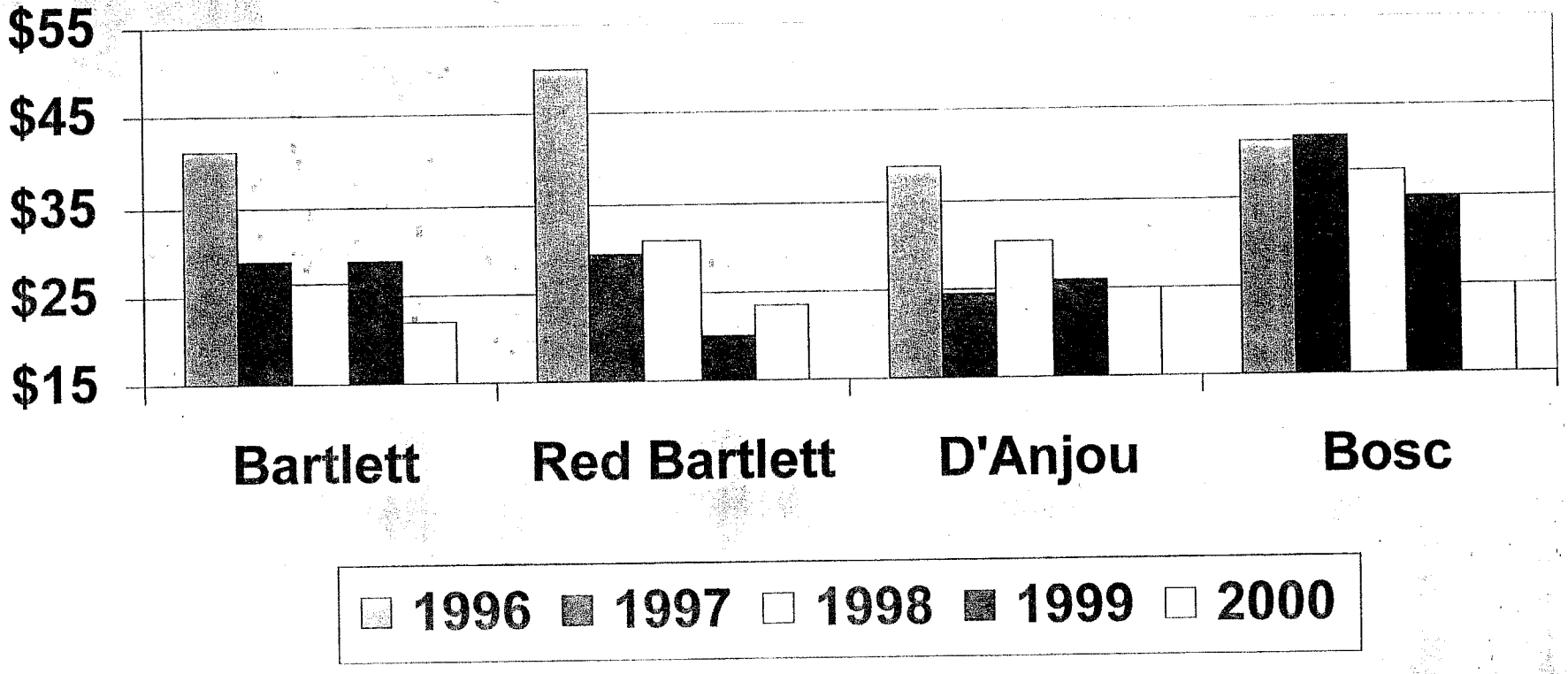


D'Anjou



COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY
World Class. Face to Face.

WA Organic Pear Prices ($/box FOB)



COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY
World Class. Face to Face.

Source: Washington Growers Clearinghouse

Organic Tree Fruit Research Needs

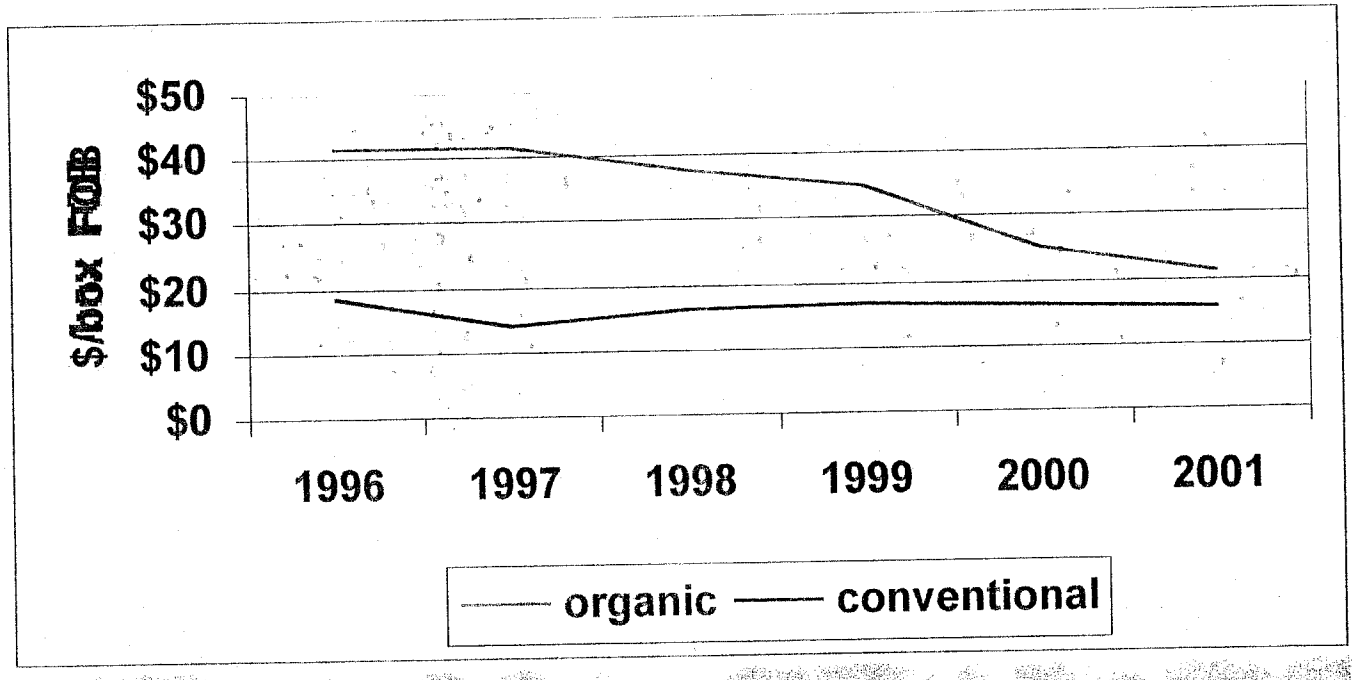
Semi-arid regions (western North America, Argentina) – weed control, insect pests, fruit thinning, fertility, replant disease

Humid regions (Michigan, NY, Europe) – scab, insect pests, weed control, fruit thinning, fertility

COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY
World Class. Face to Face.

Price Trends

Bosc



COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY
World Class. Face to Face.

Looking Ahead …

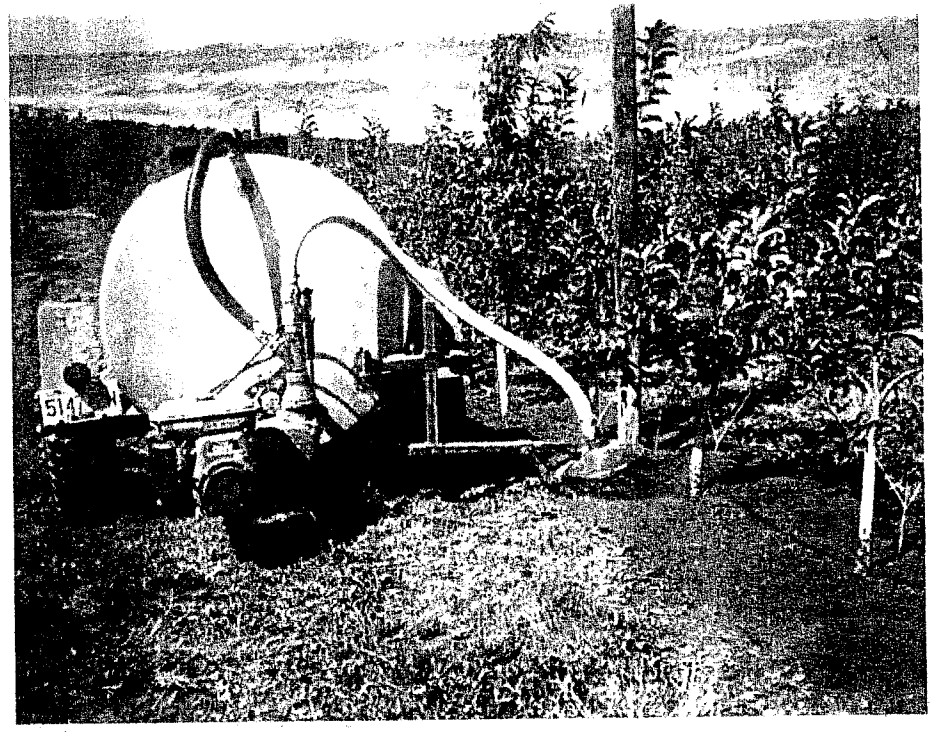
Organic:

… is on the leading edge of sustainable agriculture; not the endpoint.

… doesn't guarantee "no spray, no chemicals, no residues, no synthetics, tastier, healthier, family farm, local."

… cannot remain static and must continually improve.

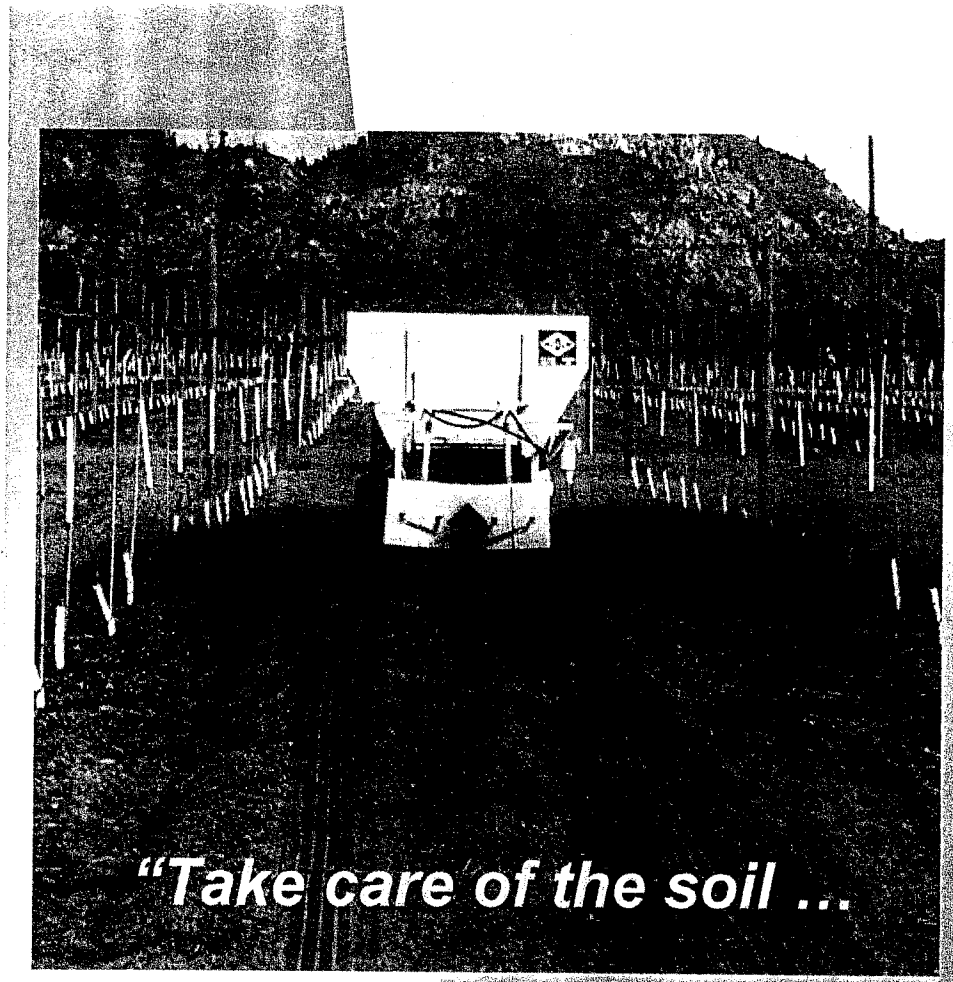
COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY
World Class. Face to Face.

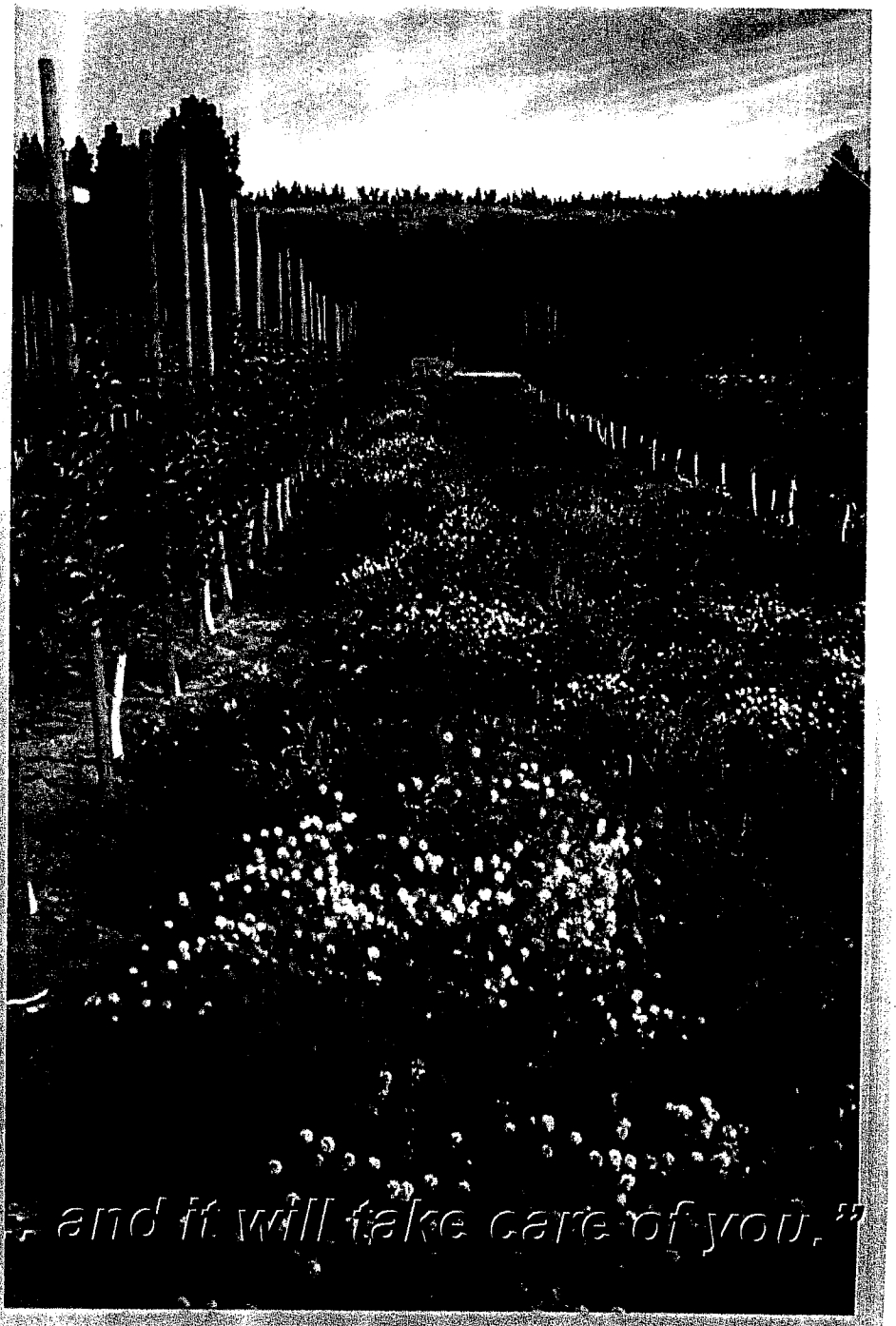


COOPERATIVE EXTENSION

WASHINGTON STATE UNIVERSITY

World Class. Face to Face.





COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY

World Class. Face to Face.

Keep Your Eye on the Consumer

- More interest in food and farm attributes outside the organic rule (labor, wildlife, food miles, energy…). 'Beyond Organic' or 'Organic Plus'
- Organic fruit and "wellness" – apples and antioxidants. Pre-sliced organic apples as a snack food alternative?
- "Taste, face and place." Where is there more consumer loyalty – local, fresh, non-organic vs. imported organic Chilean fruit?

COOPERATIVE EXTENSION
WASHINGTON STATE UNIVERSITY
World Class. Face to Face.

DETHIER, V.G., and KUCH, J.H. 1971. Electrophysiological studies of gustation in lepidopterous larvae. I. Comparative sensitivity to sugars, amino acids, and glycosides. *Z. Vergl. Physiol.* 72:343–363.

HATFIELD, L.D., FRAZIER, J.L., and FERREIRA, J. 1982. Gustatory discrimination of sugars, amino acids, and selected allelochemicals by the tarnished plant bug, *Lygus lineolaris*. *Physiol. Entomol.* 7:15–23.

Heron, R. J. 1965. The role of chemotactic stimuli in the feeding behavior of spruce budworm larvae on white spruce. *Can. J. Zool.* 43:247–269.

JERMY, T., HANSON, F.E., and DETHIER, V.G. 1968. Induction of specific food preferences in lepidopterous larvae. *Entomol. Exp. Appl.* 11:211–230.

KIMMINS, J.P. 1971. Variations in the foliar amino acid composition of flowering and non-flowering balsam fir [*Abies balsamea* (L.) Mill.] and white spruce [*Picea glauca* (Moench) Voss] in relation to outbreaks of the spruce budworm [*Choristoneura fumiferana* (Clem.)]. *Can. J. Zool.* 49:1005–1011.

MA, W.C. 1972. Dynamics of feeding responses in *Pieris brassicae* Linn. as a function of chemosensory input: A behavioural, ultrastructural and electrophysiological study. *Meded. Landbouwhogesch. Wageningen* 72(11):162 pp.

MATTSON, W.J., and HAACK, R.A. 1987. The role of drought in outbreaks of plant-eating insects. *Bioscience* 37:110–118.

MCMORRAN, A. 1965. A synthetic diet for the spruce budworm *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). *Can. Entomol.* 97:58–62.

MILLER, C.A. 1963. The spruce budworm. *Mem. Entomol. Soc. Can.* 31:12–19.

MITCHELL, B.K. 1985. Specificity of an amino acid-sensitive cell in the adult Colorado beetle, *Leptinotarsa decemlineata*. *Physiol. Entomol.* 10:421–429.

NEISCH, A.C. 1957. Pungenin: A glucoside found in leaves of *Picea pungens* (Colorado spruce). *Can. J. Biochem. Physiol.* 35:161–167.

NIE, N.H., HULL, C.H., JENKINS, J.G., STEINBRENNER, K., and BENT, D.H. 1975. Statistical Package for the Social Sciences. McGraw-Hill, New York.

SCHOONHOVEN, L.M., 1969. Amino-acid receptor in larvae of *Pieris brassicae* (Lepidoptera). *Nature* 221:1268.

SHIRAISHI, A., and KUWABARA, M. 1970. The effects of amino acids on the labellar hair chemosensory cells of the fly. *J. Gen. Physiol.* 56:768–782.

Sokal, R. R., and Rohlf, F. J. 1969. Biometry. W. H. Freedman, San Francisco.

SRIVASTAVA, P.N., and AUCLAIR, J.L. 1974. Effect of amino acid concentration on diet uptake and performance by the pea aphid, *Acyrthosiphon pisum* (Homoptera: Aphididae). *Can. Entomol.* 106:149–156.

SRIVASTAVA, P.N., and AUCLAIR, J.L., and SRIVASTAVA, U. 1983. Effect of nonessential amino acids on phagostimulation and maintenance of the pea aphid, *Acyrthosiphon pisum*. *Can. J. Zool.* 61:2224–2229.

STRUNZ, G.M., GIGUERE, P., and THOMAS, A.W. 1986. Synthesis of pungenin, a foliar constituent of some spruce species, and investigation of its efficacy as a feeding deterrent for spruce budworm [*Choristoneura fumiferana* (Clem.)]. *J. Chem. Ecol.* 12:251–260.

SUGARMAN, D., and JAKINOVICH, W., JR. 1986. Behavioural gustatory responses of adult cockroaches, *Periplaneta americana* to D and L amino acids. *J. Insect Physiol.* 32:35–41.

WENSLER, R.J., and DUDZINSKI, A.E. 1972. Gustation of sugars, amino acids and lipids by larvae of the scarabacid, *Sericesthis geminata* (Coleoptera). *Entomol. Exp. Appl.* 15:155–165.

Journal of Chemical Ecology, Vol. 14, No. 8, 1988

CHEMICAL PROTECTION OF PHEROMONES CONTAINING AN INTERNAL CONJUGATED DIENE SYSTEM FROM ISOMERIZATION AND OXIDATION

RUT IDESES and ARNON SHANI

Department of Chemistry, Ben-Gurion University of the Negev
Be'er-Sheva 84105, Israel

(Received June 1, 1987; accepted September 28, 1987)

Abstract—Conjugated diene systems are common in natural products, including pheromones. The systems are sensitive to heat, light, and oxygen, among other things. They can be protected by antioxidants and UV absorbers, which slow down *cis–trans* isomerization and oxidation. Three sex pheromones (one as an analog) containing *Z,E*, *E,Z*, and *E,E* units were studied: (*Z,E*)-9,11-C_{14}OAc, (*E,Z*)-7,9-C_{12}OAc, and (*E,E*)-10,12-C_{16}OAc. The UV absorber 2-hydroxy-4-methoxybenzophenone and the antioxidants BHT and BHA were found to be effective in solution. The protective effect of the UV absorber against photoisomerization on paper carriers was not as good as that in solution. Preliminary studies on the utilization of formulations containing these compounds and (*Z,E*)-9,11-C_{14}OAc in the mass trapping of Egyptian cotton leafworm male in cotton fields showed the new combinations to be as good as a previously used formulation with UOP 688, a compound which is unpleasant to handle.

Key Words—*Lobesia botrana*, *Spodoptera littoralis*, *Earias insulana*, conjugated dienes, sex pheromone, photoisomerization, UV absorbers, antioxidants, protection of pheromones.

INTRODUCTION

As integrated pest management (IPM) becomes more sophisticated, the main role played by pheromones will be that of monitoring and disruption of communication (confusion) via controlled release of the active ingredients in the field. The longer the desired field life of the pheromones, the greater the quantity of pheromones that will be degraded by chemical and physical processes. Evaporation is known to be a major cause of the loss of material in short-lived

0098-0331/88/0800-1657$06.00/0

Flasks were refilled to 5 ml every day to compensate for evaporation. Solid carriers (two replicates of each sample) were extracted with hexane (2 × 2 ml), evaporated and then diluted to 1 ml and injected into the GLC. All formulations (solutions or solids carriers) were subjected to diffused sunlight on the roof of the building for the greater part of the experiment, although for some of the time they were placed in direct sunlight. (Details of the light regime are given in the tables or in the text.) Samples were usually withdrawn every two to four days until no pheromone (or its isomers) could be detected by GLC; this period usually lasted five to six weeks. Thus, samples analyzed up to the third or fourth week contained enough material for detection (in general 20–40% of the starting material and isomers were present). In the absence of antioxidant, the pheromone decomposed much faster, and the last sample that contained measurable amounts is documented in the Tables.

RESULTS AND DISCUSSION

It has already been established that heat (50°C in the laboratory hood) causes thermal oxidation via $^3O_2$ and that the process can be slowed down by antioxidants such as BHA or BHT (Shani et al., 1982).

The effect of light seems to be more complicated. In the field, both direct and scattered sunlight may be present, a situation that may affect both pheromone carriers in traps and slow-release devices that are covered by leaves and other objects in the field. Moreover, the excitation of the diene system could be through direct absorption of light by its end absorption (Shani and Klug, 1980b; Shani et al., 1982) or via energy transfer from a sensitizer. In addition, in tropical or subtropical regions, exposure during the summer means that the pheromones are subjected to a combination of high-intensity sunlight and temperatures of 40°C or more. We have already found (Shani and Klug, 1980b) that the rate of direct photoisomerization in sunlight is two to three times faster in summer than in winter. These results are in keeping with the summer and winter figures for total solar energy in Be'er-Sheva (5500–6900 vs. 2300–3500 kcal/m^2/day)[1]. Therefore, in all the experiments described below, the pheromones were, in fact, exposed to a combination of heat, light, and oxygen. Thus, the protective effect of the antioxidant is crucial. The set of experiments performed by us was designed, therefore, to include all possible factors that also play a role in any study on the effect of light on isomerization of pheromones.

Effect of Antioxidants. When pheromones are subjected to heat and light, two competing chemical processes take place—thermal decomposition (oxidation) and photoisomerization. Heating alone (in the hood) caused decomposition of the pheromone within a few days (Shani et al., 1982), as was the situation in this experiment with pheromones exposed to sunlight and not protected by an antioxidant. In this case, very little isomerization took place (entries 1, 4, and 7 in Table 1). Pheromones protected by an antioxidant and hence exposed to longer periods of sunlight underwent more extensive isomerization, almost reaching the equilibrium composition, which was found to be 68–75% of the *E,E* isomer, 12–16% of each of the *Z,E* and *E,Z* isomers, and 1–3% of the *Z,Z* isomer (Shani et al., 1982; Ideses and Shani, 1986). In an earlier study (Shani and Klug, 1980b), we observed that the rate of photoisomerization is somewhat slower in the presence of the antioxidant, as compared to the process in its absence. After 11 days of sunlight, 17% of (*E,E*)-9,11-TDDA was found when

[1]We thank A. I. Kudish, Department of Chemical Engineering, Ben-Gurion University for the data.

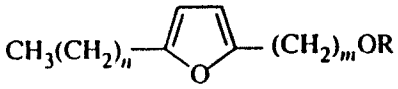
TABLE 1. PHOTOISOMERIZATION OF (*Z,E*)-9,11-C_{14}OAc (TDDA), (*E,Z*)-7,9-C_{12}OAc (DDA) AND (*E,E*)-10,12-C_{16}OAc (HDDA) EXPOSED TO SUNLIGHT IN PRESENCE OF UV ABSORBERS[a]

		Without BHA						With BHA					
				Composition (%)						Composition (%)			
Entry	Pheromone	UV absorber	Period (days)[b]	*Z,E*	*E,Z*	*Z,Z*	*E,E*	UV absorber	Period (days)	*Z,E*	*E,Z*	*Z,Z*	*E,E*
1.	TDDA		7	75	5		20		6	80	8		12
									25[c]	25	15		60
2.	TDDA	E-4360	11	82	7		11	E-4360	11	90	5		5
								E-4360	23[c]	86	7		7
3.	TDDA	E-8021	11	58	20		22	E-8021	11	73	13		14
			14	50	24		26	E-8021	14	66	16		18
								E-8021	27[c]	47	23		30
4.	DDA		3		87		13		3		85		15
									30[c]	20	36	2	42
5.	DDA	E-4360	10		90		10	E-4360	10		89		11
								E-4360	30[c]		85		15
6.	DDA	E-8021	10		85		15	E-8021	10	5	74		21
								E-8021	32[c]	9	63	1	27
7.	HDDA		10	5	5		90		10	7	8		85
									25[c]	10	10		80
8.	HDDA	E-4360	10	2	2		96	E-4360	10	2	2		96
								E-4360	30[c]	3	3		94
9.	HDDA	E-8021	10	6	6		88	E-8021	10	6	6		88
								E-8021	20[c]	9	9		82

[a]Each sample contained 100 mg of pheromone, 100 mg of antioxidant, and 100 mg of UV absorber (when mentioned) in 5 ml cyclohexane.
[b]Due to fast decomposition, the last sample withdrawn which gave measurable results is presented. Without antioxidant, the oxidation product 1 was formed in some cases (Ideses et al., 1982c).
[c]Last sample withdrawn when some 20–40% of starting material and isomers were still present in solution.

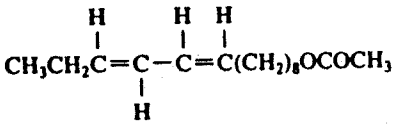
devices, but not much is known about chemical degradation under field and/or laboratory conditions. The few studies that have been performed have dealt mainly with the oxidation and isomerization of double bonds (Goto et al., 1974; Bruce and Lum, 1976, 1981; Fujiwara et al., 1977; Shani and Klug, 1980a, b; Ideses et al., 1982b, c; Shani et al., 1982; Vaintraub et al., 1983; Davis et al., 1984; Vylegzhanina et al. 1984; Guerin et al., 1984; Nesterova, 1985; Nesterova et al., 1985; Chisholm et al., 1985; Brown and McDonough, 1986; Sychev et al., 1987), with transformations of aldehydes (Weatherston et al., 1981; Shaver and Ivie, 1982; Dunkelblum et al., 1984), and with hydrolysis of acetates in the field (Shani and Klug, 1980b).

Our group (Shani and Klug, 1980a, b; Shani et al., 1982; Ideses et al., 1982b, c) has already shown that the conjugated diene systems in several sex pheromones of moths are isomerized into a mixture of the four possible geometric isomers and oxidized in the field (or under simulated field conditions in the laboratory) to a furan system (Scheme 1, structure I), via a peroxide.

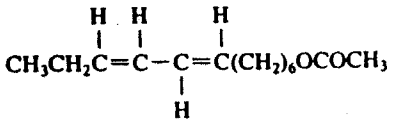


I

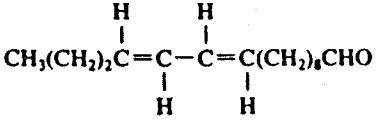
Ia: $m = 6, 8, 9$; $n = 1, 2$; R = Ac
Ib: $m = 7$; $n = 0$; R = H



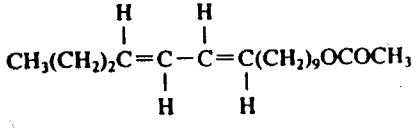
II



III



IV



V

Scheme 1.

With the aim of finding means of protecting these sensitive chemicals and prolonging their biologically active period in the field, we studied the effect of antioxidants and UV absorbers on pheromone stability both in the laboratory and under field conditions. Three internal conjugated diene systems known to be present in moth sex pheromones were studied: Z,E dienes as found in (Z,E)-9,11-tetradecadien-1-yl acetate (TDDA) (II), the main component of the sex pheromone of the female Egyptian cotton leafworm (*Spodoptera littoralis*) (Nesbitt et al., 1973; Tamaki et al., 1973); E,Z dienes as found in (E,Z)-7,9-dodecadien-1-yl acetate (DDA) (III), the sex pheromone of the female European grapevine moth (*Lobesia botrana*) (Roelofs et al., 1973; Buser et al., 1974); and E,E dienes, as found in (E,E)-*10,12*-hexadecadienal (HDAL) (IV) [studied as the corresponding ester (E,E)-10,12-hexadecadien-1-yl acetate (HDDA) (V)], the sex pheromone of the female spiny bollworm (*Earias insulana*) (Hall et al., 1980).

We report here the results of these studies and a preliminary study of the efficiency of protected pheromone carriers in cotton fields in Israel in mass trapping of Egyptian cotton leafworm males.

METHODS AND MATERIALS

Chemicals. The purity of the pheromones and the pheromone analog was determined by GLC: (Z,E)-9,11-C_{14}OAc 98%, 2% E,E-isomer; (E,Z)-7,9-C_{12}OAc 92%, 8% E, E-isomer; (E,E)-10,12-C_{16}OAc 100%. Antioxidants 3(2)-*tert*-butyl-4-hydroxyanoisole (BHA) and 2,6-di-*tert*-butyl-4-methylphenol [butylated hydroxytoluene (BHT)] were purchased from Sigma. UV absorbers 2-hydroxy-4-methoxybenzophenone [$\lambda_{max}^{C_6H_{12}}$ 288, 360 nm (ϵ = 14,770, 9,090); $\lambda_{max}^{t\text{-BuOH}}$ 286, 340 nm (12,860, 7,750)] (trade name Eusolex 4360), and a eutectic mixture of 4-isopropyldibenzoylmethane and 3-(4-methylbenzylidene)camphor [$\lambda_{max}^{C_6H_{12}}$ 312, 342 nm (ϵ = 15,790, 18,420); $\lambda_{max}^{t\text{-BuOH}}$ 299, 350 (22,470, 19,720)] (trade name Eusolex 8021) were purchased from Merck. UV spectra were determined with a Bausch & Lomb Spectronic 2000 in spectroscopic grade cyclohexane or in *tert*-butanol.

GLC analyses were performed on a Packard GLC model 417 fitted with a flame ionization detector and with either a fused silica capillary column of SP 2340 30 m × 0.25 mm, flow rate (He) 0.4 ml/min at 130–160°C or a WCOT Silar 9 25 m × 0.5 mm, flow rate (He) 2.7 ml/min at 130–160°C, depending on the pheromone studied.

All solutions contained 100 mg of a pheromone in 5 ml cyclohexane or *tert*-butanol in a corked Pyrex flask, and 50 μl were withdrawn and diluted to 0.5 ml with cyclohexane and then injected twice (±1%) into the GLC (1–2 μl).

which, having absorption bands at 288 and 360 nm, does not take part in energy transfer to the diene system. We thus may consider E-4360 as a good "filter."

It was interesting that the antioxidant (BHA) was also effective in slowing down the sensitized photoisomerization. As noted above (entries 1 and 2 in Table 3) and as has already been published (Shani et al., 1982), sensitized photoisomerization towards equilibration of the four isomers is fast, being accomplished in 30–100 min. However, when a solution of 100 mg of pheromone (TDDA or DDA) containing 10 mg of rose bengal and 100 mg of BHA was left in the sunlight, the rate of isomerization was much slower, and more than 50% of the starting isomer was detected in the tested solution after 4 hr. When the UV spectrum of an admixture of rose bengal and BHA was studied, two separate and unchanged spectra of the components were clearly observed. Still, energy transfer from rose bengal to BHA can take place since the absorption band of BHA is wide and intense at this concentration (100 mg in 5 ml solution) even beyond the 300 nm. Another explanation could be that the phenoxy radical reacts with the photosensitizer and thus decreased its efficacy as a photosensitizer. More experiments should be undertaken in order to determine the preferred mechanism.

The effect of radicals (I_2, C_6H_5SH) on the isomerization was also studied, and we found that the process with I_2, but not with the thiophenol, is slowed down by antioxidants as radical scavengers. The reaction is temperature dependent and can be considered as a catalytic process in 5–10% of iodine in solution (Ideses and Shani, in preparation).

Simulated and Real Field Conditions. The effect of the binary mixture of the antioxidants and the UV absorber was also checked in pheromone carriers, which are usually applied in the field in traps. We selected to study (*Z*,*E*)-9,11-C_{14}OAc, since this compound is used extensively in mass trapping of males of the Egyptian cotton leafworm in cotton fields in Israel. The pheromone is usually applied into the carrier as a solution of the active ingredients (the solvent evaporates in a short time and then the carriers are stored in a refrigerator until used). These carriers are installed into traps under a "cover" that protects them from direct sunlight. We thus investigated these carriers on the roof of our building in diffused sunlight. The results are shown in Table 4.

We found that the photoisomerization of pheromones in solution takes place at a slower rate than that in pheromones impregnated on a carrier. This phenomenon may be explained in terms of the observation that upon application of solution, "paper chromatography" took place and two or three zones appeared on the carrier. We cannot determine how much of overlapping of the components is effective, but it is interesting that the UV absorber is not as effective on the carrier as it is in solution, especially on the cigarette filter (entries 2 and 6 in Table 4). In fact, it seems that the antioxidant alone is almost as good as the binary mixture of antioxidant and UV absorber. This finding could be

TABLE 4. PHOTOISOMERIZATION OF (*Z*,*E*)-9,11-C_{14}OAc LOADED ON CARRIERS IN PRESENCE OF ANTIOXIDANT (BHA) AND UV ABSORBER (E-4360)[a]

		Relative amount (%)			Exposure time	Composition (%)			
Entry	Carrier	Pheromone[a]	BHA	E-4360	(days)[b]	*Z*,*E*	*E*,*Z*	*Z*,*Z*	*E*,*E*
1	Solution	100	10		17[c]	47	16		37
2	Solution	100	10	10	17	92	2		6
					21	84	6		10
3	Cardboard	100	10		18	69	12		19
					25	60	16		24
4	Cardboard	100	10	10	17	81	8		11
					24	78	10		12
5	Cigarette filter	100	10		18	64	14		22
					21	55	20		25
6	Cigarette filter	100	10	10	18	61	19		20
					21	59	20		21

[a] Each carrier was loaded with cyclohexane solution containing 2 mg of pheromone, 0.2 mg of BHA, and 0.2 mg of E-4360 (when mentioned). The solution contained 100 mg of pheromone in 5 ml cyclohexane. The carriers (two of each sample) were kept in a Pyrex dish, covered with a watch glass. All samples were kept on the roof of the building in diffused sunlight.
[b] The amount of the starting isomer was 20–40% of its original quantity. For technical reasons, analyses of samples were performed on different days.
[c] The flask was broken.

explained by the protective effect of the solid support of the carrier, which may furnish better masking from sunlight than is available in a clear solution. It is also possible that some additives in the paper act as either UV absorbers or quenchers, as the antioxidant acts in the presence of a photosensitizer (see above). Such a phenomenon was found in carriers made of rubber septa (Fujiwara et al., 1977; Brown and McDonough, 1986; Teich and Shani, unpublished results). It is important to remember that without an antioxidant, the pheromone decomposed on the carriers in a few days and could not be detected on the GLC.

The real test of the biological activity of the pheromone carriers loaded with the binary protecting mixture was conducted in kibbutz cotton fields. The usual pheromone carrier on a cigarette filter was loaded with 2 mg of (*Z*,*E*)-9,11-C_{14}OAc and 8 mg of UOP 688 (*N*-phenyl-*N*'-(1-methylheptyl)-*p*-phenylenediamine). The latter compound is a dark, viscous, and unpleasant material that may act as a protecting "solvent" for the pheromone and also for slowing down evaporation. Preliminary results of catching efficacy of different preparations are summarized in Table 5. No significant difference ($P = 0.05$, Student's *t* test) was found between each of the five experimental preparations and the standard (UOP), or among the five compounds themselves, except for two

BHT was added to the pheromone solution as opposed to 30% of the *E,E* isomer when it was not present. After 16 days of sunlight exposure, both solutions contained 35% of the *E,E* isomer. That study was performed during May 1979, when the total sunlight energy is very high (6000–6900 kcal/m^2/day), but temperatures in Be'er-Sheva are still moderate.[2] This might explain why we could still detect the isomers of TDDA after 16 days in sunlight while in the current study, which was performed in August 1985, decomposition was much faster, being complete within seven days (Table 1, entry 1). The first experiment with (*E,Z*)-7,9-C_{12}OAc (entry 4) was done in July 1981 and repeated in July–August 1985.

Effect of UV Absorbers. We studied the effect of two UV absorbers that have their main absorption bands in the region of 300 nm (see Methods and Materials). The photoisomerization of the three diene systems was followed in solution, and the results are summarized in Table 1.

The effect of the antioxidant is again demonstrated in that less pheromone is decomposed in the solution, and exposure period to sunlight is thus lengthened. The effect of the UV absorber is dramatic, as can be seen by comparing the composition in entries 2, 3, 5, 6, 8, and 9 with entries 1, 4, and 7 of Table 1. Exposure to sunlight for three to four weeks brings the isomeric mixture closer to equilibrium (see above), except in the presence of UV absorbers which slow down this process, allowing very little isomerization to take place (entries 2, 5, and 8 of Table 1). The difference between E-4360 and E-8021 (entries, 2, 5, 8 and 3, 6, 9 in Table 1) is clear. We attribute the protective effect to the intense absorption of E-4360 at 288 nm, which exactly covers the end absorption of the conjugated diene system (Shani and Klug, 1980a, b, Shani et al., 1982). The other UV absorbers (E-8021) is less effective, as its main absorption bands are at longer wavelengths, which do not mask the diene absorption. We may also assume that E-8021 does not act as a photosensitizer, since its components have essentially the same chromophor system as that of E-4360. As aromatic ketones, these chemicals probably possess a very high quantum yield for intersystem crossing in solution ($S_1 \rightarrow T_1$) (ΦISC 0.9–1.0) and, when excited, contain about the same triplet energy (ca. 70 kcal/mol) (Calvert and Pitts, 1966).

In order to learn more about the efficacy of both the antioxidant and the UV absorber on the photoisomerization process, we prepared solutions with different amounts of these two essential additives, as shown in Table 2. It is clear that a pheromone solution containing 10% of the antioxidant and 10% of the UV absorber is well protected from both thermal oxidation and photo-

[2] The average daily maximum temperatures (°C) in Be'er-Sheva during different periods of study were as follows: 1979, May 28.7, December 17.0; 1981, July 33.1; 1985, July 32.9, August 34.0. We thank Dov Mills of the Regional Meteorological Station in Be'er Sheva for the data.

TABLE 2. EFFECT OF DIFFERENT AMOUNTS OF UV ABSORBER AND ANTIOXIDANT ON PHOTOISOMERIZATION AND OXIDATION OF TDDA[a]

Entry	Pheromone (mg)	BHA (mg)	E-4360 (mg)	Period in sunlight (days)	Composition (%)				Oxidation product
					Z,E	*E,Z*	*Z,Z*	*E,E*	
1	100	100		16	55	15		30	none
2	100	100	100	16	94	3		3	none
3	100	100	10	17	90	6		4	none
4	100	10	10	17	92	2		6	none
5	100	10		17	47	16		37	none

[a] All samples were solutions in 5 ml cyclohexane.

isomerization of the internal conjugated diene system under sunlight (compare entries 2 and 4 in Table 2).

Effect of Photosensitizer. In the presence of a photosensitizer (in our case rose bengal) the photoisomerization is very fast, yielding an equilibrium mixture, and the process can be accomplished within 90 min (Shani et al., 1982) as compared with direct illumination for several weeks in sunlight without photosensitizer. The question whether the UV absorber could slow down this energy transfer and excitation was investigated, and the results are summarized in Table 3.

The effect of E-4360 in slowing down the sensitized photoisomerization is impressive, as compared with that of E-8021, the latter compound being completely ineffective (compare entries 3 and 4 in Table 3). These results are in keeping with the findings for these two UV absorbers in direct sunlight (Table 1). The photosensitizer (rose bengal) probably transfers its energy to E-4360,

TABLE 3. EFFECT OF UV ABSORBER ON PHOTOISOMERIZATION OF TDDA IN PRESENCE OF PHOTOSENSITIZER[a]

Entry	UV absorber	Exposure time (min)	Composition (%)			
			Z,E	*E,Z*	*Z,Z*	*E,E*
1		45	16	13	1	70
2		75	15	12	1	72
3	E-4360	60	65	10		25
4	E-8021	60	13	16	3	68

[a] Each sample contained 100 mg of (*Z,E*)-9,11-C_{14}OAc, 10 mg of rose bengal, and 100 mg of UV absorber in 5 ml *tert*-butanol.

in the field: the ultimate test of pheromone efficacy is the biological activity on the field, when all factors come into full expression and the weighted system is the governing power.

Acknowledgments—We wish to thank Miss Ofra Gerstein, Dr. Reuven Or, and Mr. Ya'acov Barkai for the cotton field study.

REFERENCES

BROWN, D.F., and MCDONOUGH, L.M. 1986. Insect sex pheromones: Formulations to increase the stability of conjugated diene. *J. Econ. Entomol.* 79:922–927.

BRUCE, W.A., and LUM, P.T.M. 1976. UV radiation: Effect on synthetic sex pheromone of the Indian meal moth. *Fla. Entomol.* 59:53–54.

BRUCE, W.A., and LUM, P.T.M. 1981. Insect pheromone protection from degradations by ultraviolet radiation. *J. Ga. Entomol. Soc.* 16:227–231.

BUSER, H.R., RAUSCHER, S., and ARN, H. 1974. Sex pheromone of *Lobesia botrana*: (*E*,*Z*)-7,9-dodecadienyl acetate in the female grapevine moth. *Z. Naturforsch.* 29C:781–783.

CALVERT, J.G., and PITTS, J.N. 1966. Photochemistry. John Wiley and Sons, New York.

CHISHOLM, M.D., REED, D.W., UNDERHILL, E.W., PALANISWAMY, P., and WONG, J.W. 1985. Attraction of tortricid moths of subfamily Olethreutinae to field traps baited with dodecadienes. *J. Chem. Ecol.* 11:217–230.

DAVIS, H.G., MCDONOUGH, L.M., BURDITT, A.K., JR., and BIERL-LEONHARDT, B.A. 1984. Filbertworm sex pheromone: Identification and field tests of (*E*,*E*)- and (*E*,*Z*)-8,10-dodecadien-1-ol acetates. *J. Chem. Ecol.* 10:53–61.

DUNKELBLUM, E., KEHAT, M., KLUG, J.T., and SHANI, A. 1984. Trimerization of *Earias insulana* sex pheromone, (*E*,*E*)-10,12-hexadecadienal, a phenomenon affecting trapping efficiency. *J. Chem. Ecol.* 10:421–428.

FUJIWARA, H., YASUO, S., and KIYOSHI, N. 1977. Physicochemical studies on the sex pheromones of insects. I. Behaviour of the sex pheromone of *Spodoptera litura* F, in rubber caps. *Chem. Abstr.* 86:12638c.

GOTO, G., MASUOKA, T., and HUROOA, K. 1974. Photooxidation of the sex pheromone (*Z*, *E*)-9,12-tetradecadienyl-1 acetate. *Chem. Lett.* 11:1275–1278.

GUERIN, P.M., ARN, H., BLASER, C., and LETTERE, M. 1984. (*Z*,*E*)-8,10-Dodecadien-1-ol, attractant for male *Pammene rhediella*. *Entomol. Exp. Appl.* 33:346–347.

HALL, D.R., BEEVOR, P.S., LESTER, R., and NESBITT, B.F. 1980. (*E*,*E*)-10,12-Hexadecadienal: A component of the female sex pheromone of the spiny bollworm, *Earias insulana* (Boisd). (Lepidoptera: Noctuidea). *Experientia* 36:152–153.

IDESES, R., and SHANI, A. 1986. Chemical protection of pheromones containing internal conjugated diene system, from chemodegradation. Abstracts of Papers, "Mating Disruption—Behaviour of Moths and Molecules," IOBC-WPRS Meeting, Sept. 8–12, 1986, Neustadt, Federal Republic of Germany, pp. 42–43.

IDESES, R., KLUG, J.T., SHANI, A., GOTHILF, S., and GUREVITZ, E. 1982a. Sex pheromone of the European grapevine moth *Lobesia botrana* Schiff. (Lepidoptera: Tortricidae): Synthesis and effect of isomeric purity on biological activity. *J. Chem. Ecol.* 8:195–200.

IDESES, R., SHANI, A., and KLUG, J.T. 1982b. Sex pheromone of European grapevine moth (*Lobesia botrana*): Its chemical transformations in sunlight and heat. *J. Chem. Ecol.* 8:973–980.

IDESES, R., SHANI, A., and KLUG, J.T. 1982c. Cyclic peroxide—an isolable intermediate in singlet oxygen oxidation of pheromones to furan system. *Chem. Ind. (London)* 00:409–410.

KEHAT, M., GOTHILF, S., DUNKELBLUM, E., and MAZOR, M. 1981. Sex pheromone traps as a potential means of improving control programs for the spiny bollworm, *Earias insulana*. *Phytoparasitica* 9:191–196.

NESBITT, B.F., BEEVOR, P.S., COLE, R.A., LESTER, R., and POPPI, R.G. 1973. Sex pheromones of two noctuid moths. *Nature (London), New Biol.* 244:208–209.

NESTEROVA, I.P. 1985. Photoisomerization of synthetic sex pheromones of insects. Part I. Dodecenyl acetates and *trans*-8,*trans*-10-dodecadien-1-ol. *Chem. Abstr.* 102:91379u.

NESTEROVA, I.P., RAKHTER, B.A., ROSHKA, G.K., and VOINYAK, V.I. 1985. Photoisomerization of synthetic sex pheromones of insects. Part II. Sex attractant of European grape moth. *Chem. Abstr.* 102:108170u.

ROELOFS, W., KOCHANSKY, J., CARDÉ, R., ARN, H., and RAUSCHER, S. 1973. Sex attractant of the grapevine moth *Lobesia botrana*. *Mitt. Schweiz. Entomol. Ges.* 46:71–73.

SHANI, A., and KLUG, J. 1980a. Photooxidation of (*Z*,*E*)-9,11-tetradecadienyl acetate, the main component of the sex pheromone of the female Egyptian cotton leafworm (*Spodoptera littoralis*). *Tetrahedron Lett.*, 21:1563–1564.

SHANI, A., and KLUG, J.T. 1980b. Sex pheromone of Egyptian cotton leafworm (*Spodoptera littoralis*): Its chemical transformations under field conditions. *J. Chem. Ecol.* 6:875–881.

SHANI, A., IDESES, R., KLUG, J.T., SKORKA, J., and ELIYAHU, R. 1982. Isomerization and oxidation of the conjugated double bonds system in pheromones. *Colloq. INRA.* 7:107–113.

SHAVER, T.N., and IVIE, G.W. 1982. Degradation products (Z)-11-hexadecenal and (Z)-9-tetradecenal, components of a sex pheromone of the tobacco budworm. *J. Agric. Food Chem.* 30:367–371.

SYCHEV, A., RETUNSKII, V.N., DUKA, G.G., and VAISMAN, A.E. 1987. Photochemical transformations of biologically active mono- and diene derivatives of pheromones. *Chem. Abstr.* 106:151J67r.

TAMAKI, Y., NOGUCHI, H., and YUSHIMA, T. 1973. Sex pheromone of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae): Isolation, identification and synthesis. *Appl. Entomol. Zool.* 8:200–203.

VAINTRAUB, F.P., RETUNSKII, V.N., and KEISER, L.S. 1983. Characteristics of the photochemical decomposition of codlemone, the sex pheromone of the codling moth. *Chem. Abstr.* 98:84828k.

VYLEGZHANINA, G.F., ANTOCH, E.V., NESTEROVA, I.P., and MOTTUS, E. 1984. Stability of preparative forms of attractants. *Chem. Abstr.* 102:199513x.

WEATHERSTON, I., CARLSON, P.W., GOLUB, M.A., PHELAN, S.A., and LEE, J.M. 1981. Oxidative degradation of pheromones in laboratory and field experiments. *J. Am. Oil Chem. Soc.* 58:569A.

TABLE 5. MASS TRAPPING OF EGYPTIAN COTTON LEAFWORM MALES BY DIFFERENT PHEROMONE PREPARATIONS IN COTTON FIELDS IN 1986

		Average No. of males caught in a trap per night[b]			
		Kibbutz Mefalsim			
		1st experiment		2nd experiment	Kibbutz Reshafim
Entry	Pheromone formulation[a]	Jun 22–Jul 24	Jul 24–Aug 28	Jul 24–Aug 28	Jul 4–Aug 2
1	BHA + E-4360	0.3	6.4	12.2	13.8[c]
2	E-4360	0.4	10.4	11.2	10.8
3	BHA	0.7	7.2	8.7	10.7
4	BHT	1.1	10.3	11.9	14.7[c]
5	BHT + E-4360	3.1	10.9	12.5	12.7
6	UOP 688 (standard)	0.2	6.7	9.3	6.2

[a] Each carrier was loaded with a cyclohexane solution of 2 mg of (*Z*,*E*)-9,11-C_{14}OAc, 0.2 mg of BAH or BHT, and 0.2 mg of E-4360 (when mentioned). With UOP 688, 8 mg of the material was loaded on each carrier with 2 mg of pheromone.
[b] Dry traps with long sleeves were erected at random, 50 m apart, six replicates of each sample. Catches were monitored twice a week.
[c] Significantly different from UOP 688 at $P = 0.10$ (Student's *t* test).

formulations (entries 1 and 4 in Table 5) in the experiment carried out at Kibbutz Reshafim, which were significantly different from UOP 688 (entry 6) ($P = 0.10$, Student's *t* test). These results strengthen previous observations (Teich, Shani, and Klug, unpublished results) made during the 1981–1982 seasons, which showed not only that BHA and BHT were better protecting chemicals for (*Z*,*E*)-9,11-C_{14}OAc than UOP 688, but also that they increased catching efficacy. Therefore, it should be desirable to replace UOP 688 as an antioxidant in pheromone protections with BHA and BHT, at least for technical and handling reasons.

It is interesting that both the UV absorber and the antioxidant as a single additive prolonged the trapping efficacy of the pheromone. This fact may be in keeping with our earlier findings (see Table 4) that the behavior of the solid carriers was very much like that of one (antioxidant) or two additives and not to the positive effect observed for the UV absorber in solution (Tables 1, 2, and 4). The exact quantity of the pheromone and the relative composition of its geometrical isomers remaining on the solid carriers in the field can indicate the

correlation between the chemical processes and the biological activity (Shani and Klug, 1980b). At this stage we should remember that, when analyzing the results from the field study and comparing them with the chemical transformations of the pheromone, either in solution or on solid carriers, we are comparing two different processes and results. It could be, and we indeed found earlier in two cases, that not much of the real pheromone is needed for attraction, and the other geometric isomers do not interfere with trapping (Shani and Klug, 1980b; Ideses et al., 1982a). Therefore, the chemical results obtained in the laboratory do not necessarily reflect the biological findings in the field and vice versa. As long as enough active pheromone is present on the carrier and the other isomers are inactive and do not interfere, there may be high activity in the field despite the fact that isomerization and/or oxidation has destroyed much of the pheromone. In solutions studies, the pheromones and their isomers could be detected by GLC for up to six weeks afer the start of the experiment, while in the field the carriers attracted the Egyptian cotton leafworm for up to two months. The results in Table 5 show that, in the first experiment at Kibbutz Mefalsim, trapping in the second month (24.7–28.8) was almost as high as that in the first month of the second experiment.

We may summarize the results as follows:

Thermal Decomposition–Oxidation. Addition of an antioxidant prolonged the life-span of the diene system both in solution and in the carrier from several days to 2 weeks to six to eight weeks (Shani and Klug, 1980b; Ideses et al., 1982b; this work).

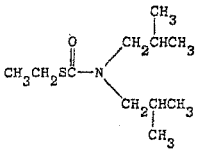
Isomerization. Addition of a UV absorber effectively slowed down isomerization in the diene system in solution and was less effective on the solid carrier. Both additives are crucial in solution, but the effect of the UV absorber becomes less important on the solid carriers.

Biological Activity. We have found that (*Z*,*E*)-9,11-C_{14}OAc attracts the Egyptian cotton leafworm in the field up to two months. The (*E*,*Z*)-7,9-C_{12}OAc isomer was found to attract the European grapevine moth in the field for up to seven weeks (Ideses et al., 1982a), which time is in the range of the chemical stability. The (*E*,*E*)-10,12-C_{16}Ald isomer is active in catching the spiny bollworm for four to five weeks (Kehat et al., 1981). The chemical behavior of this isomer was studied on the corresponding acetate, as the sensitive group is the aldehyde. The isomerization was very slow as the *E*,*E* isomer is the most stable one in the mixture.

Israeli entomologists and farmers usually change the carriers in traps for all three once in four to six weeks, although from scattered observations in the fields, some traps were found to be active for two months or more.

All these findings lead to the conclusion that results of laboratory studies and experimentation cannot necessarily be extrapolated to the real-life situation

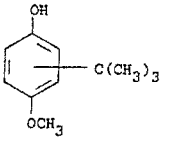
Stauffer). Metabolism: J. P. Hubbell, J. E. Casida, *J. Agr. Food Chem.* **25**, 404 (1977).



Clear liquid, bp_{21} 138°. n_D^{30} 1.4701; d 0.9417. Vapor press at 25°: 1.3×10^{-3} mm Hg. Soly in water at 25°: 45 mg/l. LD_{50} orally in rats: 4000 mg/kg, *RTECS* **Vol. I**, R. J. Lewis, Sr., R. L. Tatken, Eds. (1979) p 365.

USE: Herbicide.

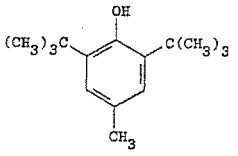
1547. Butylated Hydroxyanisole. BHA; Antrancine 12; Embanox; Nipantiox 1-F; Sustane 1-F; Tenox BHA. $C_{11}H_{16}O_2$; mol wt 180.24. C 73.30%, H 8.95%, O 17.75%. A mixture of *2-tert-butyl-4-methoxyphenol* (also called 3-*tert*-butyl-4-hydroxyanisole) and *3-tert-butyl-4-methoxyphenol* (also called 2-*tert*-butyl-4-hydroxyanisole). Prepn from *p*-methoxyphenol and isobutene: Rosenwald, **U.S.** pats. **2,459,540** and **2,470,902** (1949 to Universal Oil Products).



Waxy solid, mp 48-55°. bp_{733} 264-270°. Insoluble in water. Soluble in petr ether (Skellysolve H), in 50% alcohol (or higher), in propylene glycol; alcohols. Sol in fats and oils. Exhibits antioxidant properties and synergism with acids, BHT, propyl gallate, hydroquinone, methionine, lecithin, thiodipropionic acid, etc. LD_{50} orally in mice, rats: 2000, 2200 mg/kg, A. J. Lehman *et al., Adv. Food Res.* **3**, 197 (1951).

USE: Antioxidant, esp in foods. The American Meat Institute Foundation has proposed an antioxidant mixture known as *AMIF-72* which contains 20% of butylated hydroxyanisole, 6% of propyl gallate, and 4% of citric acid in propylene glycol.

1548. Butylated Hydroxytoluene. *2,6-Bis(1,1-dimethylethyl)-4-methylphenol; 2,6-di-tert-butyl-p-cresol;* 2,6-di-*tert*-butyl-4-methylphenol; BHT; Antrancine 8; Tenox BHT; Ionol CP; Sustane; Dalpac; Impruvol; Vianol. $C_{15}H_{24}O$; mol wt 220.34. C 81.76%, H 10.98%, O 7.26%. Prepared from *p*-cresol and isobutylene: Stillson, **U.S.** pat. **2,428,745** (1947 to Gulf); McConnell, Davis, **U.S.** pat. **3,082,258** (1963 to Eastman Kodak). Inactivator of lipid-containing mammalian and bacterial viruses: Snipes *et al., Science* **188**, 64 (1975).



Crystals, mp 70°. d_4^{20} 1.048. bp 265°. Flash pt (open cup): 260°F (127°C). Insol in water. Freely sol in toluene, sol in methanol, ethanol, isopropanol, methyl ethyl ketone, acetone, Cellosolve, petr ether, benzene, most other hydrocarbon solvents. Soly in liquid petrolatum (white oil): 0.5% w/w. More sol in food oils and fats than butylated hydroxyanisole. Good soly in linseed oil. LD_{50} orally in mice: 1040 mg/kg, *J. Am. Pharm. Assoc.* **38**, 366 (1949).

USE: Antioxidant for food, animal feed, petrol products, synthetic rubbers, plastics, animal and vegetable oils, soaps. Antiskinning agent in paints and inks.

1549. *n*-Butylbenzene. 1-Phenylbutane. $C_{10}H_{14}$; mol wt 134.21. C 89.49%, H 10.51%. $C_6H_5(CH_2)_3CH_3$. Prepn: Radziszewski, *Ber.* **9**, 261 (1876); Balbiano, *Ber.* 10, (1877); Read, Foster, *J. Am. Chem. Soc.* **48**, 1606 (1

Liquid. mp −88.5°. d_4^{20} 0.8604. bp_{760} 183.1°; bp_{400} bp_{200} 136.9°; bp_{100} 116.2°; bp_{60} 102.6°; bp_{40} 92.4°; bp_{20} bp_{10} 62.0°; bp_5 48.8°; $bp_{1.0}$ 22.7°. n_D^{20} 1.49040. Flash pt, cup: 160° F (71° C). Insol in water; miscible with alcoh ether, benzene.

1550. *sec*-Butylbenzene. *(1-Methylpropyl)benzene;* phenylbutane. $C_{10}H_{14}$; mol wt 134.21. C 89.49%, H 10. $C_6H_5CH(CH_3)CH_2CH_3$. Prepd from benzene and *n*-but chloride in presence of $AlCl_3$: Schramm, *Monatsh.* 9, (1888); by the action of sodium on γ-chloro-*sec*-butyl zene: Braun *et al., Ber.* **46**, 1277 (1913); with other produc by heating *n*- or *sec*-butyl alcohol with 80% H_2SO_4: Mey Bernhauer, *Monatsh.* **53**, 727 (1929).

Liquid. mp −82.7°. d_4^{20} 0.8608. bp_{760} 173.5°; bp_{400} bp_{200} 128.8°; bp_{100} 109.5°; bp_{60} 96.0°; bp_{40} 86.2°; bp_{20} bp_{10} 57.0°; bp_5 44.2°; $bp_{1.0}$ 18.6°. n_D^{20} 1.48980. Flash closed cup: 126° F (52° C). Insol in water; misc with hol, ether, benzene.

d-Form, $[\alpha]_D^{25}$ +26.6°: Bonner, Greenlee, *J. Am. Soc.* **81**, 3336 (1959).

l-Form, $[\alpha]_D^{25}$ −27.3°.

USE: Solvent; in organic syntheses.

1551. *tert*-Butylbenzene. *(1,1-Dimethylethyl)benzene;* 2-methyl-2-phenylpropane; trimethylphenylmethane; pseu dobutylbenzene. $C_{10}H_{14}$; mol wt 134.21. C 89.49% 10.51%. $C_6H_5C(CH_3)_3$. Prepn: Konowalow, *Bull. Soc. Chim.* [3] **16**, 865 (1896); Shoesmith, Mackie, *J. Chem. Soc.* **1928**, 2336; Meyer, Bernhauer, *Monatsh.* **53**, 727 (1929 Wilt, Abegg, *J. Org. Chem.* **33**, 923 (1968). *See also* Groo Ipatieff, *J. Am. Chem. Soc.* **57**, 2415 (1935); Ipatieff, Pines *ibid.* **58**, 1056 (1936).

Liquid. mp −58.1°. d_4^{20} 0.8669. bp_{760} 168.5°; bp_{400} bp_{200} 123.7°; bp_{100} 103.8°; bp_{60} 90.6°; bp_{40} 80.8°; bp_{20} bp_{10} 51.7°; bp_5 39.0°; $bp_{1.0}$ 13.0°. n_D^{20} 1.49235. Flash pt, cup: 140°F (60°C). Insol in water; misc with alcohol, ether benzene.

1552. *n*-Butyl Benzoate. *Benzoic acid butyl ester.* $H_{14}O_2$; mol wt 178.22. C 74.13%, H 7.92%, O 17. $C_6H_5COO(CH_2)_3CH_3$. Prepn: Newman, Fones, *J. Chem. Soc.* **69**, 1046 (1947); Justoni, **Brit.** pat. **719,891** (19 to Vismara).

Thick, oily liquid. d 1.00. mp −22°. bp 250°. Practi insoluble in water; sol in alcohol or ether. LD_{50} orall rats: 5.14 g/kg, Smyth *et al., Arch. Ind. Hyg. Occup. Me* **10**, 61 (1954).

1553. *n*-Butyl Bromide. *1-Bromobutane.* C_4H_9Br; mo wt 137.03. C 35.06%, H 6.62%, Br 58.32%. $CH_3(CH_2)_3Br$. Prepd from *n*-butyl alc and a hydrobromic-sulfuric acid mixture: Kamm, Marvel, *Org. Syn.* **vol. 1**, 5 (1921); Ska McCullough, *J. Am. Chem. Soc.* **57**, 2440 (1935).

Colorless liquid. d_4^{25} 1.2686. bp_{760} 101.3° (mp −112°) 1.4398. Insol in water; sol in alcohol, ether.

1554. *sec*-Butyl Bromide. *2-Bromobutane;* methylethyl bromomethane. C_4H_9Br; mol wt 137.03. C 35.06% 6.62%, Br 58.32%. $CH_3CH_2CHBrCH_3$. Prepn: Leve Marker, *J. Biol. Chem.* **91**, 405 (1931); Kenyon *et al. Chem. Soc.* **1935**, 1080; Skau, McCullough, *J. Am. Che Soc.* **57**, 2440 (1935); Colson *et al., J. Chem. Soc.* **1965**, 23 Prepn of optically pure isomers: Goodwin, Hudson *Chem. Soc. (B)* **1968**, 1333.

dl-Form, colorless liquid, pleasant odor. d_4^{25} 1.2530 91.2° (mp −112°). n_D^{25} 1.4344. Insol in water. Freely so alcohol, ether.

d-Form, n_D^{20} 1.4359-1.4362. α_D^{20} +42.64°.

l-Form, n_D^{20} 1.4368. α_D^{20} −43.7°.

Caution: Narcotic in high concns.

1555. *tert*-Butyl Bromide. *2-Bromo-2-methylpropane;* 2-bromoisobutane; trimethylbromomethane. C_4H_9Br; mo wt 137.03. C 35.06%, H 6.62%, Br 58.32%. $(CH_3)_3CBr$. Prepn: Brunel, *J. Am. Chem. Soc.* **39**, 1978 (1917); Bryc Smith, Howlett, *J. Chem. Soc.* **1951**, 1141; Coe *et al.*, **1954**, 2281.

more than 5,000 parts per million based on fat and oil content of the food.

[48 FR 18798, Apr. 26, 1983, as amended at 54 FR 24896, June 12, 1989]

§ 172.110 BHA.

The food additive BHA (butylated hydroxyanisole) alone or in combination with other antioxidants permitted in food for human consumption in this Subpart B may be safely used in or on specified foods, as follows:

(a) The BHA meets the following specification:

Assay (total BHA), 98.5 percent minimum. Melting point 48° C minimum.

(b) The BHA is used alone or in combination with BHT, as an antioxidant in foods, as follows:

Food	Limitations (total BHA and BHT) parts per million
Dehydrated potato shreds	50
Active dry yeast	[1] 1,000
Beverages and desserts prepared from dry mixes	[1] 2
Dry breakfast cereals	50
Dry diced glazed fruit	[1] 32
Dry mixes for beverages and desserts	[1] 90
Emulsion stabilizers for shortenings	200
Potato flakes	50
Potato granules	10
Sweetpotato flakes	50

[1] BHA only.

(c) To assure safe use of the additive:

(1) The label of any market package of the additive shall bear, in addition to the other information required by the Act, the name of the additive.

(2) When the additive is marketed in a suitable carrier, in addition to meeting the requirement of paragraph (c)(1) of this section, the label shall declare the percentage of the additive in the mixture.

(3) The label or labeling of dry mixes for beverages and desserts shall bear adequate directions for use to provide that beverages and desserts prepared from the dry mixes contain no more than 2 parts per million BHA.

§ 172.115 BHT.

The food additive BHT (butylated hydroxytoluene), alone or in combination with other antioxidants permitted in this Subpart B may be safely used in or on specified foods, as follows:

(a) The BHT meets the following specification: Assay (total BHT) 99 percent minimum.

(b) The BHT is used alone or in combination with BHA, as an antioxidant in foods, as follows:

Food	Limitations (total BHA and BHT) parts per million
Dehydrated potato shreds	50
Dry breakfast cereals	50
Emulsion stabilizers for shortenings	200
Potato flakes	50
Potato granules	10
Sweetpotato flakes	50

(c) To assure safe use of the additive:

(1) The label of any market package of the additive shall bear, in addition to the other information required by the Act, the name of the additive.

(2) When the additive is marketed in a suitable carrier, in addition to meeting the requirement of paragraph (c)(1) of this section, the label shall declare the percentage of the additive in the mixture.

§ 172.120 Calcium disodium EDTA.

The food additive calcium disodium EDTA (calcium disodium ethylene-diaminetetraacetate) may be safely used in designated foods for the purposes and in accordance with the conditions prescribed, as follows:

(a) The additive contains a minimum of 99 percent by weight of either the dihydrate $C_{10}H_{12}O_8N_2CaNa_2 \cdot 2H_2O$ or the trihydrate $C_{10}H_{12}O_8N_2CaNa_2 \cdot 3H_2O$, or any mixture of the two.

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

(1) Alone, in the following foods at not to exceed the levels prescribed, calculated as the anhydrous compound:

Food	Limitation (parts per million)	L
Cabbage, pickled	220	Promote co and textu
Canned carbonated soft drinks.	33	Promote fla retention.
Canned white potatoes	110	Promote col retention.
Clams (cooked canned)	340	Promote col retention.
Crabmeat (cooked canned).	275	Retard struvi formation; color reten
Cucumbers pickled	220	Promote colc and texture
Distilled alcoholic beverages.	25	Promote stab color, flavo product cla
Dressings, nonstandardized.	75	Preservative.
Dried lima beans (cooked canned).	310	Promote color retention.
Egg product that is hard-cooked and consists, in a cylindrical shape, of egg white with an inner core of egg yolk.	[1] 200	Preservative.
Fermented malt beverages.	25	Antigushing ag
French dressing	75	Preservative.
Mayonnaise	75	Do.
Mushrooms (cooked canned).	200	Promote color retention.
Oleomargarine	75	Preservative.
Pecan pie filling	100	Promote color retention.
Potato salad	100	Preservative.
Processed dry pinto beans.	800	Promote color retention.
Salad dressing	75	Preservative.
Sandwich spread	100	Do.
Sauces	75	Do.
Shrimp (cooked canned).	250	Retard struvite formation; pron color retention.
Spice extractives in soluble carriers.	60	Promote color an flavor retention.
Spreads, artificially colored and lemon-flavored or orange-flavored.	100	Promote color retention.

[1] By weight of egg yolk portion.

(2) With disodium EDTA (disod ethylenediaminetetraacetate) in following foods at not to exceed, combination, the levels prescribed, culated as anhydrous $C_{10}H_{12}O_8N_2Ca$

Lepidopteran Pheromones: Tolerance Exemption

[Federal Register: August 30, 1995 (Volume 60, Number 168)] [Rules and Regulations]
[Page 45060-45062]
>From the Federal Register Online via GPO Access [wais.access.gpo.gov]

ENVIRONMENTAL PROTECTION AGENCY
[OPP-300396; FRL-4971-8]

40 CFR Part 180

Lepidopteran Pheromones: Tolerance Exemption

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: This document establishes an exemption from the requirement of a food tolerance for residues of certain Lepidopteran pheromones resulting from the use of these substances independent of formulation, mode of application or physical form or shape with an annual application limitation of 150 grams active ingredient per acre (gm AI/ acre) for pest control in or on all raw agricultural commodities. This exemption pertains only to the pheromone active ingredient. Any encapsulating material needs to be a cleared inert for pesticidal uses on food crops. EPA is establishing this regulation on its own initiative.

EFFECTIVE DATE: This regulation becomes effective August 30, 1995.

ADDRESSES: Written objections and hearing requests, identified by the docket control number, OPP-300396, may be submitted to: Hearing Clerk (1900), Environmental Protection Agency, Rm. M3708, 401 M St., SW., Washington, DC 20460. A copy of any objections and hearing requests filed with the Hearing Clerk should be identified by the docket control number and submitted to: Public Response and Program Resources Branch, Field Operations Division (7506C), Office of Pesticide Programs, Environmental Protection Agency, 401 M St., SW., Washington, DC 20460. In person, bring a copy of objections and hearing requests to: Public Docket, Rm. 1132, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA.
A copy of objections and hearing requests filed with the Hearing Clerk may also be submitted electronically by sending electronic mail (e-mail) to: opp-docket@epamail.epa.gov. Copies of objections and hearing requests must be submitted as an ASCII file avoiding the use of special characters and any form of encryption. Copies of objections and hearing requests will also be accepted on disks in WordPerfect in 5.1 file format or ASCII file format. All copies of objections and hearing requests in electronic form must be identified by the docket number ``OPP-300396.'' No Confidential Business Information (CBI) should be submitted through e-mail. Electronic copies of objections and hearing requests on this rule may be filed online at many Federal Depository Libraries. Additional information on electronic submissions can be found in Unit IV. of this document.

[[Page 45061]]

FOR FURTHER INFORMATION CONTACT: By mail: Phil Hutton, Product Manager (PM-90), Biopesticides and Pollution Prevention Division (7501W), Office of Pesticide Programs, Environmental Protection Agency, 401 M St., SW., Washington, DC 20460. Office location, telephone number, and e-mail address: 5th Floor, Crystal Station 1, 2805 Crystal Drive, Arlington, VA, (703) 308-8260, e-mail: hutton.phil@epamail.epa.gov.

SUPPLEMENTARY INFORMATION: In the Federal Register of March 29, 1995 (60 FR 16128), EPA issued a notice of filings and invited comments on a pesticide petition to propose amending 40 CFR part 180 by establishing an exemption from the requirement of a food tolerance for certain Lepidopteran pheromones regardless of mode of application when used at rates less than or equal to 150 grams ai/acre/year. The Agency received no comments in response to its notice. In this document, EPA sets forth its reasons for determining that a tolerance for these pheromone products is not necessary to protect public health. For the purposes of this exemption, a Lepidopteran pheromone is defined as a naturally occurring compound, or identical or substantially similar synthetic compound, designated by the unbranched aliphatics (with a chain between 9 and 18 carbons) ending in an alcohol, aldehyde or acetate functional group and containing up to 3 double bonds in the aliphatic backbone. This definition encompasses the majority of Lepidopteran pheromones. While other types of chemical compounds have been demonstrated to be Lepidopteran pheromones and other arthropod pheromones have been recommended for tolerance exemptions, there is limitied toxicity data and exposure information available. The Agency believes the type described here represents not only the majority of Lepidopteran pheromones but also those with the most complete toxicological data base. Synthetically produced compounds that are identical to a known aliphatic Lepidopteran pheromone as described above, and those that differ only in that their molecular structures are stereochemical isomers (or ratios of such isomers) are also included in this tolerance exemption. Other Lepidopteran pheromones and other pheromones not included within the described scope will still require mammalian toxicity testing (40 CFR 158.690) if

used on food crops and are not otherwise exempt from the requirement of a tolerance.

I. Background

A pheromone (including an identical synthetic compound) is defined by EPA as a compound produced by an arthropod (insect, arachnid, or crustacean) that modifies the behavior of other individuals of the same species (40 CFR 152.25(b)). Lepidopteran pheromones are those produced by a member of the order Lepidoptera, which includes butterflies and moths. One physical-chemical feature common to all these compounds is their volatility which is the basis for the signalling and homing mechanism. The Agency has registered 17 arthropod pheromones active ingredients, 11 of which are Lepidopteran pheromones. The Agency has assumed that pheromones and other similar semiochemicals are different from conventional synthetic pesticides, and has attempted to facilitate their registration with reduced data requirements and regulatory relief efforts. Most recently the Agency has recognized that a special category of pheromone products dispensed from larger sized polymeric matrices with low annual use rates represent minimal risk for dietary and environmental exposure and has greatly eased the burden to register these items. Broadcast methods of application were not included because the Agency did not have sufficient information on the levels of exposure from pheromones applied in this manner. The Agency has since received data in this area. In addition to submitted data, the Agency utilized in its decision an internal document of the toxicology of certain Lepidopteran pheromones related by their chemical structure. For pheromone products, especially those directly applied to food, one problem has been a lack of subchronic toxicity studies and an estimate of the actual pheromone residues occurring with use. Some pheromone uses in solid matrix dispensers have been registered based on the low probability of exposure justifying the waiver of the subchronic toxicity studies, namely the 90-day feeding, the developmental toxicity and immunotoxicity studies. However, the Agency has held that sprayable formulations or other modes of application that may increase the likelihood of human exposure would still require the subchronic toxicology studies.

II. Human Health

Data has been submitted on subchronic toxicology studies done to date on compounds similar in structure to the Lepidopteran pheromones and published in the peer reviewed, public literature. The information submitted covered compounds that were from six to sixteen carbon unbranched alcohols, acetates and aldehydes. Since the Agency is basing this tolerance exemption on chemical structure, it is relevant to consider the available subchronic toxicology data for this group. The results given in these literature reports indicate that there is no significant acute toxicity associated with the primary alcohols, acetates or aldehydes mentioned (C<INF>8 to C<INF>16 unbranched aliphatics). In addition, the subchronic toxicity of an isomeric mixture of tridecenyl acetate indicated no significant signs of toxicity other than those expected with longer term exposure to high doses of a hydrocarbon. The findings of the published studies indicate that there were no significant health effects from subchronic exposures to this group of chemicals.
Studies examining the volatilization of a pheromone from a microcapsule indicates that about 70 percent of the pheromone remains after 30 days. These results indicate the pheromone is released at a slower rate than anticipated. The studies show that only a small proportion of the microcapsules actually release any pheromone or only a portion of the total pheromone loaded into the capsule is capable of ever being released. These laboratory studies indicate a potential for pheromone residues to occur in the absence of any biological or environmental factors.
In a submitted field study, however, residue analyses from field treated plants indicate no significant amounts of pheromone can be detected on the resulting fruit. The detectable residues on unwashed fruit of tomato pinworm pheromone ranged from 21-72 ppb on the day of application, decreased to 0.9-6.8 ppb on day 15, and was recorded at 0.29-1.2 ppb on day 30. Washing the tomatoes brought all the residues below the level of detection. This study demonstrates that the expected pheromone residue levels found in tomato fruit are several orders of magnitude lower than previously calculated estimates. The process of application, weathering, and other environmental degradation leads to a reduction in the active ingredient that approaches the system limit of detection in the expected 3-week lifetime of the raw agricultural product.

III. Conclusion

The Agency believes that the potential for pheromone residues is not a dietary hazard. This conclusion is based on: (1) The low acute toxicity seen in the data

[[Page 45062]]
review of the Lepidopteran pheromones registered to date; (2) the known metabolism of long-chain fatty acids that predicts these compounds would be metabolized either by <greek-b>-oxidation yielding a series of paired carbon losses or by complexing with glucuronide and excretion by the kidneys; and (3) low exposure subsequent to application from product aging, volatilization, and the results of the field residue studies.
EPA has determined that, when used in accordance with good agricultural practices, a food tolerance for the defined subset of Lepidopteran pheromones is not necessary to protect the public health. A generic exemption for this low-risk, low-exposure group of substances will facilitate the use of semiochemicals as alternatives to conventional synthetic pesticides. Therefore, EPA is establishing an exemption from the requirement of a tolerance as set forth below for the defined group of compounds with from 9 to 18 carbon atoms, regardless of formulation or mode of application, at use rates of less than 150 grams active ingredient/acre/year. It is important to note that any encapsulating material needs to be a cleared inert for pesticidal uses on food crops. To the extent that other straight chained, or non-straight chained chemicals within this group may be naturally occurring and sufficiently similar to these Lepidopteran compounds in use, they may also meet the exemption from the requirement for a food

tolerance upon review by the Agency. Any person adversely affected by this regulation may, within 30 days, file written objections and/or request a hearing with the Hearing Clerk and a copy submitted to the OPP docket for this rulemaking at the addresses given above.

IV. Rulemaking Record

A record has been established for this rulemaking under docket number ``OPP-300396" (including objections and hearing requests submitted electronically as described below). A public version of this record, including printed, paper versions of electronic comments, which does not include any information claimed as CBI, is available for inspection from 8 a.m. to 4:30 p.m., Monday through Friday, excluding legal holidays. The public record is located in Room 1132 of the Public Response and Program Resources Branch, Field Operations Division (7506C), Office of Pesticide Programs, Environmental Protection Agency, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA. Written objections and hearing requests, identified by the document control number ``OPP-300396", may be submitted to the Hearing Clerk (1900), Environmental Protection Agency, Rm. 3708, 401 M St., SW., Washington, DC 20460.
A copy of electronic objections and hearing requests filed with the Hearing Clerk can be sent directly to EPA at:

opp-docket@epamail.epa.gov

A copy of electronic objections and hearing requests filed with the Hearing Clerk must be submitted as an ASCII file avoiding the use of special characters and any form of encryption. The official record for this rulemaking, as well as the public version, as described above will be kept in paper form. Accordingly, EPA will transfer any objections and hearing requests received electronically into printed, paper form as they are received and will place the paper copies in the official rulemaking record which will also include all objections and hearing requests submitted directly in writing. The official rulemaking record is the paper record maintained at the address in ``ADDRESSES" at the beginning of this document.

V. Regulatory Assessments

The Office of Management and Budget has exempted this notice from the requirement of section 3 of Executive Order 12866. Pursuant to the requirements of the Regulatory Flexibility Act (Pub. L. 96354, 94 Stat. 1164, 5 U.S.C. 601-612), the Administrator has determined that regulations establishing new tolerances or raising tolerance levels or establishing exemptions from tolerance requirements do not have a significant economic impact on a substantial number of small entities. A certification statement to this effect was published in the Federal Register of May 4, 1981 (46 FR 24950).

Dated: August 18, 1995.
Janet L. Andersen,
Acting Director, Biopesticides and Pollution Prevention Division Office of Pesticide Programs.

Therefore, it is proposed that 40 CFR part 180 be amended as follows:

PART 180--[AMENDED]

1. The authority citation for part 180 continues to read as follows: Authority: 21 U.S.C. 346a and 371.

2. By adding Sec. 180.1153 to subpart D to read as follows:

Sec. 180.1153 Lepidopteran pheromones; exemption from the requirement of a tolerance.

Lepidopteran pheromones that are naturally occurring compounds, or identical or substantially similar synthetic compounds, designated by an unbranched aliphatic chain (between 9 and 18 carbons) ending in an alcohol, aldehyde or acetate functional group and containing up to 3 double bonds in the aliphatic backbone, are exempt from the requirement of a tolerance in or on all raw agricultural commodities. This exemption pertains to only those situations when the pheromone is applied to growing crops at a rate not to exceed 150 grams active ingredient/acre/year in accordance with good agricultural practices.

[FR Doc. 95-21037 Filed 8-29-95; 8:45 am] BILLING CODE 6560-50-F

Biopesticide Fact Sheet
Generic Factsheet for Lepidopteran Pheromones
Issued: 09/01

Fact Sheet	Technical Doc	Products	Registrants	Regulatory Activity	FR Notices	Bibliography

SUMMARY

Lepidopteran pheromones are used to disrupt the mating behavior of certain moths whose larvae destroy crops and trees. Data indicate that these compounds do not present any known risks to humans or the environment.

I. DESCRIPTION OF THE ACTIVE INGREDIENT

Pheromones are volatile chemicals produced by a given species to communicate with other individuals of the same species to change their behavior. For example, various species use pheromones to attract a mate, to mark territory, or to warn others of danger. Pheromones are usually effective in tiny amounts.

The lepidopteran group of insects includes butterflies and moths. All of the lepidopteran pheromones that EPA has approved for pesticide use are chemicals produced by female moths to attract a mate. The pesticide products contain synthetic versions of these naturally occurring compounds. Sometimes the relative amounts of several pheromone chemicals in a pesticide product determine which specific pests are controlled.

When the pesticide product releases pheromone into the air where males are looking for females, the males become confused and cannot easily locate the females. As a result, many of the females do not mate and lay eggs, and there are many fewer offspring than usual.

A list of the currently approved lepidopteran pheromones is attached

II. USE SITES, TARGET PESTS, AND APPLICATION METHODS

Use Sites: Wide variety of places where plants grow, such as agricultural and residential sites and forests.

Target pests: Specific moths whose offspring harm crops.

Application Methods: There are two major ways to disperse lepidopteran pheromones:

1) Using dispensers that slowly release the pheromone over a period of weeks. The dispensers are often attached to trees or to stakes in the field.
2) Using ground or aerial spray equipment.

III. ASSESSING RISKS TO HUMAN HEALTH

Based on low toxicity in animal testing, and expected low exposure to humans, no risk to human health is expected from the use of these pheromones. During more than 10 years of use of lepidopteran pheromones as pesticides, no adverse effects have been reported.

Whether or not a substance poses a risk to humans or other organisms depends on two

The safety record for lepidopteran pheromones has allowed the Agency to conclude that consumption of food containing residues of the pheromones presents no risk. In addition, these pheromones can be used experimentally without a permit on up to 250 acres, versus the 10-acre limit imposed on other pesticides.

factors: how toxic the substance is, and how much of it an organism is exposed to. Therefore, the EPA considers both toxicity and exposure data in determining whether to approve pesticide for use

IV. ASSESSING RISKS TO THE ENVIRONMENT

Adverse effects on nontarget organisms (mammals, birds, and aquatic organisms) are not expected because these pheromones are released in very small amounts to the environment and act on a select group of insects.

V. REGULATORY INFORMATION

As of November 1999, EPA had registered (licensed for sale) approximately 20 moth mating **pheromones as** pesticide active ingredients and more than 60 individual pesticide products containing these active ingredients. (See below for examples).

VI. FOR FURTHER INFORMATION CONTACT

Brian Steinwand

Regulatory Action Leader

Biopesticides and Pollution Prevention Division (7511C)
Phone (703) 305-7973
e-mail: steinwand.brian@epa.gov

or

Biopesticides and Pollution Prevention Division (7511C)

Office of Pesticide Programs

Environmental Protection Agency
1200 Pennsylvania Ave. N.W.
Washington, D.C. 20460
Phone (703) 308-8712
Fax (703) 308-7026

Female moth mating pheromones approved as pesticides

Pests Controlled	Use Sites	Chemical Name of Pheromone (OPP Chemical Code) Note: When 2 or more pheromones are listed in a cell, various mixtures of the pheromones have been approved for use against the pest)	CAS Number
Artichoke plume moth	Artichokes	(Z)-11-Hexadecenal (120001)	53939-28-9
Beet armyworm	Alfalfa Cotton Strawberries Vegetables (various) Tobacco	(Z,E) -9,12-Tetradecadienyl acetate (117203) (Z)-9-Tetradecen-1-ol (119409)	31654-77-0 35153-15-2
Blackheaded fire worm	Cranberries Fruit	(Z)-11-Tetradecenyl acetate (128980)	20711-10-8

Codling moth	Fruit Nuts	Lauryl alcohol (001509) Myristyl alcohol (001510) (E,E) -8,10-Dodecadien-1-ol (129028) (Z) -11-Tetradecenyl acetate (128980)	112-53-8 112-72-1 33956-49-9 20711-10-8
Codling moth	Fruit Nuts Ornamental trees / shrubs Uncultivated agricultural areas	(E,E) -8,10-Dodecadien-1-ol (129028)	33956-49-9
Diamondback moth	Manufacturing use	(Z)-11-Hexadecenyl Acetate (129101)	34010-21-4
Eastern Pine Shoot Borer	Forest trees Woodland trees	(E)-9-Dodecen-1-ol acetate	35148-19-7
Grapeberry moth	Grapes Vine fruit	(Z)-9-Dodecenyl acetate (129004)	16974-11-1
Grapeberry moth	Grapes	(Z)-11-Tetradecenyl acetate (128980) (Z)-9-Dodecenyl acetate (129004)	20711-10-8 16974-11-1
Gypsy moth	Forest trees Ornamental evergreen trees and shrubs	cis-7,8-Epoxy-2-methyloctadecane (114301)	29804-22-6
Hickory shuckworm	Fruits Nuts Uncultivated agricultural areas	(E,E) -8,10-Dodecadien-1-ol (129028)	33956-49-9
Hickory shuckworm	Fruit Nuts	Lauryl alcohol (1509) Myristyl alcohol (1510) (E,E) -8,10-Dodecadien-1-ol (129028) (Z) -11-Tetradecenyl acetate (128980)	112-53-8 112-72-1 33956-49-9 20711-10-8

Koa seed worm	Fruit Nuts	(Z)-8-Dodecen-1-yl acetate (128906) (E)-8-Dodecen-1-yl-acetate (128907) (Z)-8-Dodecen-1-ol (128908)	28079-04-1 38363-29-0 40642-40-8
Leafrollers (various)	Cranberries Fruit	(Z)-11-Tetradecenyl acetate (128980)	20711-10-8
Macadamia nut borer	Fruit Nuts	(Z)-8-Dodecen-1-yl acetate (128906) (E)-8-Dodecen-1-yl-acetate (128907) (Z)-8-Dodecen-1-ol (128908)	28079-04-1 38363-29-0 40642-40-8
Navel Orangeworm	Orange	(Z,Z)-11,13-Hexadecadienal (000711)	71317-73-2
Obliquebanded leafroller	Fruit	Lauryl alcohol (001509) Myristyl alcohol (001510) (E,E) -8,10-Dodecadien-1-ol (129028) (Z) -11-Tetradecenyl acetate (128980)	112-53-8 112-72-1 33956-49-9 20711-10-8
Omnivorous leafroller	Fruit (deciduous) Grapes Kiwi Nuts	(E)-11-Tetradecenyl acetate (129019) (Z)-11-Tetradecenyl acetate (128980)	33189-72-9 20711-10-8
Oriental fruit moth	**Fruit** **Nuts**	**(Z)-8-Dodecen-1-yl acetate (128906)** **(E)-8-Dodecen-1-yl-acetate (128907)** **(Z)-8-Dodecen-1-ol (128908)**	**28079-04-1** **38363-29-0** **40642-40-8**
Oriental fruit moth	**Fruit** **Nuts**	**(E)-5-Decenyl acetate (117703)** **(E)-5-Decen-1-ol (78038)** **(Z)-8-Dodecen-1-yl acetate (128906)** **(E)-8-Dodecen-1-yl-acetate (128907)** **(Z)-8-Dodecen-1-ol (128908)**	**38421-90-8** **56578-18-8** **28079-04-1** **38363-29-0** **40642-40-8**
Pandemis leafroller	**Fruit**	**Lauryl alcohol (001509)** **Myristyl alcohol (001510)** **(E,E) -8,10-Dodecadien-1-ol (129028)** **(Z) -11-Tetradecenyl acetate (128980)**	**112-53-8** **112-72-1** **33956-49-9** **20711-10-8**
		(E)-5-Decenyl acetate (117703)	**38421-90-8**

Peach twig borer	Fruit Nuts	(E)-5-Decen-1-ol (78038)	56578-18-8
		(Z)-8-Dodecen-1-yl acetate (128906)	28079-04-1
		(E)-8-Dodecen-1-yl-acetate (128907)	38363-29-0
		(Z)-8-Dodecen-1-ol (128908)	40642-40-8
Peach twig borer	Fruit Nuts Agricultural crops (unspecified)	(E)-5-Decen-1-ol acetate (117703)	38421-90-8
		(E)-5-Decen-1-ol (78038)	56578-18-8
Pink bollworm	Cotton	7,11-Hexadecadien-1-ol acetate (114103)	50933-33-0
Pink bollworm	Cotton	(Z,E)-7,11-Hexadecadien-1-yl acetate (114101)	53042-79-8
		(Z,Z)-7,11- Hexadecadien-1-yl acetate (114102)	52207-99-5
Sparganothis fruitworm	Cranberries	(E)-11-Tetradecen-1-ol acetate (129019)	33189-72-9
Tomato pinworm	Eggplant Tomato Vegetables (fruiting)	(Z)-4-Tridecen-1-yl acetate (121901)	65954-19-0
		(E)-4-Tridecen-1-yl acetate (121902)	72269-48-8

DISCLAIMER: ***The information in this Pesticide Fact Sheet is a summary only.***

Biopesticide Home | OPP Home | EPA Home | Comments
Site Map | Search OPP | Search EPA

www.epa.gov/pesticides/biopesticides/factsheets/fs-generic_lep.htm
updated September 4, 2001

Commodity	Parts per million
Corn grain, fodder, and forage	0.1

[FR Doc. 93-29833 Filed 12-7-93; 8:45 am]
BILLING CODE 6560-50-F

40 CFR Part 180

[OPP-300292A; FRL-4646-3]

RIN 2070-AB78

Inert Ingredients of Semiochemical Dispensers; Tolerance Exemption

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: EPA is establishing an exemption from the requirement of a tolerance for residues of all inert ingredients of semiochemical dispenser products formulated with, and/or contained in, dispensers made of polymeric matrix materials, including the monomers, plasticizers, dispersing agents, antioxidants, UV protectants, stabilizers, and other inert ingredients. The exemption applies when the dispensers are used as carriers in pesticide formulations applied to growing crops only and when the dispensers are large enough to be removed from the site. EPA is issuing this regulation on its own initiative.

EFFECTIVE DATE: This regulation becomes effective on December 8, 1993.

ADDRESSES: Written objections and hearing requests, identified by the document control number, [OPP-300292A], may be submitted to: Hearing Clerk (1900), Environmental Protection Agency, 401 M St., SW., Washington, DC 20460. A copy of any objections and hearing requests filed with the Hearing Clerk should be identified by the document control number and submitted to: Public Response and Program Resources Branch, Field Operations Division (7506C), Office of Pesticide Programs, Environmental Protection Agency, 401 M St., SW., Washington, DC 20460. In person, bring copy of objections and hearing request to: Rm. 1132, CM #2, 1921 Jefferson Davis Hwy., Arlington, VA 22202. Fees accompanying objections shall be labeled "Tolerance Petition Fees" and forwarded to: EPA Headquarters Accounting Operations Branch, OPP (Tolerance Fees), P.O. Box 360277M, Pittsburgh, PA 15251.

FOR FURTHER INFORMATION CONTACT: Connie Welch, Registration Support Branch (7505W), Environmental Protection Agency, 401 M St., SW., Washington, DC 20460. Office location and telephone number: 2800 Crystal Dr., 6th Fl., North Tower, Arlington, VA 22202, (703)-308-8320.

SUPPLEMENTARY INFORMATION: In the Federal Register of August 18, 1993 (58 FR 43830), EPA issued a proprosal to amend 40 CFR part 180 by establishing an exemption from the requirement of a tolerance for residues of components of semiochemical dispensers made of solid matrix polymeric materials (including the monomers, plasticizers, and other ingredients), when these dispensers are large enough to be removed from the site as inert ingredients (carriers) in pesticide formulations applied to growing crops only.

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

Four comments were received in response to the proposed rule. Three of the four commenters requested that the wording of the regulation regarding the components covered should be clarified to match the coverage described in the preamble. The language suggested by all three commenters is as follows:

> § 180.1122(a). All inert ingredients of semiochemical dispenser products formulated with and/or contained in dispensers made of solid matrix polymeric materials (including the monomers, plasticizers, dispersing agents, antioxidants, UV protectants, stabilizers and other inert ingredients), are exempted from the requirement of a tolerance when used in pesticide formulations for application to growing crops only. These dispensers shall conform to the following specifications: * * *.

The Agency has adopted the suggested language, with a minor change.

One commenter suggested an additional definition under § 180.1122(d) for semiochemical dispenser component. Since the term "component" has been removed from the regulation, this definition is not necessary.

Another commenter noted that the term "solid polymeric matrix" appeared to exclude "twist-tie" dispensers which contain a lumen in which the active ingredient and certain inerts initially reside, although the Agency used this dispenser as an example of what is to be included. The Agency has replaced the term "solid matrix polymeric" with "polymeric matrix" to clarify that "twist tie" dispensers are included in the regulation.

All of the commenters requested that the exemption be expanded to include broadcast application formulations. One commenter noted that certain broadcast formulations were less likely to lead to buildup of plastics in the environment. Another commenter requested that the Agency exempt all substances with molecular weights greater than 1,000 since EPA notes in exempting certain polymeric substances from a tolerance requirement that substances with such high molecular weights are not absorbed through the gastrointestinal tract and therefore "are generally incapable of eliciting a toxic response" even if ingested.

The Agency agrees that there may be certain advantages to some broadcast applications of semiochemicals over those products covered by the current exemption. However, the current exemption was developed independent of consideration of the toxicity of components based upon an evaluation that these dispensers had a low potential for contact with food and therefore were unlikely to lead to residues. Broadcast applications have a greater potential for residues, and the components of such products must be evaluated for toxicity. While many of the components used in these formulations may qualify for exemption as polymers, the Agency must make that determination on a case-by-case basis. The criteria used to make the determination include high molecular weight and other characteristics. The Agency has greatly shortened the time required to obtain exemption-from-tolerance for such polymers, but is not prepared to discontinue reviewing them.

Another comment noted that the size at which a dispenser could be removed from the field might vary. It noted that 1.25 inch polymeric fibers which can be hand applied to the stakes of staked tomatoes could, in theory, be removed, but to do so would be very difficult. As noted above, the generic exemption is based on the unlikelihood of the dispensers coming into contact with food and leave residues. The conditions include size, proximity to the raw agricultural commodity (RAC), and

method of application. The fiber dispensers applied to stakes of staked tomatoes would be covered by the exemption, because of their lack of proximity to the RAC and their discrete method of application, but broadcast application of a similar fiber would not.

Another commenter objected to the use of the term "point-source" which in pheromone terminology is used to differentiate pheromone formulations which provide a strong release of pheromones such that the mode of action could be that of "false trail following" rather than "habituation" or "adaptation." The Agency did not intend the term "point-source" to imply the technical definition of pheromone terminology. The Agency has changed the definition to read "* * * to provide discrete application of the semiochemical(s) into the environment," and has similarly modified § 180.1122 (a)(2). This commenter also noted that it is incorrect to state that these semiochemicals are applied at less than peak naturally occurring background levels, but that the amount of pheromone in the atmosphere at any given time is less than peak naturally occurring levels. The Agency agrees and was merely referring to the time-release nature of the dispensers. The Agency believes that providing an exemption for the inert ingredients will facilitate development of appropriate time-release products and reduce the regulatory burden for this technology.

One commenter noted that these dispensers could cause environmental problems. Although EPA agrees that this could be the case, the Agency believes that these products are far better from an environmental perspective than the conventional alternatives. EPA encourages removal of the dispensers and development of biodegradable forms.

Another commenter suggested that the word "receptor" in the definition of semiochemical be changed to "receiving" since "receptor" has narrow, specific meanings related to particular types of proteins and to sensory nerve endings. This change is being made.

Finally, one commenter suggested exempting everything included under 21 CFR parts 173 to 178 and 40 CFR 180.1001(c) and (d), 180.1028, 180.1037, 180.1038, and 180.1062 and all inerts previously approved by the Agency for all types of semiochemical formulations. All of these substances will be exempted for use in dispensers covered by this regulation. In addition, the Agency plans to issue broader exemptions for those substances considered to be "minimal risk" inerts in the future, so that they may be used in a variety of products rather than being limited to specific uses. However, EPA does not have sufficient information to issue the broad regulation proposed by the commenter at this time.

Based on the information considered, the Agency concludes that tolerances are not necessary to protect the public health for the inert ingredients in the semiochemical dispenser products. Therefore, the tolerance exemptions are established as set forth below.

Any person adversely affected by this regulation may, within 30 days after publication of this document in the **Federal Register**, file written objections and/or a request for a hearing with the Hearing Clerk, at the address given above (40 CFR 178.20). A copy of the objections and/or hearing requests filed with the Hearing Clerk should be submitted to the OPP docket for this rulemaking. The objections submitted must specify the provisions of the regulation deemed objectionable and the grounds for the objections. 40 CFR 178.25. Each objection must be accompanied by the fee prescribed by 40 CFR 180.33(i). If a hearing is requested, the objections must include a statement of the factual issue(s) on which a hearing is requested, the requestor's contentions on each such issue, and a summary of any evidence relied upon by the objector. 40 CFR 178.27. 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 issue(s) in the manner sought by the requestor would be adequate to justify the action requested.

The Office of Management and Budget has exempted this rule from the requirements of section 3 of Executive Order 12866. Pursuant to the requirements of the Regulatory Flexibility Act (Pub. L. 96-354, 94 Stat. 1164, 5 U.S.C. 601-612), the Administrator has determined that regulations establishing new tolerances or food additive regulations or raising tolerance levels or food additive regulations or establishing exemptions from tolerance requirements do not have a significant economic impact on a substantial number of small entities. A certification statement of this effect was published in the **Federal Register** of May 4, 1981 (46 FR 24950).

List of Subjects in 40 CFR Part 180

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

Dated: November 15, 1993.

Douglas D. Campt,

Director, Office of Pesticide Programs.

Therefore, it is proposed that 40 CFR part 180 be amended as follows:

PART 180—[AMENDED]

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

Authority: 21 U.S.C. 346a and 371.

2. By adding new § 180.1122 to subpart D, to read as follows:

§ 180.1122 Inert ingredients of semiochemical dispensers; exemptions from the requirement of a tolerance.

(a) All inert ingredients of semiochemical dispenser products formulated with, and/or contained in, dispensers made of polymeric matrix materials (including the monomers, plasticizers, dispersing agents, antioxidants, UV protectants, stabilizers, and other inert ingredients) are exempted from the requirement of a tolerance when used as carriers in pesticide formulations for application to growing crops only. These dispensers shall conform to the following specifications:

(1) Exposure must be limited to inadvertent physical contact only. The design of the dispenser must be such as to preclude any contamination by its components of the raw agricultural commodity (RAC) or processed foods/feeds derived from the commodity by virtue of its proximity to the RAC or as a result of its physical size.

(2) The dispensers must be applied discretely. This exemption does not apply to components of semiochemical formulations applied in a broadcast manner either to a crop field plot or to individual plants.

(b) A semiochemical dispenser is a single enclosed or semi-enclosed unit that releases semiochemical(s) into the surrounding atmosphere via volatilization and is applied in a manner to provide discrete application of the semiochemical(s) into the environment.

(c) Semiochemicals are chemicals that are emitted by plants or animals and modify the behavior of receiving organisms. These chemicals must be naturally occurring or substantially

identical to naturally occurring semiochemicals.

[FR Doc. 93-29834 Filed 12-7-93; 8:45 am]

BILLING CODE 6560-50-F

40 CFR Part 180

[OPP-300279B; FRL-4743-8]

RIN 2070-AB78

2-[Methyl[(Perfluoroalkyl)Alkyl(C_2-C_8)Sulfonyl] Amino]Alkyl(C_2-C_8)Acrylate-Alkyl(C_2-C_8)Methacrylates-N-Methylolacrylamide Copolymer; Tolerance Exemption

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: This document establishes an exemption from the requirement of a tolerance for residues of 2-[methyl[(perfluoroalkyl)alkyl(C_2-C_8)sulfonyl]amino]alkyl(C_2-C_8)acrylate-alkyl(C_2-C_8)methacrylates-*N*-methylolacrylamide copolymer when used as an inert ingredient (water repellant agent) in pesticide formulations applied to animals. This regulation was requested by SmithKline Beecham Animal Health. The proposal elicited a comment stating that if the copolymer had a particular structure it would be subject to gastrointestinal (GI) metabolism resulting in the formation of toxic metabolites, and the comment is addressed in this document.

EFFECTIVE DATE: Effective on December 8, 1993.

ADDRESSES: Written objections and hearing requests, identified by the document control number, [OPP-300279B], may be submitted to: Hearing Clerk (1900), Environmental Protection Agency, Rm. M3708, 401 M St., SW., Washington, DC 20460. A copy of any objections and hearing requests filed with the Hearing Clerk should be identified by the document control number and submitted to: Public Response and Program Resources Branch, Field Operations Division (7506C), Office of Pesticide Programs, Environmental Protection Agency, 401 M St., SW., Washington, DC 20460. In person, bring copy of objections and hearing requests to: Rm. 1132, CM #2, 1921 Jefferson Davis Hwy., Arlington, VA 22202. Fees accompanying objections shall be labeled "Tolerance Petition Fees" and forwared to: EPA Headquarters Accounting Operations Branch, OPP (Tolerance Fees), P.O. Box 360277M, Pittsburgh, PA 15251.

FOR FURTHER INFORMATION CONTACT: By mail: Rosalind L. Gross, Registration Support Branch, Registration Division (7505W), Office of Pesticide Programs, Environmental Protection Agency, 401 M St., SW., Washington, DC 20460. Office location and telephone number: 6th Floor, North Tower, 2800 Crystal Drive, Arlington, VA 22202, (703)-308-8354.

SUPPLEMENTARY INFORMATION: In the **Federal Register** of March 10, 1993 (58 FR 13239), EPA issued a proposed rule that gave notice that SmithKline Beecham Animal Health, 1600 Paoli Pike, P.O. Box 2650, West Chester, PA, 19380-6014, had submitted pesticide petition (PP) 2E4147 requesting that the Administrator, pursuant to section 408(e) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 346a(e)), propose to amend 40 CFR part 180 by establishing an exemption from the requirement of a tolerance for residues of 2-[methyl [(perfluoroalkyl) sulfonyl] amino]alkyl (C_2-C_8) acrylate-alkyl (C_2-C_8) methacrylates-*N*-methylolacrylamide copolymer when used as an inert ingredient (water repellant agent) in pesticide formulations applied to animals.

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

As part of the EPA policy statement on inert ingredients published in the **Federal Register** of April 22, 1987 (52 FR 13305), the Agency established data requirements which will be used to evaluate the risks posed by the presence of an inert ingredient in a pesticide formulation. Exemptions from some or all of the requirements may be granted if it can be determined that the inert ingredient will present minimal or no risk.

One comment was received in response to the proposed rule. The comment stated that if the copolymer had a particular structure it would be subject to gastrointestinal (GI) metabolism resulting in the formation of toxic metabolites. The comment reported that when certain perfluorinated sulfonyl copolymers were fed to ants, delayed toxicity was seen from a single feeding and caused concern regarding the exemption from the requirement of a tolerance for 2-[methyl[(perfluoroalkyl)sulfonyl] amino]alkyl(C_2-C_8) acrylate-alkyl (C_2-C_8) methacrylates-*N*-methylolacrylamide copolymer. The comment was withdrawn when it was learned that the perfluorinated sulfonyl copolymer had methylene units *alpha* and *beta* to the sulfonyl group, indicating that the presence of methylene units adjacent to the sulfonyl group altered the toxicological properties of the copolymer enough that the original comment was no longer relevant.

Although the comment was withdrawn, the possibility of GI absorption and/or metabolism of the copolymer caused the Agency to reevaluate the risks to human health and the environment from the proposed use of this copolymer. EPA finds it has no evidence that a copolymer with an average molecular weight of 50,000 and low water solubility would be absorbed or metabolized in the GI tract. Additionally, EPA acknowledges the name of the copolymer in the proposed rule was vague, resulting in potential confusion regarding the precise chemical structure of the copolymer. Therefore, the name of the copolymer in the final rule will be changed to more accurately reflect its chemical structure. The name of the copolymer in the final rule will appear as 2-[methyl [(perfluoroalkyl) alkyl(C_2-C_8) sulfonyl] amino]alkyl(C_2-C_8) acrylate-alkyl (C_2-C_8)methacrylates-*N*-methylolacrylamide copolymer.

Based upon the information considered and discussed in the proposed rule and here, EPA concludes that the tolerance exemption for residues of 2-[methyl [(perfluoroalkyl) alkyl (C_2-C_8)sulfonyl] amino]alkyl (C_2-C_8) acrylate-alkyl (C_2-C_8) methacrylates *N*-methylolacrylamide copolymer will protect the public health. Therefore, the exemption from the requirement of a tolerance for the copolymer will be established as set forth below.

Any person adversely affected by this regulation may, within 30 days after publication of this document in the **Federal Register**, file written objections and/or a request for a hearing with the Hearing Clerk, at the address given above (40 CFR 178.20). A copy of the objections and/or hearing requests filed with the Hearing Clerk should be submitted to the OPP docket for this rulemaking. The objections submitted must specify the provisions of the regulation deemed objectionable and the grounds for the objections (40 CFR 178.25). Each objection must be accompanied by the fee prescribed by

Coast Guard's position that these new operating rules will provide for the reasonable needs of navigation. This is the extent of the Coast Guard's authority in issuing drawbridge operating requirements. Issues related to the safety of train operations are the responsibility of the Federal Railroad Administration. For this reason, these considerations are not included in the criteria authorized to be used by the Coast Guard in determining whether specific drawbridge operating requirements should be implemented. These concerns have been forwarded to the Federal Railroad Administration for appropriate consideration and action.

Based on the comments submitted and the Coast Guard response to these comments, the Coast Guard is publishing the requirements as proposed.

Federalism

This action has been analyzed in accordance with the principles and criteria contained in Executive Order 12612, and it has been determined that the final rulemaking does not have sufficient federalism implications to warrant the preparation of a Federalism Assessment.

Economic Assessment and Certification

This final rule is not considered a significant regulatory action under Executive Order 12866 and is not significant under the Department of Transportation Regulatory Policies and Procedures (44 FR 11034: February 26, 1979).

The economic impact of this action is expected to be so minimal that a full regulatory evaluation is unnecessary. The basis for this conclusion is that during the regulated period there will be very little inconvenience to vessels using the waterway. In addition, mariners requiring the bridge openings are repeat users of the waterway and giving the bridge owner advance notice should involve little or no additional expense to them. Since the economic impact of this regulation is expected to be minimal, the Coast Guard certifies that it will not have a significant economic impact on a substantial number of small entities.

Environment

This final rulemaking has been thoroughly reviewed by the Coast Guard and it has been determined to be categorically excluded from further environmental documentation in accordance with section 2.B.2.g.5 of Commandant Instruction M16475.1B. A Categorical Exclusion Determination statement has been prepared and placed in the rulemaking docket.

List of Subjects in 33 CFR Part 117

Bridges.

Regulations

In consideration of the foregoing, part 117 of title 33, Code of Federal Regulations, is amended as follows:

PART 117—DRAWBRIDGE OPERATION REGULATIONS

1. The authority citation for part 117 continues to read as follows:

Authority: 33 U.S.C. 499; 49 CFR 1.46; 33 CFR 1.05–1(g).

2. Section 117.439 is revised to read as follows:

§ 117.439 Des Allemands Bayou.

(a) The draw of the S631 bridge, mile 13.9 at Des Allemands, shall open on signal if at least four hours notice is given.

(b) The draw of the Southern Pacific Railroad bridge, mile 14.0, shall open on signal Monday through Friday from 7 a.m to 3 p.m. At all other times the draw shall open on signal if at least 4 hours notice is given.

Dated: March 16, 1994.

J.C. Card,

Rear Admiral, U.S. Coast Guard, Commander, Eighth Coast Guard District.

[FR Doc. 94–7537 Filed 3–29–94; 8:45 am]

BILLING CODE 4910–14–M

ENVIRONMENTAL PROTECTION AGENCY

40 CFR Part 180

[OPP–300314A; FRL–4761–9]

RIN 2070–AB78

Anthropod Pheromones; Tolerance Exemption

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: EPA is establishing an exemption from the requirement of a tolerance for residues of arthropod pheromones resulting from the use of these substances in retrievably sized polymeric matrix dispensers with an annual application limitation of 150 grams active ingredient per acre (gm AI/acre) for pest control in or on all raw agricultural commodities (RAC). EPA is establishing this regulation on its own initiative.

EFFECTIVE DATE: This regulation becomes effective March 30, 1994.

ADDRESSES: Written objections and hearing requests, identified by the document control number, [OPP-300314A], may be submitted to: Hearing Clerk (1900), Environmental Protection Agency, Rm. M3708, 401 M St., SW., Washington, DC 20460. A copy of any objections and hearing requests filed with the Hearing Clerk should be identified by the document control number and submitted to: Public Response and Program Resources Branch, Field Operations Division (7506C), Office of Pesticide Programs, Environmental Protection Agency, 401 M St., SW., Washington, DC 20460. In person, bring copy of objections and hearing requests to: Rm. 1132, CM #2, 1921 Jefferson Davis Hwy., Arlington, VA 22202. Fees accompanying objections shall be labeled "Tolerance Petition Fees" and forwarded to: EPA Headquarters Accounting Operations Branch, OPP (Tolerance Fees), P.O. Box 360277M, Pittsburgh, PA 15251.

FOR FURTHER INFORMATION CONTACT: By mail: Phil Hutton, Product Manager (PM) 18, Registration Division (7505C), Environmental Protection Agency, 401 M St., SW., Washington, DC 20460. Office location and telephone number: Rm. 213, CM #2, 1921 Jefferson Davis Hwy., Arlington, VA 22202, (703)-305-7690.

SUPPLEMENTARY INFORMATION: In the **Federal Register** of December 8, 1993 (58 FR 64538), EPA issued a proposed rule to amend 40 CFR part 180 by establishing an exemption from the requirement of a tolerance for all arthropod pheromones used in solid matrix dispensers at rates less than or equal to 150 grams active ingredient (AI)/acre/year. A pheromone is defined by EPA as a compound produced by an arthropod that modifies the behavior of other individuals of the same species (40 CFR 152.25(b)(1)). Solid matrix dispensers as defined in the proposal include, but are not limited to, the following: Rubber septa dispensers, trilaminate sheets, tapes, tags, wafers, macrocapillary devices which are placed by hand in the field and are of such size and construction that they are readily recognized. Formulations not inlcuded in this exemption are as follows: Liquid flowables, microcapsules, microcapillary straws; granular powder, flakes, or confetti formulations which are sprayed or broadcast over the crop area; and cigaret filters or unprotected ropes which generally contain the active ingredient on the outer surface of the unit. In the proposal, EPA set forth its reasons for determining that a tolerance for these

pheromone products is not necessary to protect public health.

EPA is choosing to change the term "solid matrix dispnesers" to "retrievably sized polymeric matrix dispensers" for consistency with 40 CFR 180.1122, which exempts from the requirement of a tolerance the inert materials of these dispensers (58 FR 64493). The Agency does not believe this change will change the intent or scope of the original definition of a dispenser. EPA intendes the term "retrievably sized polymeric matrix dispensers " to include such dispensers as the following: Rubber septa dispensers; trilaminate sheeps; tapes; tags, microcapillary devices such as long tubes or fibers; twist ties; and protected ropes. Each of these dispensers is placed by hand in the field and is of such size and contstruction that it is readily recognized and retrievable. Dispensers with similar characteristics would also come within this term. In the preamble to the proposal, EPA included "wafers" in the list of dispensers which would qualify under this exemption. EPA now believes use of this example is ambiguous because wafers could be of a size that could or could not be retrieved. To emphasize that this exemption only applies to retrievable dispensers, EPA has intentionally omitted wafers from the examples given above of qualifying dispensers.

Three comments were received in response to the proposed rule. All the responses were generally favorable to the generic tolerance proposal, with two of the comments finding the proposal too restrictive. These comments suggested the Agency should further broaden the scope and the exemption to allow the tolerance exemption to extend beyond polymeric, retrievably sized dispenser formulations to include broadcast applications.

The Agency dose not have a toxicology data base for arthropod pheromones that addresses the potential risk of repeated, direct dietary exposure to the active ingredient possible with formulations such as sprayables which can be incorporated into food. The Agency believes that restricting the exemption to retrievably sized dispensers will severely limit the possibility of direct dietary exposure to the active ingredient and that such a limitation is necessary to protect the public health. Producers of new pheromone formulations not exempted by this proposal, including smaller-sized granules, may request a tolerance exemption. Producers of previously registered pheromone products wishing to utilize formulations other than those mentioned in this rule may petition for an amendment to the existing tolerance exemption for a registered active ingredient, if they can demonstrate that the new formulation does not increase the likely dietary exposure.

One of the commenters believed that the proposed rule was too restrictive for lepidopteran pheromones. On the other hand, the same commenter believed that the proposed rule was too lenient for other types of pheromones. This commenter questioned how the Agency could exempt all arthropod pheromones from a requirement for a tolerance when several groups of arthropods are known to produce pheromone compounds that are chemicals structurally so diverse as to be of unknown toxicity. The Agency agrees that the scope of the tolerance exemption is broad and does exempt arthropod pheromones for which there is not an extensive data base including pheromones with chemical structures unrelated to the majority of pheromone active ingredients registered to date. Nevertheless, the Agency believes the restrictions in the proposed rule along with aspects of pheromone biology mitigate concerns about the wider scope of this tolerance exemption.

First, the proposal incorporates features that would limit the direct dietary exposure to the arthropod pheromones used as pesticides by requiring the formulation to be restricted to larger dispensers. This formulation restriction will limit exposure to an active ingredient resulting from the small amount that volatilizes from the dispenser and subsequently may deposit on food crops. Due to its size, the dispenser itself, with or without any remaining active ingredient, is not likely to become incorporated into food. Second, the Agency believes that an annual rate limitation of 150 grams AI/acre and a restriction to retrievably sized dispensers are likely to limit the dietary exposure to what is no greater than that found naturally in food as a result of heavy infestations of the pest arthropods. An arthropod species becomes a pest only if its populations reach levels that impede economic returns. The Agency believes there already has been dietary exposure to the arthropod pheromones deposited after volatilization from natural heavy pest infestations that could be shown to control such pest species. The dietary exposure to these natural pheromones that results from registered pheromones and those used in traps to date has not adversely affected public health. No commenters found the annual 150 grams AI/acre limit objectionable.

One commenter noted that it appeared that the Agency was basing its tolerance exemption on wildlife exposure risks. The primary reason for exempting arthropod phermones in these formulations is based on human health considerations. The public health is protected from unnecessary direct exposure to pheromone active ingredients in food if these compunds are released from larger retrievable dispensers. In addition, although not directly relevant to this tolerance exemption, the Agency believes that wildlife risks would be minimal with use of these dispensers.

A commenter claimed that the Agency was demonstrating a bias against products developed from certain food crops such as grains and row crops by the proposed tolerance exemption. EPA disagrees. Larger dispenser formulations can be adapted to row crops by incorporating pheromones dispensers into stakes or other supports. The Agency believes that other pheromone formulations such as sprayables and microencapsulated products should be developed when the crop use demands these parameters and does not intend to burden producers of these products unnecessarily. However, the fate and subsequent dietary exposure of these smaller dispensers, some perhaps still charged with the pheromone active ingredient, must be addressed during a request for a tolerance exemption.

The same commenter requested that the Agency change the language of the exmeption to indicate that many other organisms besides arthropods produce pheromones but provided no information to support expanding the exemption to other types of pheromones. Without more information, EPA is unwilling to expand the exemption in this manner at this time.

Based on the information considered, the Agency concludes that tolerances for these pheromone products are not necessary to protect the public health. Therefore, the tolerance exemptions are established as set forth below.

Any person adversely affected by this regulation may, within 30 days after publication of this document in the Federal Register, file written objections and/or request a hearing with the Hearing Clerk, at the address given above (40 CFR 178.20). A copy of the objections and/or hearing requests filed with the Hearing Clerk should be submitted to the OPP docket for this rulemaking. The objections submitted must specify the provisions of the regulation deemed objectionable and the grounds for the objections (40 CFR 178.25). Each objection must be accompanied by the fee prescribed by

40 CFR 180.33(i). If a hearing is requested, the objections must include a statement of the factual issue(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). 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 issue(s) in the manner sought by the requestor would be adequate to justify the action requested (40 CFR 178.32).

Under Executive Order 12866 (58 FR 51735, Oct. 4, 1993), the Agency must determine whether the regulatory action is "significant" and therefore subject to review by the Office of Management and Budget (OMB) and the requirements of the Executive Order. Under section 3(f), the order defines a "significant regulatory action" as an action that is likely to result in a rule: (1) Having an annual effect on the economy of $100 million or more, or adversely and materially affecting a sector of the economy, productivity, competition, jobs, the environment, public health or safety, or State, local, or tribal governments or communities (also referred to as "economically significant"); (2) creating serious inconsistency or otherwise interfering with an action taken or planned by another agency; (3) materially altering the budgetary impacts of entitlement, grants, user fees, or loan programs or the rights and obligations or recipients thereof; or (4) raising novel legal or policy issues arising out of legal mandates, the President's priorities, or the principles set forth in this Executive Order.

Pursuant to the terms of the Executive Order, EPA has determined that this rule is not "significant" and is therefore not subject to OMB review.

Pursuant to the requirements of the Regulatory Flexibility Act (Pub. L. 96-354, 94 Stat. 1164, 5 U.S.C. 601-612), the Administrator has determined that regulations establishing new tolerances or raising tolerance levels or establishing exemptions from tolerance requirements do not have a significant economic impact on a substantial number of small entities. A certification statement to this effect was published in the Federal Register of May 4, 1981 (46 FR 24950).

List of Subjects in 40 CFR Part 180

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

Dated: March 21, 1994.

Susan H. Wayland,

Acting Director, Office of Pesticide Programs.

Therefore, 40 CFR part 180 is amended as follows:

PART 180—[AMENDED]

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

Authority: 21 U.S.C. 346a and 371.

2. By adding new § 180.1124 to subpart D, to read as follows:

§ 180.1124 Arthropod pheromones; exemption from the requirement of a tolerance.

Arthropod pheromones, as described in § 152.25(b) of this chapter, when used in retrievably sized polymeric matrix dispensers are exempt from the requirement of a tolerance in or on all raw agricultural commodities when applied to growing crops only at a rate not to exceed 150 grams active ingredient/acre/year in accordance with good agricultural practices.

[FR Doc. 94-7368 Filed 3-29-94; 8:45 am]
BILLING CODE 6560-50-F

---

40 CFR Part 180

[PP 9F3798/R2047; FRL-4762-6]

RIN No. 2070-AB78

Pesticide Tolerance for Lactofen (1-(Carboethoxy)Ethyl-5-(2-Chloro-4-(Trifluoromethyl)Phenoxy)-2-Nitrobenzoate)

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: This rule extends a time-limited tolerance for residues of the herbicide lactofen (1-(carboethoxy)ethyl-5-(2-chloro-4-(trifluoromethyl)phenoxy)-2-nitrobenzoate) and its metabolites containing the diphenyl ether linkage on the raw agricultural commodity (RAC) cottonseed at 0.05 part per million (ppm) to December 31, 1995. This regulation was requested by Valent U.S.A. Corp. and continues the maximum permissible level for residues of the herbicide in or on this RAC.

EFFECTIVE DATE: This time-limited regulation becomes effective on March 30, 1994.

ADDRESSES: Written objections and requests for a hearing, identified by the document control number, [PP 9F3798/R2047], may be submitted to: Hearing Clerk (1900), Environmental Protection Agency, Rm. 3708, 401 M St., SW., Washington, DC 20460. A copy of any objections and hearing requests filed with the Hearing Clerk should be identified by the document control number and submitted to: Public Response and Program Resources Branch, Field Operations Division (7506C), Office of Pesticide Programs, Environmental Protection Agency, 401 M St., SW., Washington, DC 20460. In person, bring copy of objections and hearing requests to: Rm. 1132, CM #2, 1921 Jefferson Davis Hwy., Arlington, VA 22202. Fees accompanying objections shall be labeled "Tolerance Petition Feeds" and forwarded to: EPA Headquarters Accounting Operations Branch, OPP (Tolerance Fees), P.O. Box 360277M, Pittsburgh, PA 15251.

FOR FURTHER INFORMATION CONTACT: By mail: Joanne I. Miller, Product Manager (PM 23), Registration Division (7505C), Office of Pesticide Programs, Environmental Protection Agency, 401 M St., SW., Washington, DC 20460. Office location and telephone number: Rm. 255, CM #2, 1921 Jefferson Davis Highway, Arlington, VA 22202, (703)-305-7830.

SUPPLEMENTARY INFORMATION: EPA issued a time-limited tolerance in the **Federal Register** of June 14, 1990 (55 FR 24084), under section 408(e) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 346a(e)) for residues of the herbicide lactofen 1-(carboethoxy)ethyl-5-(2-chloro-4-(trifluoromethyl)phenoxy)-2-nitrobenzoate) and its associated metabolites containing the diphenyl ether linkage on the raw agricultural commodity (RAC) cottonseed at 0.05 part per million (ppm). This tolerance was requested by Valent U.S.A. Corp. (formerly Chevron Chemical Co.), 1333 N. California Blvd., P.O. Box 8025, Walnut Creek, CA 94596-8025, and establishes the maximum permissible level for residues of the herbicide in or on this RAC.

This tolerance was issued as a time-limited tolerance because EPA required additional information on a cottonseed processing study and required animal metabolism studies. EPA's review of the processing study resulted in a preliminary determination that concentration does not occur in processed food, but additional information on the study was required to confirm that determination. Information was submitted, and the determination was confirmed. The

REGISTRATION ELIGIBILITY DOCUMENT

Isomate - BAW Pheromone
(PC Codes 117203 and 119409)

U.S. Environmental Protection Agency
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division
Isomate - BAW Pheromone
(PC Codes 117203 and 119409)

Table of Contents

8. Cumulative Effects
9. 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. Conditional/Unconditional Registration
2. CODEX Harmonization
3. Nonfood Re/Registrations
4. Risk Mitigation
5. 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
   End-Use Product Environmental Hazards Labeling
3. Application Rate

D. LABELING

V. Actions Required by Registrants

VI. Appendix A

I. Executive Summary

A. IDENTITY

The end use product, Isomate-BAW Pheromone, contains 55% (Z,E)-9,12-Tetradecadienyl acetate and 24.8% Z-9-Tetradecen-1-ol. The components of this end use product are adequately identified and the product chemistry data submitted satisfies the requirements for product identity.

B. USE/USAGE

This end use product will be used to disrupt the mating activity of the male beet armyworm in numerous agricultural crops. Refer to Section II, B for a complete listing of use sites/crops.

C. RISK ASSESSMENT

1. Human Health Risk Assessment

a. Toxicological Endpoints

Mammalian toxicity data have been submitted and adequately satisfy data requirements to support registration. Submitted data indicate that the Technical Grade of the Active Ingredient (TGAI) should be classified as Toxicity IV for acute oral, dermal, and inhalation toxicity. The TGAI was found to cause mild ocular irritation in a eye irritation study (Toxicity Category III) and very slight to well defined dermal irritation which cleared by 96 hours in a dermal irritation study (Toxicity Category IV). In addition, the TGAI was shown not to be a contact sensitizer in the Magnusson-Kligman maximization assay.

b. Human Exposure

Because the active ingredients contained in Isomate - BAW Pheromone are slowly released in very small amounts by dispenser, the potential for dermal, eye and inhalation exposure to pesticide handlers and to the general public is expected to be negligible. Further, the Agency has concluded that the potential for dietary exposure is expected to be minimal based on volatility of the compounds, the low application rates and known metabolism of similar compounds.

c. Risk Assessment

The potential risks to humans are considered negligible based on low exposure and the lack of significant toxicological concerns. 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 this compound when label instructions are followed.

2. Ecological Risk Assessment

a. Ecological Toxicity Endpoints

No toxic endpoints were identified.

b. Ecological Exposure

Nontarget organism requirements data were waived based on the proposed use pattern and lack of exposure.

c. Risk Assessment

Because Isomate - BAW Pheromone is enclosed in a solid matrix dispenser and is slowly released by volatilization, transport and exposure is expected to be very limited. This system is generally accepted as posing minimal to no exposure and risk to non-target terrestrial and aquatic species.

D. DATA GAPS / LABELING RESTRICTIONS

There are no data gaps.

II. Overview

A. ACTIVE INGREDIENT OVERVIEW

Isomate-BAW Pheromone is a mixture of (Z,E)-9,12-Tetradecadienyl acetate and (Z)-9-Tetradecen-1-ol.

(Z,E)-9,12-Tetradecadienyl acetate

Molecular Formula: $C_{16}H_{28}O_2$

Structure: $CH_3CO_2(CH_2)_8CH{=}CHCH{=}CHC_2H_5$

Chemical Class: Pheromone

CAS Registry Number: 31654-77-0

OPP Chemical Code: 117203

(Z)-9-Tetradecen-1-ol

Molecular Formula: $C_{14}H_{28}O$

Structure: $CH_3(CH_2)_3CH{=}CH(CH_2)_8OH$

Chemical Class: Pheromone

CAS Registry Number: 35153-15-2

OPP Chemical Code: 119409

Isomate - BAW Pheromome

Trade Name: Isomate - BAW Pheromone

Basic Manufacturer: Biocontrol Limited
16010 NE 36 th Avenue
Ridgefield, WA 98642

B. USE PROFILE

The following is information on the proposed uses with an overview of use sites and application methods.

Type of Pesticide:

Biochemical (pheromone, mating disruptant)

Use Sites:

Terrestrial food and feed crops (alfalfa, asparagus, beans, beets, cabbage, celery, cole crops, cotton, cucumbers, groundnuts, lettuce, onions, peas, peppers, soybeans, strawberries, sweet potatoes and tomatoes)

Terrestrial nonfood crop (tobacco)

Target Pests:

Beet Armyworm (*Spodoptera exigua*)

Formulation Types:

Formulated into a slow release plastic dispenser

Method and Rates of Application:

Dispenser is hand applied to stakes placed uniformly in the crops before the emergence of insects. Application rates vary from 100 - 200 dispensers per acre (23.9 - 47.9 grams ai per acre).

Use Practice Limitations:

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

Avoid placing dispensers in contact with the soil.

Do not exceed 150 grams ai per acre per year.

Timing:

Dispenser release pheromone for 60-90 days depending on temperature. Apply product prior to adult flight, early enough to disrupt mating communications within the field.

C. ESTIMATED USAGE

None used yet since this will be the first registered product.

D. CLASSIFICATION

Because (Z,E)-9,12-Tetradecadienyl acetate and (Z)-9-Tetradecen-1-ol are synthetic compounds that mimic naturally occurring substances of insect origin and function by a non-toxic mode of action, these compounds can be classified as biochemical pesticides.

E. REGULATORY HISTORY

The following is a list of actions have been taken by the Agency to provide regulatory relief for pheromones:

-Exemption from the requirement of a tolerance for inert ingredients of semiochemical dispenser products formulated with and/or contained in dispensers made of polymeric matrix materials. (published in 40 CFR 180.1122).

-Exemption from the requirement of a tolerance for arthropod pheromones when used in retrievably sized polymeric matrix dispensers and used at a rate not to exceed 150 grams active ingredient per acre per year (published in 40 CFR 180.1124).

-Exemption from the requirement of a tolerance for certain Lepidopteran pheromones (that are naturally occurring compounds, or identical or substantially similar synthetic compounds, designated by an unbranched aliphatic chain [between 9 and 18 carbons] ending in an alcohol, aldehyde or acetate functional group and containing up to 3 double bonds in the aliphatic backbone) when used on all raw agricultural commodities at a rate not to exceed 150 grams active ingredient per acre per year (published in 40 CFR 180.1153).

In regard to the registration of Isomate - BAW Pheromone, on July 7, 1998, the Agency received an application from Biocontrol Limited to register this product.

A notice of receipt of the application for registration for Isomate - BAW Pheromone was published in the Federal Register (volume 63, Number 241, page 69281) on December 16, 1998. No comments were received on this notice.

F. DATA REQUIREMENTS

Recognizing the low toxicity (Toxicity categories III and IV) and low expected exposure to humans from contact with pheromones in point source applications (e.g., in solid matrix dispenser), the Agency has waived the requirement for certain mammalian toxicity studies, such as subchronic (90-day) oral and inhalation toxicity, immunotoxicity, and developmental toxicity. Due to the low use rate and target species specificity, the Agency has been using a variety of measures to facilitate the development and registration of pheromone products.

To expedite the registration of lepidopteran pheromone products, the Agency usually only considers product chemistry data, and if needed, inert clearance data for pesticidal uses of these compounds on food crops. If toxicity data are submitted, the Agency will evaluate (usually by summary review) the toxicity of the product to determine if the toxicity levels and categories correspond to other pheromone products.

The registrant has submitted product chemistry, acute toxicity and mutagenicity studies to support the product registration. The data requirements for granting this registration under Section 3(c)(5) of FIFRA have been reviewed by the Biopesticides and Pollution Prevention Division (BPPD). Based on submitted information, the Agency foresees no unreasonable adverse effects to human health and the environment from the use of this chemical and recommends an unconditional registration of these new active ingredients for the proposed uses.

G. FOOD CLEARANCES/TOLERANCES

Isomate - BAW Pheromone fits the criteria for the lepidopteran pheromone listed in Section II, E above and is, therefore, exempt from the requirement of a tolerance when used on all raw agricultural commodities and when applied to growing crops at a rate not to exceed 150 grams active ingredient per acre per year.

III. Science Assessment

A. PHYSICAL/CHEMICAL PROPERTIES ASSESSMENT

1. Product Identity and Mode of Action

a. Product Identity:

The TGAI is a colorless or transparent liquid with a mild, fatty-fruity odor. It has a boiling point of between 120-135 °C, a specific gravity of 0.878 - 0.880, and a pH of 5.3. The technical is soluble in n-hexane, cyclohexane, benzene, toluene, methylene chloride, chloroform, ethanol, ethyl ether, acetone and acetonitrile. The end use product contains 55% (Z,E)-9,12-Tetradecadienyl acetate and 24.8% (Z)-9-Tetradecen-1-ol by weight.

b. Mode of Action: Disrupts mating activity.

2. Food Clearances/Tolerances

These lepidopteran pheromones are exempt from the requirement of a tolerance under 40 CFR 180.1124 and 40 CFR 180.1153.

3. Physical And Chemical Properties Assessment

The data requirements for physical and chemical characteristics that support the registration are summarized in Table 1.

Table 1. Product chemistry data requirements

GUIDELINE NO.	STUDY	RESULTS	MRID NO.
151B-10 151B-11 151B-12	Product identity; Manufacturing process; Discussion of formulation of unintentional ingredients	Satisfied requirements	446060-01 446060-01 446060-01

GUIDELINE NO.	STUDY	RESULTS	MRID NO.
151B-13	Analysis of samples	Satisfied requirement	446060-01, 448709-01
151B-15	Certification of limits	Satisfied requirement	446060-01, 448709-01
151B-16	Analytical Method	Satisfied requirement	446060-01, 448709-01
151B-17	PHYSICAL / CHEMICAL PROPERTIES of TGAI		
151B-17a	Color	colorless or light yellow transparent	446060-01
151B-17b	Physical State	liquid	446060-01
151B-17c	Odor	mild and fatty-fruity	446060-01
151B-17d	Melting point	N/A	
151B-17e	Boiling point	120-135 ° C	446060-01
151B-17f	Density/Specific gravity	0.878 - 0.880	446060-01
151B-17g	Solubility	soluble in n-hexane, cyclohexane, benzene, toluene, methylene chloride, chloroform, ethanol, ethyl ether, acetonitrile, THF, acetone insoluble in DMSO and ethylene glycol	446060-01
151B-17h	Vapor Pressure	7.0×10^{-4}mm Hg at 20°C	446060-01
151B-17i	pH	5.3	446060-01
151B-17j	Stability	N/A	

GUIDELINE NO.	STUDY	RESULTS	MRID NO.
151B-17k	Flammability	163°C	446060-01
151B-17l	Storage stability	When stored for 12 months at room temperature and at 5°, total loss is 3.01% and 0.87% respectively.	446060-01
151B-17m	Viscosity	6.42 at 20°C	446060-01
151B-17n	Miscibility	N/A	
151B-17o	Corrosion characteristics	N/A	

B. HUMAN HEALTH ASSESSMENT

1. Toxicology Assessment

As discussed in Section II (f), no toxicity data were required to support this registration.
The Agency has evaluated by summary review the submitted toxicity data. The results are provided below.

a. Acute Toxicity

The registrant submitted acceptable acute toxicity studies for the TGAI. The acute oral LD_{50} in rats was >5000 mg/kg (Toxicity Category IV); the acute dermal LD_{50} in rats was >5000 mg/kg (Toxicity Category IV); the acute inhalation LC_{50} in rats was > 5.0 mg/L (Toxicity Category IV); caused mild ocular irritation in rabbits which cleared by 48 hours post-instillation (Toxicity Category III); caused very slight to well-defined dermal irritation in rabbits which cleared by 96 hours postdosing (Toxicity Category IV); and was shown not to be a contact sensitizer in guinea pigs using the Magnusson-Kligman maximization test.

b. Mutagenicity and Developmental Toxicity

The registrant submitted an acceptable mammalian mutagenicity study for the technical. Based on the data obtained from the *Salmonella typhimurium/Escherichia coli* microsome reverse mutation assay, the product did not induce positive increases in the number of revertants when tester strain cell cultures were

dosed with the active ingredient at 50 to 5000 μg/culture plate; cell toxicity was observed at the 5000 μg/culture plate dose. The study demonstrated that the technical is not a mutagen.

Mammalian toxicity data submitted are summarized in Table 2.

Table 2. Toxicity data requirements for the TGAI

GUIDELINE NO.	STUDY	RESULTS	MRID NO.
TIER I			
152-10	Acute oral toxicity	LD50 >5000 mg/kg in rats (Toxicity Category IV)	446060-02
152-11	Acute dermal toxicity	LD50 >5000 mg/kg in rats (Toxicity Category IV)	446060-03
152-12	Acute inhalation toxicity	LC50 >5.0 mg/L in rats (Toxicity Category IV)	446060-04
152-13	Primary eye irritation	mild irritation in rabbits (Toxicity Category III)	446060-05
152-14	Primary dermal irritation	very slight to well defined irritation in rabbits (Toxicity Category IV)	446060-06
152-15	Dermal sensitization	not a sensitizer in guinea pigs (Magnusson-Kligman maximization test)	446060-07
152-16	Hypersensitivity incidents	Any incident must be reported if observed	

GUIDELINE NO.	STUDY	RESULTS	MRID NO.
152-17	Genotoxicity - *Salmonella typhimurium* gene mutation assay	Not mutagenic	446060-08
152-18	Cellular immune response	Waived	

c. Subchronic Toxicity

Data from subchronic toxicology studies that evaluate compounds similar in structure to the lepidopteran pheromones have been published in the scientific literature (Daughtrey *et al.* 1990. Subchronic toxicity evaluation of tridecyl acetate in rats. *Fundam. Appl. Toxicol.* 14: 104-112). The data and/or information submitted included compounds that were from six- to sixteen- carbon unbranched alcohols, acetates and aldehydes. The Agency's analysis of these compounds indicate that there were no significant signs of toxicity in rats other than those expected with longer-term exposure to high doses of a hydrocarbon. The findings were indicative of an overall low degree of systemic toxicity following subchronic oral administration of tridecyl acetate at doses up to 1 g/kg body weight. In addition, for most of the hematology parameters there were no statistically significant differences between control and treated groups. Similarly, there were no significant differences from control for the majority of measured serum chemistry values. It should be noted that no significant acute toxicity effects were observed with the primary alcohols, acetates or aldehydes evaluated. Based on these test results, an exemption from tolerance, low application rates (<150 g/a.i./acre/year), and nominal potential exposure, the Agency has waived data requirements for immunotoxicity, chronic toxicity and oncogenicity studies. No additional information and/or data will be required .

d. Chronic Exposure and Oncogenicity Assessment

Chronic and oncogenicity studies are not required. Refer to discussion of subchronic toxicity section (c) above.

e. Effects on the Endocrine Systems

The Agency is not requiring information on the endocrine effects of these compounds at this time. BPPD has considered, among other relevant factors, available information concerning whether these biochemical compounds may have an effect in humans similar to an effect produced by a

naturally occurring estrogen or other endocrine effects. There is no known evidence so far that these active ingredients act as endocrine disrupters in humans. No adverse effects to the endocrine system are known or expected.

2. Dose Response Assessment

No toxicological endpoints are identified.

3. Dietary Exposure and Risk Characterization

These compounds are incorporated into dispensers and are not directly applied to the growing plants. Therefore, dietary exposure to these compounds is expected to be minimal.

The Agency has concluded that residues on treated crops are not a dietary hazard for the following reasons: low acute mammalian toxicity in lepidopteran pheromones registered to date, the known metabolism of long chain fatty acids, low application rates, and nominal human exposure due to application method and to volatilization.

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

Human exposure and risk to these compounds is expected to be minimal in occupational, residential, school and day care settings.

a. Occupational Exposure and Risk Characterization

Based on the use pattern, the potential for dermal, eye and inhalation exposure to pesticide handlers is expected to be negligible. No adverse health effects to workers are expected from the use of this product. According to Regulation (PR) Notice 93-7, "Labeling Revision Required by the Worker Protection Standard (WPS)," WPS does not apply to attractants used in insect traps. Since Isomate - BAW Pheromone is to be used in a solid matrix device, it is exempt from WPS labeling requirements.

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

No indoor residential, school or day care uses currently appear on the proposed labels.

5. Drinking Water Exposure and Risk Characterization

Exposure is not expected from an accumulation of Isomate - BAW Pheromone in the aquatic environment due to the application method. The Agency does not anticipate exposure to residues of Isomate - BAW Pheromone in drinking water.

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

The Agency has concluded that the potential for exposure to lepidopteran pheromone residues (including Isomate -BAW Pheromone) is not a dietary hazard to the general population, including infants and children. This decision is based on: low mammalian toxicity, known metabolism of similar compounds, and the history of safe use of similar lepidopteran pheromones. Available data indicate that for food uses of pheromones, the exemption from the requirement of a tolerance is appropriate and adequately protects human health, including that of infants and children.

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

(Z,E)-9,12-Tetradecadienyl acetate and (Z)-9-Tetradecen-1-ol are synthetic compounds which mimic naturally occurring substances of insect origin with a non-toxic mode of action to target pests. Submitted data confirm the low oral, dermal and inhalation toxicity of this combination product. Based on this information, the Agency has concluded that aggregate exposure to lepidopteran pheromones such as the components of Isomate - BAW Pheromone over a lifetime will not pose appreciable risks to human health. In addition, the toxicity and exposure data are sufficiently complete to adequately address the potential for additional sensitivity of infants and children to residues of these compounds. The Agency concludes that there is reasonable certainty of no harm to infants and children from aggregate exposure to residues of (Z,E)-9,12-Tetradecadienyl acetate and (Z)-9-Tetradecen-1-ol.

8. Cumulative Effects

Isomate - BAW Pheromone, the combination product of (Z,E)-9,12-Tetradecadienyl acetate and (Z)-9-Tetradecen-1-ol, is not toxic and therefore would not be expected to have cumulative effects from common mechanisms of toxicity.

9. Risk Characterization

The Agency has considered Isomate - BAW Pheromone in light of the relevant safety factors and 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 this compound when label instructions are followed.

C. ENVIRONMENTAL ASSESSMENT

1. Ecological Effects Hazard Assessment

All Tier I ecological effects data requirements are waived based on the proposed use pattern and lack of exposure.

2. Environmental Fate and Ground Water Data

Environmental fate and groundwater data are not required for biochemical pesticides unless adverse effects on nontarget species are observed as a result of acute testing for ecological effects (Tier I).

3. Ecological Exposure and Risk Characterization

Because Isomate - BAW Pheromone is enclosed in a solid matrix dispenser and is slowly released by volatilization, transport and exposure is expected to be very limited. This system is generally accepted as posing minimal to no exposure and risk to non-target terrestrial and aquatic species.

D. EFFICACY DATA

Registrants are required to conduct and have available, upon request, efficacy studies to support the registration of lepidopteran pheromones. The Agency is not requiring the submission and review of efficacy studies to support this product at this time.

IV. Risk Management Decision

A. DETERMINATION OF ELIGIBILITY FOR REGISTRATION

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

To satisfy criteria "A" above, Isomate - BAW Pheromone is not expected to cause unreasonable adverse effects when used according to label instructions. Criteria "B" is satisfied by the current label and by the data presented in this document. It is believed that the components of Isomate - BAW Pheromone will not cause any unreasonable adverse effects, will act as a sex attractant by disrupting the mating activity of male beet armyworm as claimed satisfying Criteria "C". Criteria "D" is satisfied in that the toxicological properties of this product are less toxic than any other conventional pesticide product currently in use.

Therefore, Isomate - BAW Pheromone is eligible for registration. Registered use is listed in Table 4, Appendix A.

B. REGULATORY POSITION

1. Conditional/Unconditional Registration

All data requirements are fulfilled and BPPD recommends unconditional registration of Isomate - BAW.

2. CODEX Harmonization

There are no Codex harmonization considerations since a tolerance exemption is in place to cover the use of Isomate - BAW Pheromone in all raw agricultural commodities.

3. Nonfood Re/Registrations

There are no nonfood issues at this time.

4. Risk Mitigation

Since there are no risk issues, risk mitigation measures are not required at this time.

5. Endangered Species Statement

Currently, the Agency is developing a program (The Endangered Species Protection Program) to identify all pesticides whose use may cause potential adverse impacts on endangered and threatened species and their habitats. To aid in the identification of threatened and endangered species and their habitats, several companies have formed an Endangered Species Task Force (EST) under the direction of the American Crop Protection Association (ACPA). Moreover, the EST will assist in providing species location information at the subcounty level, and particularly if an endangered species occurs in areas where pesticides would be used. This information will be useful once the Endangered Species Protection Program has been implemented.

Prior to the implementation of the Endangered Species Protection Program, the Agency will not impose specific labeling on those pesticides that pose risks to threatened and endangered species and their habitats but will defer imposing specific labeling language until implementation of the Program.

C. LABELING RATIONALE

It is the Agency's position that the labeling for Isomate - BAW Pheromone containing (Z,E)-9,12-Tetradecadienyl acetate and (Z)-9-Tetradecen-1-ol complies with the current pesticide labeling requirements.

1. Human Health Hazard

a. Worker Protection Standard

This product does not come under the provisions of the Worker Protection Standard (WPS).

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 Isomate - BAW Pheromone, the combination product of (Z,E)-9,12-Tetradecadienyl acetate and (Z)-9-Tetradecen-1-ol and concludes that the proposed precautionary labeling (i.e. Signal Word, Statement of Practical Treatment and other label statements) adequately mitigate the risks associated with the proposed uses.

End-Use product Precautionary Labeling: for this end use product, Isomate-BAW Pheromone, the following labeling is required:

"Keep out of Reach of Children"

First aid:

"If in Eyes: Flush eyes with plenty of water. Get a physician if irritation persists".

"If on Skin: Wash with plenty of soap and water. Get medical attention if irritation persists."

Precautionary Statements:

"Hazards to Humans and Domestic Animals"

"CAUTION"

"Causes moderate eye irritation. Avoid contact with skin, eyes or clothing. Wash thoroughly with soap and water after handling."

2. Environmental Hazards Labeling

End-Use Product Environmental Hazards Labeling:

"Do not apply directly to water, to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of this product. Do not discharge into lakes, streams, ponds or public waters unless in accordance with NPDES permit. For guidance contact your regional office of the Environmental Protection Agency."

3. Application Rate

It is the Agency's position that the labeling for the pesticide product, Isomate - BAW Pheromone, containing (Z,E)-9,12-Tetradecadienyl acetate and (Z)-9-Tetradecen-1-ol complies with the current pesticide labeling requirements. The tolerance exemption requires that rate of this product not exceed 150 grams active ingredient per acre per year. The label contains the statement "Do not exceed 150 grams of ai per acre per year."

D. LABELING

(1) Product name: Isomate - BAW Pheromone

Active Ingredient:

Z,E-9,12-Tetradecadien-1-yl Actetate	55.0%
Z-9-Tetradecen-1-ol	24.8%
Other Ingredients	20.2%
Total	100.00%

Signal word is "Caution ".
The product shall contain the following information:

- Product Name
- Ingredient Statement
- Registration Number
- "Keep Out of Reach of Children"
- Signal Word (Caution)

V. Actions Required by Registrants

Reports of incidences of adverse effects to humans or domestic animals under FIFRA, Section 6(a)2 and incidents of hypersensitivity under 40 CFR Part 158.690(c), guideline reference number 152-16. There are no data requirements, label changes and other responses necessary for the reregistration of the end-use product since the product is being registered after November 1984 and is, therefore, not subject to reregistration. There are also no existing stocks provisions at this time.

VI. Appendix A

Table 4 lists the use sites for the product. The label for the product is also attached.

Table 4. Non-food Use Site Registration/Reregistration

Isomate- BAW Pheromone Terrestrial Food and Feed Sites: Alfalfa, Asparagus, Beans, Beets, Cabbage, Celery, Cole Crops, Cotton, Cucumbers, Groundnuts, Lettuce, Onions, Peas, Peppers, Soybeans, Strawberries, Sweet Potatoes, Tomatoes Terrestrial Nonfood: Tobacco	Official date registered:

BIOCONTROL®

ISOMATE®-C PLUS

ACTIVE INGREDIENTS:	
(E, E)-8, 10-Dodecadien-1-ol	53.0 %
Dodecanol	29.7 %
Tetradecanol	6.0 %
INERT INGREDIENTS	11.3 %
TOTAL	100.0 %

Keep out of reach of children

WARNING

PRECAUTIONARY STATEMENTS
HAZARDS TO HUMANS AND DOMESTIC ANIMALS

WARNING

Causes skin irritation. May cause eye injury. Do not get in eyes, on skin, or on clothing. Wear eye protection (such as goggles or safety glasses) and gloves when handling. Do not touch eyes while handling or attaching the dispensers. Wash thoroughly with soap and water after handling.

ENVIRONMENTAL HAZARDS

Do not discharge into lakes, streams, ponds or public waters unless in accordance with NPDES permit. For guidance contact your regional office of the Environmental Protection Agency.

PACIFIC BIOCONTROL CORPORATION
14615 NE 13th Court, Suite A
Vancouver, WA 98685 U.S.A.
Telephone (360) 571-2247
1-800-999-8805

MADE IN JAPAN

NET CONTENTS:
400 Dispenser Units
One dispenser contains 0.008 fl oz or 205 mg
Total content of package: 3.2 fl oz or 82 gm

EPA Est. No: 47265-JP-01
EPA Reg. No: 53575-6

STORAGE AND DISPOSAL

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

Pesticide Storage	Store unopened package at temperatures below 40°F in a dry location. Product may be stored in cold storage facilities used for food storage.
Pesticide Disposal	Pesticide that cannot be used according to label instructions must be disposed of according to applicable Federal, State and Local procedures.
Container Disposal	Dispose of empty dispensers by burning or burying with prunings in winter. If burned, stay out of smoke. Foil envelopes can be disposed of as household refuse.
General	Consult Federal, State or Local disposal authorities for approved alternative procedures.

DIRECTIONS FOR USE

IT IS A VIOLATION OF FEDERAL LAW TO USE THIS PRODUCT IN A MANNER INCONSISTENT WITH ITS LABELING.

It is *critical* that ISOMATE-C PLUS is applied as directed.

1. Crop	Apple, pear, walnut, quince, prune, plum, peach, pecan and nectarine.
2. Pest	Codling moth *(Cydia pomonella)*, hickory shuckworm *(Cydia caryana)*.
3. Rate	400 dispensers per acre or 1000 dispensers per hectare. Apply double rate of dispensers to edges of orchard.
4. Application	Apply dispensers securely to lateral branches in upper third of tree canopy. Can be applied efficiently from moving trailer.
5. Timing	Apply prior to moth emergence in spring. Dispensers release pheromone for 120-140 days. Reapply dispensers to crops with long field seasons (i.e. more than 120 days).
6. Precautions	Isomate-C Plus suppresses codling moth and hickory shuckworm from mating. However, if a major source of mated female moths of these species is present adjacent to the treated field, migration of these moths may significantly reduce the level of control achieved. Sources are likely to be unsprayed apple, pear, quince, walnut, plum, crabapple trees or other host species within 300 yards of the treated field. This can be overcome by: a. Treatment of **entire blocks** and not just sections of large conventionally treated fields that frequently serve as sources of mated females. b. Treatment of infestation sources with Isomate-C Plus (e.g. a strip at least 50 yards wide nearest the treated field). c. Treatment of infestation source with an effective insecticide. Other pests must be monitored on a regular schedule so that timely intervention with conventional insecticides is possible.

WARRANTY AND LIMITATION OF DAMAGES

All statements concerning the use of this product apply only when used as directed. The Manufacturer makes no warranties, expressed or implied, concerning this product or its use, which extend beyond the description on the label. Read all directions carefully.

BIOCONTROL®

PB-ROPE L

ACTIVE INGREDIENTS:

(Z, Z)-7, 11-Hexadecadien-1-yl Acetate	46.7 %
(Z, E)-7, 11-Hexadecadien-1-yl Acetate	44.1 %
INERT INGREDIENTS	9.2 %
TOTAL	100.0 %

Keep out of reach of children
CAUTION

PRECAUTIONARY STATEMENTS
HAZARDS TO HUMANS AND DOMESTIC ANIMALS

CAUTION

STATEMENT OF PRACTICAL TREATMENT

Avoid contact with eyes. In case of contact, immediately flush with water. Get medical attention if irritation persists. Wash hands with soap and water after handling.

ENVIRONMENTAL HAZARDS

Do not discharge into lakes, streams, ponds or public waters unless this product is specifically identified and addressed in a NPDES permit. For guidance contact your regional office of the Environmental Protection Agency.

PACIFIC BIOCONTROL CORPORATION
14615 NE 13th Court, Suite A
Vancouver, WA 98685 U.S.A.
Telephone (360) 571-2247
1-800-999-8805

MADE IN JAPAN

NET CONTENTS:
500 Dispenser Units
One dispenser contains 0.0056 fl oz or 147 mg
Total content of package: 2.8 fl oz or 73.5 gm

EPA Est. No: 47265-JP-01
EPA Reg. No: 53575-15

STORAGE AND DISPOSAL

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

Pesticide Storage	Store in original unopened package at temperatures below 40°F in a dry location.
Pesticide Disposal	Pesticide that cannot be used according to label instructions must be disposed of according to applicable Federal, State and Local government procedures. Contact the State pesticide or EPA Hazardous Waste representative at nearest EPA regional office.
Container Disposal	Dispose of containers in a sanitary landfill, or by incineration, or, if allowed by State or Local authorities, by burning. If burned, stay out of smoke.

DIRECTIONS FOR USE

IT IS A VIOLATION OF FEDERAL LAW TO USE THIS PRODUCT IN A MANNER INCONSISTENT WITH ITS LABELING.

It is *critical* that PB-ROPE L is applied as directed.

1. Crop	Cotton.
2. Pest	Pink bollworm *(Pectinophora gossypiella).*
3. Rate	100-200 dispensers per acre or 250-500 dispensers per hectare.
4. Application	i. Twist dispensers loosely around the main stem of cotton near the bottom of the plant. ii. Apply immediately prior to moth emergence in the field or adjoining area. iii. Dispensers must be applied uniformly throughout the treated acreage to obtain a reduction in mating.
5. Timing	Attach dispensers at pinsquare.
6. Precautions	PB-Rope L suppresses mating of pink bollworms. However, if a major source of mated female pink bollworm moths is present in adjacent areas, migration of these moths may significantly reduce the level of control achieved. Sources are likely to be cotton (sprayed or unsprayed) or other host species within 300 yards of the treated field. This may be counteracted by: a. Treatment of **entire blocks** and not just sections of large conventionally treated fields. b. Treatment of infestation sources with PB-Rope L (e.g. a strip at least 250 yards wide nearest the treated field). c. Treatment of infestation sources with an effective insecticide. Supplementary applications of insecticide are advised when PB-Rope L is used to control very high populations of pink bollworms. Other pests must be monitored so that timely intervention with insecticides is possible.

WARRANTY AND LIMITATION OF DAMAGES

All statements concerning the use of this product apply only when used as directed. The Manufacturer makes no warranties, expressed or implied, concerning this product or its use, which extend beyond the description on the label. Read all directions carefully.

BIOCONTROL®

ISOMATE®-M 100

ACTIVE INGREDIENTS:	
Z-8-Dodecen-1-yl Acetate	88.5 %
E-8-Dodecen-1-yl Acetate	5.7 %
Z-8-Dodecen-1-ol	1.0 %
INERT INGREDIENTS	4.8 %
TOTAL	100.0 %

Keep out of reach of children

CAUTION

STATEMENT OF PRACTICAL TREATMENT:

IF ON SKIN: Wash with plenty of soap and water. Get medical attention.

IF IN EYES: Flush eyes with plenty of water. Get medical attention if irritation persists.

PRECAUTIONARY STATEMENTS
HAZARDS TO HUMANS AND DOMESTIC ANIMALS

CAUTION

Harmful if absorbed through skin. Causes eye irritation. Avoid contact with skin, eyes or clothing. Wash thoroughly with soap and water after handling.

ENVIRONMENTAL HAZARDS

Do not apply directly to water, to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of this product. Do not discharge into lakes, streams, ponds or public waters unless in accordance with NPDES permit. For guidance contact your regional office of the Environmental Protection Agency.

PACIFIC BIOCONTROL CORPORATION
14615 NE 13th Court, Suite A
Vancouver, WA 98685 U.S.A.
Telephone (360) 571-2247 or 1-800-999-8805

MADE IN JAPAN

NET CONTENTS:
400 Dispenser Units
One dispenser contains 0.0094 fl oz or 243.8 mg
Total content of package: 3.75 fl oz or 97.5 gm

EPA Est. No: 47265-JP-01
EPA Reg. No: 53575-19

STORAGE AND DISPOSAL

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

Pesticide Storage	Store in original unopened package at temperatures below 40°F in a dry location. Product may be stored in cold storage facilities used for food storage.
Pesticide Disposal	Pesticide that cannot be used according to label instructions must be disposed of according to applicable Federal, State and Local government procedures. Contact the State pesticide or EPA Hazardous Waste representative at nearest EPA regional office.
Container Disposal	Dispose of dispensers in sanitary landfill or by incineration, or if allowed by State and Local authorities, by burning. If burned, stay out of smoke. Foil envelopes can be disposed of as household refuse.

DIRECTIONS FOR USE

IT IS A VIOLATION OF FEDERAL LAW TO USE THIS PRODUCT IN A MANNER INCONSISTENT WITH ITS LABELING.

It is *critical* that ISOMATE-M 100 is applied as directed.

1. Crop	Peach, nectarine, almond, apricot, plum, apple, quince and macadamia.
2. Pest	Oriental fruit moth *(Grapholita molesta)*, macadamia nut borer *(Cryptophlebia ombrodelta)*, koa seed worm *(Cryptophlebia illepida)*.
3. Rate	100-150 dispensers per acre or 250-375 dispensers per hectare (0.9 fl oz or 23.4 gm a.i. per application).
4. Application	Apply dispensers in at least upper third of tree, preferably within 2-3 feet of treetop. Apply dispensers within canopy and on branches to maximize shade protection. Apply dispensers securely on lateral branches. Dispensers twisted too tightly may girdle branches. Can be applied efficiently from moving trailer or with a pole applicator.
5. Timing	Apply prior to moth emergence in the spring. Dispensers release pheromone for up to 90 days. In crops with long field seasons or in orchards with high pest populations, a second application is recommended. If subsequent applications are required, apply prior to the start of subsequent flights.
6. Precautions	Isomate-M 100 suppresses oriental fruit moth from mating. However, if a major source of mated female moths of this species is present adjacent to the treated field, migration of these moths may significantly reduce the level of control achieved. Sources are likely to be unsprayed peach, nectarine, almond, apricot, plum, apple, quince and macadamia trees or other host species within 300 yards of the treated field. This can be overcome by: a. Treatment of **entire blocks** and not just sections of large conventionally treated fields that frequently serve as sources of mated females. b. Treatment of infestation sources with Isomate-M 100 (e.g. a strip at least 50 yards wide nearest the treated field). c. Treatment of infestation source with an effective insecticide. d. Treatment of 4-6 rows along border of pheromone treated orchard with insecticide. Other pests must be monitored on a regular schedule so that timely intervention with conventional insecticides is possible.

WARRANTY AND LIMITATION OF DAMAGES

All statements concerning the use of this product apply only when used as directed. The Manufacturer makes no warranties, expressed or implied, concerning this product or its use, which extend beyond the description on the label. Read all directions carefully.

BIOCONTROL®

ISOMATE®-CM/LR PHEROMONE

ACTIVE INGREDIENTS:

Z-11-Tetradecenyl acetate	45.8 %
E, E-8, 10-Dodecadien-1-ol	39.2 %
Dodecanol	6.6 %
Tetradecanol	1.0 %
INERT INGREDIENTS	7.4 %
TOTAL	100.0 %

Keep out of reach of children

CAUTION

STATEMENT OF PRACTICAL TREATMENT:

IF ON SKIN: Wash with plenty of soap and water. Get medical attention if irritation persists.

IF IN EYES: Flush eyes with plenty of water. Get medical attention if irritation persists.

PRECAUTIONARY STATEMENTS
HAZARDS TO HUMANS AND DOMESTIC ANIMALS

CAUTION

Harmful if absorbed through skin. Causes eye irritation. Avoid contact with skin, eyes or clothing. Wash thoroughly with soap and water after handling.

ENVIRONMENTAL HAZARDS

Do not apply directly to water, to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of this product. Do not discharge into lakes, streams, ponds or public waters unless in accordance with NPDES permit. For guidance contact your regional office of the Environmental Protection Agency.

PACIFIC BIOCONTROL CORPORATION
16010 NE 36th Avenue, Suite A
Ridgefield, WA 98642 U.S.A.
Telephone (360) 574-9726
1-800-999-8805

MADE IN JAPAN

NET CONTENTS:
400 Dispenser Units
One dispenser contains 0.01 fl oz or 264 mg
Total content of package: 4.1 fl oz or 105 gm

EPA Est. No: 47265-JP-01
EPA Reg. No: 53575-20

STORAGE AND DISPOSAL

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

Pesticide Storage	Store in original unopened package at temperatures below 40°F in a dry location. Product may be stored in cold storage facilities used for food storage.
Pesticide Disposal	Pesticide that cannot be used according to label instructions must be disposed of according to applicable Federal, State and local government procedures. Contact the State pesticide or EPA Hazardous Waste representative at nearest EPA regional office.
Container Disposal	Dispose of dispensers in sanitary landfill or incineration, or if allowed by State and local authorities, by burning. If burned, stay out of smoke. Foil envelopes can be disposed of as household refuse.

DIRECTIONS FOR USE

IT IS A VIOLATION OF FEDERAL LAW TO USE THIS PRODUCT IN A MANNER INCONSISTENT WITH ITS LABELLING.

It is *critical* that ISOMATE-CM/LR is applied as directed.

1. Crop	Apple and pear.
2. Pest	Codling moth *(Cydia pomonella)*, oblique banded leafroller *(Choristoneura rosaceana)*, pandemis leafroller *(Pandemis pyrusana)*.
3. Rate	400 dispensers per acre or 1000 dispensers per hectare (3.9 fl oz or 102 gm a.i. per application). Apply double rate of dispensers to edges of orchard.
4. Application	Apply dispensers in at least upper third of tree, preferably within 2-3 feet of treetop. Apply dispensers within canopy and on branches to maximize shade protection. Apply dispensers securely on lateral branches. Dispensers twisted too tightly may girdle branches. Can be applied efficiently from moving trailer or with a pole applicator.
5. Timing	Apply prior to codling moth emergence in the spring. Dispensers release pheromone for 100 -120 days. In crops with long field seasons (i.e. more than 120 days) or in orchards with high pest populations, a second application is recommended. If subsequent applications are required, apply prior to the start of subsequent flights.
6. Precautions	Isomate-CM/LR suppresses codling moth and leafroller moth from mating. However, if a major source of mated female moths of these species is present adjacent to the treated field, migration of these moths may significantly reduce the level of control achieved. Sources are likely to be unsprayed apple, pear, peach, apricot, plum, and cherry trees or other host species within 300 yards of the treated field. This can be overcome by: a. Treatment of **entire blocks** and not just sections of large conventionally treated fields that frequently serve as sources of mated females. b. Treatment of infestation sources with Isomate-CM/LR (e.g. a strip at least 50 yards wide nearest the treated field). c. Treatment of infestation source with an effective insecticide. d. Treatment of 4-6 rows along border of pheromone treated orchard with insecticide. Other pests must be monitored on a regular schedule so that timely intervention with conventional insecticides is possible.

WARRANTY AND LIMITATION OF DAMAGES

All statements concerning the use of this product apply only when used as directed. The Manufacturer makes no warranties, expressed or implied, concerning this product or its use, which extend beyond the description on the label. Read all directions carefully.

BIOCONTROL®

ISOMATE®-BAW PHEROMONE

ACTIVE INGREDIENTS:	
(Z,E)-9,12-Tetradecadien-1-yl Acetate	55.0 %
Z-9-Tetradecen-1-ol	24.8 %
OTHER INGREDIENTS	20.2 %
TOTAL	100.0 %

Keep out of reach of children

CAUTION

STATEMENT OF PRACTICAL TREATMENT:

IF ON SKIN: Wash with plenty of soap and water. Get medical attention if irritation persists.

IF IN EYES: Flush eyes with plenty of water. Get a physician if irritation persists.

PRECAUTIONARY STATEMENTS
HAZARDS TO HUMANS AND DOMESTIC ANIMALS

CAUTION

Causes moderate eye irritation. Avoid contact with skin, eyes or clothing. Wash thoroughly with soap and water after handling.

ENVIRONMENTAL HAZARDS

Do not apply directly to water, to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of this product. Do not discharge into lakes, streams, ponds or public waters unless in accordance with NPDES permit. For guidance contact your regional office of the Environmental Protection Agency.

PACIFIC BIOCONTROL CORPORATION
14615 NE 13th Court, Suite A
Vancouver, WA 98685 U.S.A.
Telephone (360) 571-2247
1-800-999-8805

MADE IN JAPAN

EPA Est. No: 47265-JP-01
EPA Reg. No: 53575-21

NET CONTENTS:
500 Dispenser Units
One dispenser contains 0.0097 fl oz or 252.65 mg
Total content of package: 4.86 fl oz or 126.3 gm

STORAGE AND DISPOSAL

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

Pesticide Storage	Store in original unopened package at temperatures below 40°F in a dry location. Only unopened or unbroken dispenser packages may be stored in cold storage facilities used for food storage. Care must be taken to avoid contamination of food or feed items.
Pesticide Disposal	Pesticide that cannot be used according to label instructions must be disposed of according to applicable Federal, State and Local government procedures. Contact the State pesticide or EPA Hazardous Waste representative at nearest EPA regional office.
Container Disposal	Dispose of dispensers in sanitary landfill or by incineration, or if allowed by State and Local authorities, by burning. If burned, stay out of smoke. Foil envelopes can be disposed of as household refuse.

DIRECTIONS FOR USE

IT IS A VIOLATION OF FEDERAL LAW TO USE THIS PRODUCT IN A MANNER INCONSISTENT WITH ITS LABELING.

It is *critical* that ISOMATE-BAW is applied as directed.

1. Crop	Alfalfa, asparagus, beans, beets, cabbage, celery, cole crops, cotton, cucumbers, lettuce, onions, peanuts, peas, peppers, soybeans, strawberries, sweet potatoes, tomatoes, tobacco.
2. Pest	Beet armyworm *(Spodoptera exigua).*
3. Rate	Minimum of 100 dispensers per acre (0.9 fl oz or 23.9 gm a.i. per acre) or 250 dispensers per hectare. Maximum of 200 dispensers per acre (1.8 fl oz or 47.9 gm a.i. per acre) or 500 dispensers per hectare. Do not exceed 150 gm a.i. per acre per year.
4. Application	Attach dispensers to stakes placed uniformly within the treated field. Stakes should be at the canopy level of the crop. Avoid placing dispensers in contact with the soil. At 100 dispensers per acres, stakes should be placed approximately every 20 feet apart. Treat all border rows. Increase the number of dispensers on upwind side of the fields or along borders adjacent to other beet armyworm hosts. Higher rates may be needed in smaller fields or in windy conditions.
5. Timing	Monitor with pheromone traps and crop inspection. Apply product prior to adult flight, early enough to disrupt mating communication within the field. Consult your local pest control advisor for proper timing. Dispenser releases pheromone for 60-90 days depending on temperature.
6. Precautions	Isomate-BAW suppresses mating between beet armyworms. However, if a major source of mated female moths of this species is present adjacent to the treated field, migration of these moths may significantly reduce the level of control achieved. A common source of mated females is unsprayed host species within 300 yards of the treated field. This can be overcome by: a. Treatment of **entire blocks** and not just sections of large conventionally treated fields that frequently serve as sources of mated females. b. Treatment of infestation sources with Isomate-BAW (e.g. a strip at least 50 yards wide nearest the treated field). c. Treatment of infestation source with an effective insecticide. Supplementary applications of insecticide are advised when Isomate-BAW is used to control very high populations of beet armyworm, and when beet armyworm larvae are imported into the field on transplants. All pests must be monitored so that timely intervention with insecticides is possible.

WARRANTY AND LIMITATION OF DAMAGES

All statements concerning the use of this product apply only when used as directed. The Manufacturer makes no warranties, expressed or implied, concerning this product or its use, which extend beyond the description on the label. Read all directions carefully.

BIOCONTROL®

ISOMATE®-OBLR/PLR PLUS

ACTIVE INGREDIENTS:	
Z-11-Tetradecen-1-yl Acetate	88.97 %
OTHER INGREDIENTS	11.03 %
TOTAL	100.00 %

226.82 mg active ingredients per dispenser

Keep out of reach of children

CAUTION

FIRST AID

If on Skin or Clothing	• Take off contaminated clothing. • Rinse skin immediately with plenty of water for 15-20 minutes. • Call a poison control center or doctor for treatment advice.
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. You may also contact 1-800-999-8805 for further questions.

PRECAUTIONARY STATEMENTS
HAZARDS TO HUMANS AND DOMESTIC ANIMALS

CAUTION

Harmful if absorbed through skin. Causes moderate eye irritation. Avoid contact with skin, eyes or clothing. Wash thoroughly with soap and water after handling.

ENVIRONMENTAL HAZARDS

Do not apply directly to water, to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of this product. Do not discharge into lakes, streams, ponds or public waters unless in accordance with NPDES permit. For guidance contact your regional office of the Environmental Protection Agency.

PACIFIC BIOCONTROL CORPORATION
14615 NE 13th Court, Suite A, Vancouver, WA 98685 U.S.A.
Telephone (360) 571-2247 or 1-800-999-8805

MADE IN JAPAN
EPA Est. No: 47265-JP-01
EPA Reg. No: 53575-24

NET CONTENTS: 400 Dispenser Units
One dispenser contains 0.0099 fl oz or 255.5 mg
Total content of package: 3.95 fl oz or 102.2 gm

STORAGE AND DISPOSAL
Do not contaminate water, food or feed by storage or disposal

Pesticide Storage	Store in original unopened package at temperatures below 40°F in a dry location. Only unopened or unbroken dispenser packages may be stored in cold storage facilities used for food storage. Care must be taken to avoid contamination of food or feed items.
Pesticide Disposal	Wastes resulting from this product may be disposed of on site or at an approved waste disposal facility.
Container Disposal	Dispose of dispensers in sanitary landfill or by incineration, or if allowed by State and Local authorities, by burning. If burned, stay out of smoke. Foil envelopes can be disposed of as household refuse.

DIRECTIONS FOR USE
It is a violation of Federal law to use this product in a manner inconsistent with its labeling.
It is *critical* that ISOMATE-OBLR/PLR PLUS is applied as directed.

1. Crop	Apple, pear, apricot, cherry, peach and plum.
2. Pest	Obliquebanded leafroller *(Choristoneura rosaceana)* and pandemis leafroller *(Pandemis pyrusana)*.
3. Rate	Minimum of 200 dispensers per acre (1.75 fl oz or 45.36 gm a.i. per acre) or 500 dispensers per hectare. Maximum of 400 dispensers per acre or 1000 dispensers per hectare (3.5 fl oz or 90.73 gm a.i. per acre). Apply double rate of dispensers to edges of orchard. Do not exceed 150 gm a.i. per acre per year
4. Application	Apply dispensers securely to lateral branches in upper third of tree canopy, preferably within 2-3 feet of treetop. Dispensers twisted too tightly may girdle branches. Can be applied efficiently from a moving trailer or with a pole applicator.
5. Timing	Apply prior to leafroller emergence in the spring. Dispensers release pheromone for up to 150 days depending on temperature. In crops with long field seasons (i.e. more than 150 days), a second application is recommended. If subsequent applications are required, apply prior to the start of subsequent flights. Consult your local pest control advisor for proper timing.
6. Note	Isomate-OBLR/PLR Plus suppresses the obliquebanded and pandemis leafrollers from mating. However, if a major source of mated female moths of these species is present adjacent to the treated field, migration of these moths may significantly reduce the level of control achieved. Sources are likely to be unsprayed apple, pear, peach, apricot, plum, prune and cherry trees or other wild plant host species within 300 yards of the treated field. This can be reduced by: a. Treatment of **entire blocks** and not just sections of large conventionally treated fields that frequently serve as sources of mated females. b. Treatment of infestation sources with Isomate-OBLR/PLR Plus (e.g. a strip at least 50 yards wide nearest the treated field). c. Treatment of infestation source with an effective insecticide. d. Treatment of 4-6 rows along border of pheromone treated orchard with insecticide. Supplementary applications of insecticide are advised when Isomate-OBLR/PLR Plus is used to control high populations of leafrollers. All pests must be monitored so that timely intervention with insecticides is possible.

WARRANTY AND LIMITATION OF DAMAGES

All statements concerning the use of this product apply only when used as directed. The Manufacturer makes no warranties, expressed or implied, concerning this product or its use, which extend beyond the description on the label. Read all directions carefully.

Revision Date: February 2002

BIOCONTROL®

ISOMATE®-C TT

ACTIVE INGREDIENTS:	
(E, E)-8, 10-Dodecadien-1-ol	53.0 %
Dodecanol	29.7 %
Tetradecanol	6.0 %
OTHER INGREDIENTS	11.3 %
TOTAL	100.0 %

382.4 mg active ingredients per dispenser

Keep out of reach of children

WARNING

FIRST AID

If on Skin or Clothing	• Take off contaminated clothing. • Rinse skin immediately with plenty of water for 15-20 minutes. • Call a poison control center or doctor for treatment advice.
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. You may also contact 1-800-999-8805 for further questions.

PRECAUTIONARY STATEMENTS
HAZARDS TO HUMANS AND DOMESTIC ANIMALS

WARNING

Harmful if absorbed through skin. Causes eye irritation. Avoid contact with skin, eyes or clothing. Wash thoroughly with soap and water after handling.

ENVIRONMENTAL HAZARDS

Do not apply directly to water, to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of this product. Do not discharge into lakes, streams, ponds or public waters unless in accordance with NPDES permit. For guidance contact your regional office of the Environmental Protection Agency.

PACIFIC BIOCONTROL CORPORATION
14615 NE 13th Court, Suite A, Vancouver, WA 98685 U.S.A.
Telephone (360) 571-2247 or 1-800-999-8805

MADE IN JAPAN
EPA Est. No: 47265-JP-01
EPA Reg. No: 53575-25

NET CONTENTS: 400 Dispenser Units
One dispenser contains 0.017 fl oz or 431.4 mg
Total content of package: 6.81 fl oz or 172.56 gm

STORAGE AND DISPOSAL

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

Pesticide Storage	Store in original unopened package at temperatures below 40°F in a dry location. Product may be stored in cold storage facilities used for food storage.
Pesticide Disposal	Pesticide that cannot be used according to label instructions must be disposed of according to applicable Federal, State and Local government procedures. Contact the State pesticide or EPA Hazardous Waste representative at nearest EPA regional office.
Container Disposal	Dispose of dispensers in sanitary landfill or by incineration, or if allowed by State and Local authorities, by burning. If burned, stay out of smoke. Foil envelopes can be disposed of as household refuse.

DIRECTIONS FOR USE

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

It is *critical* that ISOMATE-C TT is applied as directed.

1. Crop	Apple, pear, walnut, quince, peach, prune, plum, peach, pecan and nectarine.
2. Pest	Codling moth *(Cydia pomonella)*, hickory shuckworm *(Cydia caryana)*.
3. Rate	200 dispensers per acre or 500 dispensers per hectare (3.0 fl oz or 76.5 gm a.i. per application). Apply double rate of dispensers to edges of orchard. Do not exceed 150 gm a.i. per acre per year.
4. Application	Apply dispensers in upper third of tree, preferably within 2-3 feet of treetop. Apply dispensers within canopy. Apply dispensers securely on branches. Can be applied efficiently from moving trailer or with a pole applicator.
5. Timing	Apply prior to codling moth emergence in the spring. Dispensers release pheromone for 120-140 days depending on temperature. In crops with long field seasons (i.e. more than 120 days), a second application is recommended. If subsequent applications are required, apply prior to the start of subsequent flights. Consult your local pest control advisor for proper timing.
6. Note	Isomate-C TT suppresses codling moth and hickory shuckworm from mating. However, if a major source of mated female moths of these species is present adjacent to the treated field, migration of these moths may significantly reduce the level of control achieved. Sources are likely to be unsprayed apple, pear, quince, walnut, plum, crabapple trees or other host species within 300 yards of the treated field. This can be overcome by: a. Treatment of **entire blocks** and not just sections of large conventionally treated fields that frequently serve as sources of mated females. b. Treatment of infestation sources with Isomate-C TT (e.g. a strip at least 50 yards wide nearest the treated field). c. Treatment of infestation source with an effective insecticide. d. Treatment of 4-6 rows along border of pheromone treated orchard with insecticide. Supplementary applications of insecticide are advised when Isomate-C TT is used to control very high populations of codling moth or hickory shuckworm. All pests must be monitored so that timely intervention with insecticides is possible.

WARRANTY AND LIMITATION OF DAMAGES

All statements concerning the use of this product apply only when used as directed. The Manufacturer makes no warranties, expressed or implied, concerning this product or its use, which extend beyond the description on the label. Read all directions carefully.

BIOCONTROL®

ISOMATE®-M ROSSO

ACTIVE INGREDIENTS:	
Z-8-dodecen-1-yl acetate	88.5 %
E-8-dodecen-1-yl acetate	5.7 %
Z-8-dodecen-1-ol	1.0 %
OTHER INGREDIENTS	4.9 %
TOTAL	100.0 %

Keep out of reach of children

CAUTION

FIRST AID

If on Skin or Clothing
- Take off contaminated clothing.
- Rinse skin immediately with plenty of water for 15-20 minutes.
- Call a poison control center or doctor for treatment advice.

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. You may also contact 1-800-999-8805 for further questions.

PRECAUTIONARY STATEMENTS
HAZARDS TO HUMANS AND DOMESTIC ANIMALS

CAUTION

Harmful if absorbed through skin. Causes eye irritation. Avoid contact with skin, eyes or clothing. Wash thoroughly with soap and water after handling.

ENVIRONMENTAL HAZARDS

Do not apply directly to water, to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of this product. Do not discharge into lakes, streams, ponds or public waters unless in accordance with NPDES permit. For guidance contact your regional office of the Environmental Protection Agency.

PACIFIC BIOCONTROL CORPORATION
14615 NE 13th Court, Suite A
Vancouver, WA 98685 U.S.A.
Telephone (360) 571-2247 or 1-800-999-8805

MADE IN JAPAN
EPA Est. No: 47265-JP-01
EPA Reg. No: 53575-26

NET CONTENTS: 400 Dispenser Units
One dispenser contains 0.01 fl oz or 264.3 mg
Total content of package: 4.06 fl oz or 105.7 gm

STORAGE AND DISPOSAL

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

Pesticide Storage	Store in original unopened package at temperatures below 40°F in a dry location. Product may be stored in cold storage facilities used for food storage.
Pesticide Disposal	Pesticide that cannot be used according to label instructions must be disposed of according to applicable Federal, State and Local government procedures. Contact the State pesticide or EPA Hazardous Waste representative at nearest EPA regional office.
Container Disposal	Dispose of dispensers in sanitary landfill or by incineration, or if allowed by State and Local authorities, by burning. If burned, stay out of smoke. Foil envelopes can be disposed of as household refuse.

DIRECTIONS FOR USE

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

It is *critical* that ISOMATE-M ROSSO is applied as directed.

1. Crop	Peach, nectarine, almond, apricot, plum, apple, quince and macadamia.
2. Pest	Oriental fruit moth *(Grapholita molesta)*, macadamia nut borer *(Cryptophlebia ombrodelta)*, koa seed worm *(Cryptophlebia illepida)*.
3. Rate	200 dispensers per acre or 500 dispensers per hectare (1.94 fl oz or 50.5 gm a.i. per application). Do not exceed 150 gm a.i. per acre per year.
4. Application	Apply dispensers in upper third of tree, preferably within 2-3 feet of treetop. Apply dispensers within canopy and on branches to maximize shade protection. Apply dispensers securely on lateral branches. Dispensers twisted too tightly may girdle branches. Can be applied efficiently from moving trailer or with a pole applicator.
5. Timing	Apply prior to moth emergence in the spring. Dispensers release pheromone for up to 120 days depending on temperature. In crops with long field seasons (i.e. more than 120 days), a second application is recommended. If subsequent applications are required, apply prior to the start of subsequent flights. Consult your local pest control advisor for proper timing.
6. Precautions	Isomate-M Rosso suppresses orient fruit moth, macadamia nut borer and koa seed worm from mating. However, if a major source of mated female moths of these species is present adjacent to the treated field, migration of these moths may significantly reduce the level of control achieved. Sources are likely to be unsprayed peach, nectarine, almond, apricot, plum, apple, quince and macadamia trees or other host species within 300 yards of the treated field. This can be overcome by: a. Treatment of **entire blocks** and not just sections of large conventionally treated fields that frequently serve as sources of mated females. b. Treatment of infestation sources with Isomate-M Rosso (e.g. a strip at least 50 yards wide nearest the treated field). c. Treatment of infestation source with an effective insecticide. d. Treatment of 4-6 rows along border of pheromone treated orchard with insecticide Supplementary applications of insecticide are advised when Isomate-M Rosso is used to control very high populations of oriental fruit moth, macadamia nut borer or koa seed worm. All pests must be monitored so that timely intervention with insecticides is possible.

WARRANTY AND LIMITATION OF DAMAGES

All statements concerning the use of this product apply only when used as directed. The Manufacturer makes no warranties, expressed or implied, concerning this product or its use, which extend beyond the description on the label. Read all directions carefully.

MATERIAL SAFETY DATA SHEET

1. CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

Product Name :Sumilizer BHT-R
Chemical Name :2,6-Di-tert-butyl-p-cresol

MANUFACTURER :

Sumitomo Chemical Co.,Ltd.
27-1,Shinkawa,2-Chome,
Chuo-ku, Tokyo 104, Japan

EMERGENCY TELEPHONE NUMBERS :

Sumitomo Chemical Co.,Ltd.
TEL +81-3-5543-5641(Japan)
FAX +81-3-5543-5916(Japan)

2. COMPOSITION/INFORMATION ON INGREDIENTS

COMPONENT	CAS No.	%	OSHA PEL	ACGIH TLV
#Sumilizer BHT-R	128-37-0	100	10mg/m^3	10mg/m^3

#Hazardous with the meaning of 29 C.F.R.Part 1910.1200.

3. HAZARDS IDENTIFICATION

EMERGENCY OVERVIEW :White crystalline powder that is no immediate hazard.
Avoid contact with skin and eyes. Avoid breathing dust.

POTENTIAL HEALTH EFFECTS:

INHALATION :No known effect.

EYE CONTACT :Mechanical irritation will probably develop after contact with this material.

SKIN CONTACT :Non-irritating.

INGESTION :Practically non-toxic in normal industrial use.

CHRONIC :Listed in Group 3(not classifiable as to their carcinogenicity to humans) by IARC.

MSDS continued on next page.(US)

4. EMERGENCY AND FIRST AID MEASURES

INHALATION :If exposure to dust causes irritation or distress, remove subject to fresh air. Give oxygen or artificial respiration if needed. Get medical attention.

SKIN CONTACT :Immediately flush skin with plenty of water. Remove clothing. Get medical attention if irritation develops or persists. Wash clothing before reuse.

EYE CONTACT :Immediately flush eyes with plenty of water for at least 15 minutes,and contact a physician.

INGESTION :If swallowed, immediately get medical attention.

5. FIRE-FIGHTING MEASURES AND EXPLOSION HAZARD DATA

FLASH POINT :127℃

AUTOIGNITION TEMPERATURE :420℃

DUST EXPLOSION LIMITS :Lower :15g/m3

EXTINGUISHING MEDIA :Use carbon dioxide or dry chemical for small fires; universal foam or water spray for large fires.

SPECIAL FIRE-FIGHTING PROCEDURES :Fire fighters should be provided with positive-pressure self-contained breathing apparatus and other protective equipment.

UNUSUAL FIRE and EXPLOSION HAZARDS :Not known.

HAZARDOUS DECOMPOSITION PRODUCTS :May generate CO when heated to burning.

6. ACCIDENTAL RELEASE MEASURES

GENERAL : Eliminate all ignition source.
Consult an expert on the disposal of recovered material. Ensure disposal is in compliance with government requirements and ensure conformity of local disposal regulations. Notify the appropriate authorities immediately. Take all additional action necessary to prevent and remedy the adverse effects of the spill.

LAND SPILL : Sweep up or shovel into sealable containers and then wash out with water. Prevent washings from entering waterways.

MSDS continued on next page.(US)

7. HANDLING AND STORAGE

PRECAUTIONS :Avoid contact with skin and eyes. Avoid breathing dust.
Use with adequate ventilation. Ground and bond containers when transferring material.
Store out of direct sunlight .Keep away from all ignition source.
Keep containers closed when not in use.

8. EXPOSURE CONTROLS AND PERSONAL PROTECTION

ENGINEERING CONTROLS (VENTILATION) : Use local ventilation at places where dust can be released into the workplace air. Keep dust concentrations below the recommended TLV.

ACGIH TLV for =10 mg/m^3
OSHA PEL for =10mg/m^3

PERSONAL PROTECTION

RESPIRATORY :Not required for occasional handling if adequate ventilation is available.
A respirator is recommended for prolonged handling or exposure.

PROTECTIVE GLOVES : Wear rubber gloves.

EYE PROTECTION : Wear safety goggles or equivalent eye protection.

OTHER : Wear appropriate protective clothing to prevent skin contact.

WORK/HYGIENIC PRACTICES : Always clean protective equipment and workplace.

9. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE	:White crystalline powder	ODOR	:Odorless
MELTING POINT	:70℃	SPECIFIC GRAVITY	:1.048(20℃/4℃)
BOILING POINT	:265℃	SOLUBILITY in water	:Practically insoluble
VAPOR DENSITY	:Not known	pH	:Not known
PERCENT VOLATILE	:Not known	VAPER PRESSURE	:6.5mmHg(120℃)

10. STABILITY AND REACTIVITY

STABILITY : Stable on normal condition. HAZARDOUS POLYMERIZATION : Will not occur.

CONDITIONS TO AVOID :High temperature

INCOMPATIBILITY :Not known

HAZARDOUS DECOMPOSITION PRODUCTS : May generate CO when heated to burn.

MSDS continued on next page.(US)

11. TOXICOLOGICAL INFORMATION

ACUTE INHALATION EFFECTS :No data available.

SKIN EFFECT :This material is classified as non-irritant based on the test results in rabbits according to the OSHA standard.(1)

EYE EFFECT :This material is classified as non-irritant based on the test results in rabbits according to the OSHA standard.(1)

ACUTE ORAL EFFECTS :The oral LD_{50} in rats is 2,340~2,530mg/kg. (1)

SUBCHRONIC AND CHRONICTOXICITY :
This material produce liver enlargement and hemorrhagic death after continuous oral administration in rats. It is recognized that these effects are not produced in humans.(1)
No adverse effects are occurred after oral administration of total 45~244g/kg during 12 months in dogs. (1)

CARCINOGENICITY:
Carcinogenicity had not been observed after continuous treatment of this material in diet for 104 and 96 weeks in rats and mice, respectively.(1)
This material is listed in Group3(not classifiable as to their carcinogenicity to humans) by IARC.
NTP or OSHA have not classified it as carcinogenicity.

MUTAGENICITY :Negative results have been reported in Ames test,Rec-assay,DNA-repair test in rat liver cells, in vitro chromosomal aberration test in CHL cells, in vivo micronucleus test and dominant lethal test in mice.
Some positive results have been reported in UDS test in rats and in vitro chromosomal aberration test in CHO cells. (1)

TERATOGENICITY: No teratogenic effects have been observed in rats and mice. (1)

REPRODUCTION :Developmental delay of offspring was observed in reproduction studies in rats and mice.(1)

This material had been evaluated for its toxicological properties and classified as GRAS by FDA. (9CFR§182.3173)

12. ECOLOGICAL INFORMATION

FISH ACUTE TOXICITY :LC50 is 6.2ppm(48hr) in killifish. (1)
BIODEGRADABILITY :Not biodegradability. (1)

13. DISPOSAL CONSIDERATIONS

To be incinerated by adequate method.Dispose in accordance with federal, state and local regulations. The owner of the materials responsible for proper waste disposal.

MSDS continued on next page.(US)

14. TRANSPORT INFORMATION

Ground and bond containers when transferring material. Keep containers tightly closed during transport.

15. REGULATORY INFORMATION (not meant to be all inclusive)

TSCA (Toxic Substance Control Act) : Listed on TSCA

16. OTHER INFORMATION

REVISION SUMMARY :Revised due to amendment of contents in section 9 on 31/March/1998.
Revised due to amendment of contents in section 11 on 16/December/1998.

REFERENCES :(1)Technical information of Sumitomo Chemical Co., Ltd.

The information is believed to be accurate and represents the best information currently available to us. However, no guarantee or warranty of any kind, expressed or implied, is made with respect to the information contained herein.

End of MSDS (US)

National Toxicology Program

Good Science for Good Decisions

BUTYLATED HYDROXYTOLUENE

CASNO: 128-37-0

FORMULA: C15-H24-O

SYNONYMS/COMMON NAMES

- 2,6-BIS(1,1-DIMETHYLETHYL)-4- METHYLPHENOL (9CI)
- BHT

KNOWN USES:

- ANTIOXIDANT FOR PETROLEUM AND FOOD PRODUCTS; ANIMAL FEED; FOOD PACKAGING

CHEMICAL HEALTH AND SAFETY INFORMATION:

SHORT-TERM TOXICITY (definition)

- 90 DAY (Dosed-Feed) (C03598), COMPLETED
  - RATS:FISCHER 344; MICE:B6C3F1
  - DOSE: R: 0,6200,50000, M: 0,3100,50000 PPM/5 PER GROUP

LONG-TERM CARCINOGENICITY (definition)

- 2 YEAR (Dosed-Feed) (C03598)
  - **TR-150** (NTIS # PB298539) (PEER REVIEW 12/78)
  - RATS:FISCHER 344; MICE:B6C3F1
  - CARCINOGENESIS RESULTS **(see RESULTS definitions)**
    - MALE RATS = NEGATIVE
    - FEMALE RATS = NEGATIVE
    - MALE MICE = NEGATIVE
    - FEMALE MICE = NEGATIVE
  - DOSE: 0,3000,6000 PPM/50 PER GROUP

GENETIC TOXICOLOGY (definition)

- IN VITRO CYTOGENETICS
  - NEGATIVE (CHROMOSOME ABERRATIONS)
  - NEGATIVE (SISTER CHROMATID EXCHANGES)
- MOUSE LYMPHOMA
  - POSITIVE
- MICRONUCLEUS
  - NEGATIVE
- SALMONELLA
  - NEGATIVE

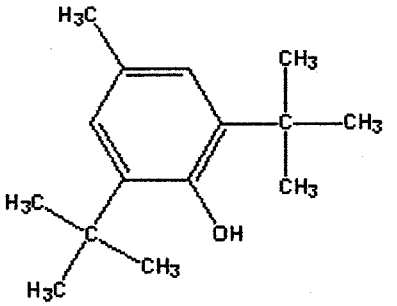
NTP Study Overviews and General Protocols

NTP Home Page

Last Updated 04/12/02

-=-

TR-150
Bioassay of Butylated Hydroxytoluene (BHT) for Possible Carcinogenicity (CAS No. 128-37-0)



Chemical Formula: $C_{15}H_{24}O$ - 3D Structure

The phenolic antioxidant butylated hydroxytoluene (BHT) was patented in 1947 and received approval for use as a food additive and preservative by the Food and Drug Administration (FDA) in 1954. Since 1959, BHT has been generally recognized as safe (GRAS) for use in foods and is one of the most commonly used antioxidants in foods containing fats.

A bioassay of BHT for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and B6C3F_1 mice.

Groups of 50 rats and 50 mice of each sex were administered BHT at one of two doses, either 3,000 or 6,000 ppm; the rats for 105 weeks and the mice for 107 or 108 weeks. Matched controls consisted of 20 untreated rats and 20 untreated mice of each sex. All surviving animals were killed at the end of administration of the test chemical.

Mean body weights of the dosed rats and mice were lower than those of the corresponding controls and were dose related throughout most of the bioassay. Survival was not affected significantly in the dosed groups of rats or mice, and the survival was 60% or greater in all dosed or control groups of rats and mice of each sex at the end of the bioassay. Sufficient number of animals were at risk for the development of late-appearing tumors.

Alveolar/bronchiolar carcinomas or adenomas occurred in the female mice at a significant incidence in the low-dose group (P=0.009) but not in the high dose group, and the incidences were not significantly dose related (control 1/20, low-dose 16/46, high-dose 7/50). Thus, these lung tumors in the female cannot clearly be related to the administration of the BHT. No tumors occurred in either male or female rats at incidences that were significantly higher in dosed groups than in corresponding control groups. Nonneoplastic lesions that may have been related to the administration of the test chemical included focal alveolar histiocytosis at increased incidences in the dosed female rats and various lesions of the liver at

increased incidences in the dosed male mice.

It is concluded that under the conditions of this bioassay, BHT was not carcinogenic for F344 rats or $B6C3F_1$ mice.

Levels of Evidence of Carcinogenicity:

Male Rats: **Negative**
Female Rats: **Negative**
Male Mice: **Negative**
Female Mice: **Negative**

Synonyms: 2,6-di-tert-butyl-p-cresol, BHT

Target Organs from 2-year Studies

Report Date: 1979

NTIS# PB29-8539/AS

Return to Long Term Abstracts

-=-

National Toxicology Program

Good Science for Good Decisions

Target Organs and Levels of Evidence
NTP Technical Report Number 150

Produced from Chemtrack Database 09/19/01

CHEMICAL/ CAS NUMBER	PEER REVIEW DATE	PRIMARY USES	ROUTE/EXPOSURE LEVELS	STUDY LABORATORY
BUTYLATED HYDROXYTOLUENE **128-37-0**	12/13/78	ANTIOXIDANT FOR PETROLEUM AND FOOD PRODUCTS; ANIMAL FEED; FOOD PACKAGING	Dosed-Feed 0,3000,6000 PPM/50 PER GROUP	Frederick Cancer Research Facility

LEVELS OF EVIDENCE OF CARCINOGENICITY--ORGAN/TISSUE (NEOPLASM):

MR: NEGATIVE
FR: NEGATIVE
MM: NEGATIVE
FM: NEGATIVE

NTP Home Page

-=-

genital herpes - Cognitive Enhancement Research Institute

genital herpes - Cognitive Enhancement Research Institute

Carcinogenicity

BHT has been shown to have both carcinogenic and anti-carcinogenic activity in animal studies. Several experimental approaches have been investigated, varying from massive single injections of BHT to chronic daily exposure.

In experiments with BHT alone, the incidence of cancer seems either to be unaffected or much reduced (Clegg 1965, Hirose 1981, Shirai 1982), especially in cancer prone animals. An early study demonstrating carcinogenic activity of BHT could not be replicated by subsequent studies, the erroneous result being attriuted to the presence of aflatoxin contamination of the animal feed.

In animal experiments with BHT and known carcinogens, BHT acts as both a promoter and antipromoter of carcinogenesis, depending upon the experimental conditions. Carcinogenesis is usually decreased when the BHT is administered prior to or concurrent with the carcinogen (Ulland 1973, Goodman 1976, Clapp 1979, McCay 1980, King 1981, Williams 1983). When BHT is administered after carcinogenic exposure, the incidence of cancer is frequently increased (Peraino 1977, Witschi 1981, Imaida 1982, Williams 1983). The increases are greatest with hepatically metabolized carcinogens where large single doses of BHT are administered post-exposure. Compared to phenobarbital, another enzyme inducer, BHT is a "weak enhancer" and then "only at near-toxic doses" (Maeura 1984).

Because BHT is both a promoter and anti-promoter, the type of carcinogens that people are exposed to will determine whether its net effect is to increase or decrease incidence of cancer. Because cruciferous vegetables (cabbage, cauliflower, brussels sprouts, broccoli) are known to induce liver enzymes similarly to BHT -- and also lower general cancer incidence epidemiologically -- we can infer that BHT is likely to lower net cancer risks.

In the Soviet Union during the 50s and 60s, BHT (called ionol by the Soviets) was extensively studied as an anti-tumor compound (Emanuel 1963, 1973) culminating in its approval as a treatment for bladder cancer.

BHT and Clotting

BHT also causes a lowering of prothrombin index in rats at doses as low as 0.017% of diet after one week (Takahashi 1978). This effect also attenuates with time. After four weeks, prothrombin index was lowered only at 0.25% and 0.50% BHT. At levels of 1.0-1.5%, dietary BHT causes hemorrhagic death in male rats (Takahashi 1981, 1978b, 1976b). This effect may be due to inhibition of phylloquinone epoxide reductase (Takahashi 1981c) which allows accumulation of a prothrombin precursor in the microsomes of treated rats. The hemorrhagic effect is completely blocked by phylloquinone (Vitamin K) or phylloquinone oxide (Suzuki 1979, Takahashi 1979).

There has been no published data concerning this effect in man or monkey, but we have received numerous anecdotal reports of delayed clotting in humans. It is possible that supplemental Vitamin K may prove of benefit. Special caution is indicated with concurrent use of other substances with anti-coagulant activity, like aspirin or EPA (eicosapentaenoic acid), especially during the titration phase of BHT therapy.

Steven Wm. Fowkes (fowkes@ceri.win.net)
voice: 415-321-CERI -- Cognitive Enhancement Research Institute
fax: 415-323-3864
PO Box 4029, Menlo Park, CA 94026 USA

From: wduffer@aol.com (WDuffer)
Subject: BHT = Life preservative
Date: 25 Oct 1994

Interesting stuff, BHT and BHA. Not only super anti-oxidants, but apparently anti-carcinagins. Here are some excerpts from todays NY Times.

New York Times 25 October, 1994 Science Times Section

By The Associated Press Two widely used food preservatives increased levels of a natural cancer fighter in laboratory animals and appear to do the same in humans, a researcher says. Advocates of natural foods have long objected to the use of preservatives, but Dr. Andrew Dannenberg of Cornell Medical College found that the preservatives BHA and BHT "revved up" the gene for an enzyme that helps destroy carcinogens before they lead to tumors. When the genes are cranked up, they produce more of the enzyme, providing better protection against cancer-causing substances in the environment, Dr. Dannenberg reported last month at the International Conference on Cancer Prevention at Rockefeller University in New York. The gene produces an enzyme called UDP-glucuronosyltransferase, or UGT. The study found elevated levels of the enzyme in the liver, kidneys and small intestines of rats fed higher doses of BHA and BHT than are normally found in foods, Dr. Dannenberg said. He then found preliminary evidence that the substances do the same thing in humans. Dr. Dannenberg said he had also found that sulforaphane, an anti-cancer agent recently isolated in broccoli, exerts its action partly in the same way, by energizing the gene for UGT.

-- end--

In 1968, in an AMA magazine (Today's Health?) it was reported that in a toxicology test of BHT, the lab mice were living 30% longer on a 1% of diet by weight of BHT over the controls.

Will Duff

Order | BHT page 1 | page 3 | page 4 | Lysine | colloidal-silver pg 1

Home
Amino Acids | Antibiotics | Arthritis | Asthma/Allergies | Brain/Mental | Breast Health | Cancer | Carpal Tunnel | Colds/Flu | Cold Sores | Diabetes | Digestive | Eczema/Psoriasis | Enzymes | Fibromyalgia/Chronic Fatigue | Growth Hormone Releasers | Headaches/Migraines | Heart | Herbs | Hormones | Immune | Liver | Minerals | Mood Enhancement | Multi-Formulas | Muscular | Pain | Pill Box | Prostate | Protein | Services | Sex Drive | Skin | Sleep | Tendonitis | Urinary | Vitamins | Weight Loss | Yeast Infections

View Shopping Cart

SmartBodyz Nutrition
1051 West Seventh, Suite 325
Fort Worth, Texas 76102
Toll Free: 187SmartBodyz (1-877-627-8263)
Telephone: 817-335-1982
Fax: 817-870-9018
Email: webmaster@smartbodyz.com

SEARCH HERE for anything in our stores

Miscellaneous Links

BUTYLATED HYDROXYTOLUENE (BHT)

First draft prepared by
Ms Elizabeth Vavasour
Toxicological Evaluation Division, Bureau of Chemical Safety
Food Directorate, Health Protection Branch, Health Canada
Ottawa, Ontario, Canada

1. EXPLANATION

Butylated hydroxytoluene (BHT) was previously evaluated by the Committee at its sixth, eighth, ninth, seventeenth, twentieth, twenty-fourth, twenty-seventh, thirtieth, and thirty-seventh meetings (Annex 1, references 6, 8, 11, 32, 41, 53, 62, 73, and 94). At the thirty-seventh meeting, the temporary ADI of 0 - 0.125 mg/kg bw, established at the thirtieth meeting, was extended pending the results of a study designed to elucidate the role of hepatic changes in the development of hepatic carcinomas observed in Wistar rats following *in utero* and lifetime exposure to BHT.

The results of the above study were reviewed at the present meeting. In addition, new data relating to the previously-noted effects of BHT on the lung, liver, kidney, clotting mechanisms and promotion/inhibition of carcinogenesis, new long-term and reproductive toxicity studies, genotoxicity assays and human observations were reviewed.

The following consolidated monograph is a compilation of studies from the previous monographs and monograph addenda and those reviewed for the first time at the present meeting.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution, and excretion

2.1.1.1 Mice

A single oral dose of ^{14}C-BHT given to male and female mice resulted in rapid absorption and distribution of ^{14}C to the tissues. Excretion of ^{14}C was mainly in the faeces (41-65%) and urine (26-50%), with lesser amounts in expired air (6-9%). The half-life for a single dose in the major tissues studied (stomach, intestines, liver, and kidney) was 9-11 h. When daily doses were given for 10 days, the half-life for ^{14}C in the tissues examined was 5-15 days. Metabolism was characterized by oxidation at one or both of the *tert*-butyl groups, followed by formation of the glucuronide conjugate, and excretion in the urine, or by excretion of the free acid in the faeces. More than 43 metabolites were present in the urine and faeces of mice (Matsuo *et al.*, 1984).

2.1.1.2 Rats

When ^{14}C-BHT was administered to rats, 80-90% of the ^{14}C was excreted in the urine and faeces within 96 h, and less than 0.3% of the ^{14}C was in expired air. Most of the ^{14}C-BHT was excreted as the free acid in faeces with lesser amounts in the urine. More than 43 metabolites were present in the urine and faeces of rats (Matsuo *et al.*, 1984).

Male F344 rats were fed BHA/BHT mixtures at levels of 0/0, 0.5/0.05, 1.0/0.1, or 2.0/0.2% in the diet and the levels of the compounds were determined in adipose tissue after 1, 2, or 4 months. The BHT levels found in adipose tissue were 1.4, 2.9, or 7.8 mg/kg, respectively, in the dosed animals. On an equivalent dose basis, BHT accumulated to ten times the level of BHA. However, neither showed any progressive accumulation with time. Considering the rat adipose tissue data, the mean intake of BHT by humans and the corresponding level of BHT found in adipose tissue from 6 humans (0.12 mg/kg), previous observations that accumulation of BHT in the adipose tissue on a dose/body weight basis is greater in humans than in rats were confirmed (Conacher *et al.*, 1986).

Groups of male and female rats were maintained on diets containing 0 or 0.5% BHT for a period of 35 days, and then for a period on diets free of BHT. During the period on the test diets, groups of rats were killed at 5-day intervals and fat and liver removed for BHT analysis. During the period on BHT-free diets, rats were killed at 2-day intervals to measure loss of BHT from the fat and liver. There was no clear evidence of progressive accumulation of BHT in fat during the period of administration of the test compound. BHT levels in the fat reached a maximum level (55 mg/kg in males, 65 mg/kg in females) within 10 days of exposure to BHT. Thereafter there was considerable fluctuation in the observed levels. The levels of BHT in liver were very low, the maximum BHT levels being about 5 mg/kg in males, and 1.5 mg/kg in females. The biological half-life of BHT in fat and liver was estimated to be 7 to 10 days (Gage, 1964).

killed 24 h after the final dose. The range of the total dose accounted for was 92 to 104% in males and 93 to 99% in females. There was an indication of sex difference in the route of excretion, females excreting 19-43% of the radioactivity in urine and males only 3-15%. Eight days after administration of 5 doses, 92% of the radioactivity had been excreted by males and 97% by females. Subcutaneous administration of graded doses of BHT to female rats revealed substantial faecal excretion but the rate of excretion decreased with increasing dose. There was no evidence of accumulation of ^{14}C-BHT in the body under the conditions of repeated oral dosage (Tye *et al.*, 1965).

Rats were given single oral doses (1-100 mg/rat) of ^{14}C-BHT and approximately 80 to 90% of the dose was recovered within four days in the urine and faeces. Of the total radioactivity, 40% and 25% appeared in the urine of females and males, respectively. After 4 days, approximately 3.8% of the dose was retained, mainly in the alimentary tract. A substantial portion of the radioactivity was found in the bile collected from two rats (one male, one female) over a period of 40 h. The relatively slow excretion of BHT is probably attributable to enterohepatic circulation rather than to tissue retention (Daniel & Gage, 1965).

The liver and body fat of rats fed a diet containing 0.5% BHT for 35 days were analyzed. The concentration of BHT in the liver never rose above 5 mg/kg in males and 1.5 mg/kg in females. In the body fat, the level fluctuated around 30 mg/kg in males and 45 mg/kg in females. Fat from rats returned to BHT-free diet showed a progressive fall in the concentration of BHT, the half-life being about 7 to 10 days.

The daily excretion of radioactivity in urine and faeces was studied in rats given an oral dose of ^{14}C-labelled BHT (12 mg/kg bw). Excretion became negligible by the sixth day after administration when about 70% of the injected dose had been recovered. Less than 1% was excreted as carbon dioxide in the expired air. About 50% of the radioactivity was excreted in the bile during the 24-hour period following the oral dose (Daniel & Gage, 1965).

The BHT content of fat and liver of rats given diets containing 0.5 or 1% BHT for periods up to 35 and 50 days, respectively, were analyzed. With 0.5% BHT in the diet, a level of approximately 30 mg/kg in the fat was reached in males and 45 mg/kg in females, with approximately 1-3 mg/kg in the liver. With 1% BHT, the level in the fat was 50 mg/kg in males and 30 mg/kg in females. On cessation of treatment, the level of BHT in fat fell with a half-life of 7 to 10 days (Daniel & Gage, 1965).

The level of BHT in the fat reached a plateau at approximately 100 mg/kg after 3 to 4 days when daily doses of 500 mg/kg bw were given by intubation. A daily dose of 200 mg/kg bw for one week produced a level of about 50 mg/kg (Gilbert & Golberg, 1965).

Rats were given doses of 100 µg of ^{3}H-labelled BHT intraperitoneally and the urinary output of radioactivity was measured for 4 consecutive days. After 4 days of dosing, 34.5% of the injected radioactivity was recovered in urine (Ladomery *et al.*, 1963). The same dose of BHT (100/µg) labelled with ^{14}C was given to rats, and 34% of the radioactivity was excreted in the urine in the first four days, in close agreement with the previous result using tritiated BHT (Ladomery *et al.*, 1967a).

After a single parenteral dose (100 µg) of ^{14}C-BHT, rats excreted 3235% of the radioactivity in the urine, and 35-37% in the faeces, in a 4-day period. The intestinal contents together with the gut wall contained most of the remaining activity. Biliary excretion was rapid, and the radioactive material in bile was readily absorbed from the gut, suggesting a rapid enterohepatic circulation (Ladomery *et al.*, 1967b).

White male Wistar rats (290-350 g) were administered [^{14}C]-labelled BHT, or its alcohol [BHT-CH_2OH] or its aldehyde [BHT-CHO] or acid [BHT-COOH] derivative by i.v. or i.p. injection. The overall excretion of BHT and its related compounds excreted in urine and faeces was studied for a 5-day period, and biliary excretion monitored for 120426 h after i.p. injection. For the low doses of the compounds tested (100 µg), there were no significant differences in the total recovery of ^{14}C during the 5 days urinary and faecal excretion and 120-126 h biliary excretion. However, there were differences in ratio of urinary to faecal excretion of ^{14}C. The major metabolite present in early bile after i.p. injection of the labelled compounds was BHT-COOH or its ester glucuronide. Late bile after acid hydrolysis showed BHT-COOH to be the major ^{14}C component (Holder *et al.*, 1970a).

Temporal concentration changes in BHT and BHT-quinone methide were studied following a single oral dose of 800 mg BHT/kg bw in rats, which were monitored in plasma, the GI tract, and some adipose tissues over a 48-hour period using GLC. Groups of 4 or 5 male Sprague-Dawley rats were sacrificed at 0.5, 1, 3, 6, 12, 18, 24, 30, 36 or 48 h after administration of BHT. The amount of BHT in the gastric contents stayed constant for 12 h, then declined rapidly to 24 h, followed by a gradual decline to 48 h. BHT was not detected in the plasma at any of the time points, while BHT-quinone methide showed a rapid rise between 12 and 18 h after administration, followed by a gradual decline to 48 h. BHT was detected in epididymal, subcutaneous, perirenal and brown dorsal adipose tissue soon after administration. A peak concentration was noted in epididymal and subcutaneous fat at 18 h, but did not show this trend in perirenal or brown dorsal fat.

In a second experiment, in which groups of 5 or 6 rats were sacrificed 4-7 or 24-27 h after administration of BHT, a single oral dose of 800 mg/kg bw resulted in a significant increase in the weight of stomach contents in both fasted and non-fasted rats at 4-7 h. By 24-27 h after administration, there was no difference in the weight of stomach contents between non-fasted treated animals and controls. This study suggested that high single doses of BHT caused a delay in gastric emptying which was reflected in the delay in plasma levels of metabolite.

In another experiment, 800 mg/kg bw BHT was instilled intraduodenally to 5 anaesthetized rats and 30 minutes later the concentrations of BHT and its metabolites were measured in blood from the portal vein and descending aorta, and in liver and epididymal adipose tissue. BHT and/or BHT radical was detected in all but the descending aorta, while BHT-quinone methide was not present in any of the samples collected at 30 minutes after administration (Takahashi, 1990).

2.1.1.3 Chickens

One-day old chicks were given ^{14}C-BHT at a level of 200 mg/kg in the feed for 10 weeks. At broiler age, edible portions had residues amounting to 1-3 mg/kg of BHT and metabolites. Similar diets given to laying hens produced residues in eggs of 2 mg/kg after 7 days, the level thereafter remaining constant (Frawley *et al.*, 1965a).

When feed containing 500 or 100 mg/kg BHT was given to laying hens, residues of 20 and <5 mg/kg were found in the fat fraction of eggs, respectively. In the broiler chicken, over a period of 21 weeks,

BUTYLATED HYDROXYTOLUENE (BHT)

First draft prepared by
Ms Elizabeth Vavasour
Toxicological Evaluation Division, Bureau of Chemical Safety
Food Directorate, Health Protection Branch, Health Canada
Ottawa, Ontario, Canada

1. EXPLANATION

Butylated hydroxytoluene (BHT) was previously evaluated by the Committee at its sixth, eighth, ninth, seventeenth, twentieth, twenty-fourth, twenty-seventh, thirtieth, and thirty-seventh meetings (Annex 1, references 6, 8, 11, 32, 41, 53, 62, 73, and 94). At the thirty-seventh meeting, the temporary ADI of 0 - 0.125 mg/kg bw, established at the thirtieth meeting, was extended pending the results of a study designed to elucidate the role of hepatic changes in the development of hepatic carcinomas observed in Wistar rats following *in utero* and lifetime exposure to BHT.

The results of the above study were reviewed at the present meeting. In addition, new data relating to the previously-noted effects of BHT on the lung, liver, kidney, clotting mechanisms and promotion/inhibition of carcinogenesis, new long-term and reproductive toxicity studies, genotoxicity assays and human observations were reviewed.

The following consolidated monograph is a compilation of studies from the previous monographs and monograph addenda and those reviewed for the first time at the present meeting.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution, and excretion

2.1.1.1 Mice

A single oral dose of ^{14}C-BHT given to male and female mice resulted in rapid absorption and distribution of ^{14}C to the tissues. Excretion of ^{14}C was mainly in the faeces (41-65%) and urine (26-50%), with lesser amounts in expired air (6-9%). The half-life for a single dose in the major tissues studied (stomach, intestines, liver, and kidney) was 9-11 h. When daily doses were given for 10 days, the half-life for ^{14}C in the tissues examined was 5-15 days. Metabolism was characterized by oxidation at one or both of the *tert*-butyl groups, followed by formation of the glucuronide conjugate, and excretion in the urine, or by excretion of the free acid in the faeces. More than 43 metabolites were present in the urine and faeces of mice (Matsuo *et al.*, 1984).

2.1.1.2 Rats

When ^{14}C-BHT was administered to rats, 80-90% of the ^{14}C was excreted in the urine and faeces within 96 h, and less than 0.3% of the ^{14}C was in expired air. Most of the ^{14}C-BHT was excreted as the free acid in faeces with lesser amounts in the urine. More than 43 metabolites were present in the urine and faeces of rats (Matsuo *et al.*, 1984).

Male F344 rats were fed BHA/BHT mixtures at levels of 0/0, 0.5/0.05, 1.0/0.1, or 2.0/0.2% in the diet and the levels of the compounds were determined in adipose tissue after 1, 2, or 4 months. The BHT levels found in adipose tissue were 1.4, 2.9, or 7.8 mg/kg, respectively, in the dosed animals. On an equivalent dose basis, BHT accumulated to ten times the level of BHA. However, neither showed any progressive accumulation with time. Considering the rat adipose tissue data, the mean intake of BHT by humans and the corresponding level of BHT found in adipose tissue from 6 humans (0.12 mg/kg), previous observations that accumulation of BHT in the adipose tissue on a dose/body weight basis is greater in humans than in rats were confirmed (Conacher *et al.*, 1986).

Groups of male and female rats were maintained on diets containing 0 or 0.5% BHT for a period of 35 days, and then for a period on diets free of BHT. During the period on the test diets, groups of rats were killed at 5-day intervals and fat and liver removed for BHT analysis. During the period on BHT-free diets, rats were killed at 2-day intervals to measure loss of BHT from the fat and liver. There was no clear evidence of progressive accumulation of BHT in fat during the period of administration of the test compound. BHT levels in the fat reached a maximum level (55 mg/kg in males, 65 mg/kg in females) within 10 days of exposure to BHT. Thereafter there was considerable fluctuation in the observed levels. The levels of BHT in liver were very low, the maximum BHT levels being about 5 mg/kg in males, and 1.5 mg/kg in females. The biological half-life of BHT in fat and liver was estimated to be 7 to 10 days (Gage, 1964).

doses of 44 mg/kg bw BHT on alternate days and rats in each group were killed 24 h after the final dose. The range of the total dose accounted for was 92 to 104% in males and 93 to 99% in females. There was an indication of sex difference in the route of excretion, females excreting 19-43% of the radioactivity in urine and males only 3-15%. Eight days after administration of 5 doses, 92% of the radioactivity had been excreted by males and 97% by females. Subcutaneous administration of graded doses of BHT to female rats revealed substantial faecal excretion but the rate of excretion decreased with increasing dose. There was no evidence of accumulation of ^{14}C-BHT in the body under the conditions of repeated oral dosage (Tye *et al.*, 1965).

Rats were given single oral doses (1-100 mg/rat) of ^{14}C-BHT and approximately 80 to 90% of the dose was recovered within four days in the urine and faeces. Of the total radioactivity, 40% and 25% appeared in the urine of females and males, respectively. After 4 days, approximately 3.8% of the dose was retained, mainly in the alimentary tract. A substantial portion of the radioactivity was found in the bile collected from two rats (one male, one female) over a period of 40 h. The relatively slow excretion of BHT is probably attributable to enterohepatic circulation rather than to tissue retention (Daniel & Gage, 1965).

The liver and body fat of rats fed a diet containing 0.5% BHT for 35 days were analyzed. The concentration of BHT in the liver never rose above 5 mg/kg in males and 1.5 mg/kg in females. In the body fat, the level fluctuated around 30 mg/kg in males and 45 mg/kg in females. Fat from rats returned to BHT-free diet showed a progressive fall in the concentration of BHT, the half-life being about 7 to 10 days.

The daily excretion of radioactivity in urine and faeces was studied in rats given an oral dose of ^{14}C-labelled BHT (12 mg/kg bw). Excretion became negligible by the sixth day after administration when about 70% of the injected dose had been recovered. Less than 1% was excreted as carbon dioxide in the expired air. About 50% of the radioactivity was excreted in the bile during the 24-hour period following the oral dose (Daniel & Gage, 1965).

The BHT content of fat and liver of rats given diets containing 0.5 or 1% BHT for periods up to 35 and 50 days, respectively, were analyzed. With 0.5% BHT in the diet, a level of approximately 30 mg/kg in the fat was reached in males and 45 mg/kg in females, with approximately 1-3 mg/kg in the liver. With 1% BHT, the level in the fat was 50 mg/kg in males and 30 mg/kg in females. On cessation of treatment, the level of BHT in fat fell with a half-life of 7 to 10 days (Daniel & Gage, 1965).

The level of BHT in the fat reached a plateau at approximately 100 mg/kg after 3 to 4 days when daily doses of 500 mg/kg bw were given by intubation. A daily dose of 200 mg/kg bw for one week produced a level of about 50 mg/kg (Gilbert & Golberg, 1965).

Rats were given doses of 100 µg of ^{3}H-labelled BHT intraperitoneally and the urinary output of radioactivity was measured for 4 consecutive days. After 4 days of dosing, 34.5% of the injected radioactivity was recovered in urine (Ladomery *et al.*, 1963). The same dose of BHT (100/µg) labelled with ^{14}C was given to rats, and 34% of the radioactivity was excreted in the urine in the first four days, in close agreement with the previous result using tritiated BHT (Ladomery *et al.*, 1967a).

After a single parenteral dose (100 µg) of ^{14}C-BHT, rats excreted 3235% of the radioactivity in the urine, and 35-37% in the faeces, in a 4-day period. The intestinal contents together with the gut wall contained most of the remaining activity. Biliary excretion was rapid, and the radioactive material in bile was readily absorbed from the gut, suggesting a rapid enterohepatic circulation (Ladomery *et al.*, 1967b).

White male Wistar rats (290-350 g) were administered [^{14}C]-labelled BHT, or its alcohol [BHT-CH_2OH] or its aldehyde [BHT-CHO] or acid [BHT-COOH] derivative by i.v. or i.p. injection. The overall excretion of BHT and its related compounds excreted in urine and faeces was studied for a 5-day period, and biliary excretion monitored for 120426 h after i.p. injection. For the low doses of the compounds tested (100 µg), there were no significant differences in the total recovery of ^{14}C during the 5 days urinary and faecal excretion and 120-126 h biliary excretion. However, there were differences in ratio of urinary to faecal excretion of ^{14}C. The major metabolite present in early bile after i.p. injection of the labelled compounds was BHT-COOH or its ester glucuronide. Late bile after acid hydrolysis showed BHT-COOH to be the major ^{14}C component (Holder *et al.*, 1970a).

Temporal concentration changes in BHT and BHT-quinone methide were studied following a single oral dose of 800 mg BHT/kg bw in rats, which were monitored in plasma, the GI tract, and some adipose tissues over a 48-hour period using GLC. Groups of 4 or 5 male Sprague-Dawley rats were sacrificed at 0.5, 1, 3, 6, 12, 18, 24, 30, 36 or 48 h after administration of BHT. The amount of BHT in the gastric contents stayed constant for 12 h, then declined rapidly to 24 h, followed by a gradual decline to 48 h. BHT was not detected in the plasma at any of the time points, while BHT-quinone methide showed a rapid rise between 12 and 18 h after administration, followed by a gradual decline to 48 h. BHT was detected in epididymal, subcutaneous, perirenal and brown dorsal adipose tissue soon after administration. A peak concentration was noted in epididymal and subcutaneous fat at 18 h, but did not show this trend in perirenal or brown dorsal fat.

In a second experiment, in which groups of 5 or 6 rats were sacrificed 4-7 or 24-27 h after administration of BHT, a single oral dose of 800 mg/kg bw resulted in a significant increase in the weight of stomach contents in both fasted and non-fasted rats at 4-7 h. By 24-27 h after administration, there was no difference in the weight of stomach contents between non-fasted treated animals and controls. This study suggested that high single doses of BHT caused a delay in gastric emptying which was reflected in the delay in plasma levels of metabolite.

In another experiment, 800 mg/kg bw BHT was instilled intraduodenally to 5 anaesthetized rats and 30 minutes later the concentrations of BHT and its metabolites were measured in blood from the portal vein and descending aorta, and in liver and epididymal adipose tissue. BHT and/or BHT radical was detected in all but the descending aorta, while BHT-quinone methide was not present in any of the samples collected at 30 minutes after administration (Takahashi, 1990).

2.1.1.3 Chickens

One-day old chicks were given ^{14}C-BHT at a level of 200 mg/kg in the feed for 10 weeks. At broiler age, edible portions had residues amounting to 1-3 mg/kg of BHT and metabolites. Similar diets given to laying hens produced residues in eggs of 2 mg/kg after 7 days, the level thereafter remaining constant (Frawley *et al.*, 1965a).

When feed containing 500 or 100 mg/kg BHT was given to laying hens, residues of 20 and <5 mg/kg were found in the fat fraction of eggs, respectively. In the broiler chicken, over a period of 21 weeks,

< 5 mg/kg on the 100 mg/kg diet (Van Stratum & Vos, 1965).

2.1.1.4 Humans

Four human male subjects were administered a single dose of approximately 40 mg ^{14}C-labelled BHT. About 75% of the administered radioactivity was excreted in the urine. About 50% of the dose appeared in the urine in the first 24 h, followed by a slower phase which probably represented the release of the compounds or their metabolites stored in tissues. In humans, the bulk of the radioactivity was excreted as the ether insoluble glucuronide of a metabolite in which the ring methyl group and one *tert*-butyl methyl group were oxidized to carboxyl groups, and a methyl group on the other *tert*-butyl group was also oxidized, probably to an aldehyde group. BHT-acid, free and conjugated, was a minor component of the urine and the mercapturic acid was virtually absent. The rapidity of the first phase of the urinary excretion in humans suggested that there was no considerable enterohepatic circulation as had been observed in the rat (Daniel *et al.*, 1967).

Reported values of BHT in body fat were 0.23 ± 0.15 mg/kg (11 individuals, residents of the United Kingdom) and 1.30 ± 0.82 mg/kg (12 individuals, residents of the United States of America) (Collings & Sharratt, 1970).

Based on reported BHT levels in human fat in Japan, United Kingdom and USA and the calculated dietary intakes of BHT, a bioconcentration factor in humans (BCF, wet weight basis) of 0.36 was calculated for BHT. This BCF was 45 times higher than that calculated for the rat. In comparison, the BCF for total DDT was calculated at 1279 (Geyer *et al.*, 1986).

The disposition of single oral doses of BHT was compared in humans and rats. A single oral dose of 0.5 mg/kg bw of BHT was ingested by 7 healthy male volunteers after fasting overnight. Blood samples were taken after 0, 15, 30, 45, 60, 75, 90, 120, 150, 180 and 240 minutes. Total urine and faeces were collected for 2 days. In another experiment, 5 healthy female volunteers ingested 0.25 mg/kg bw of BHA and one week later 0.25 mg/kg bw of BHT. After another week, 0.25 mg/kg bw of BHT plus 0.25 mg/kg bw of BHA were given simultaneously. After each dosing, blood samples were taken as described above. Similar experiments were conducted in male Wistar rats, except that the doses used were 20, 63, or 200 mg/kg bw of BHT. In rats, peak plasma concentrations of BHT (0.2, 0.3, and 2.3 µg/ml) were seen after 2.6 h. Simultaneous administration of BHA produced significantly lower plasma concentrations between 0.5 and 3 h. Large variations were seen in humans in plasma levels of BHT. The mean peak plasma level was 0.09/µg/ml, reached after 1.5 h. The plasma concentrations were not influenced by simultaneous administration of BHA. In the rat, approximately 2% of the dose was excreted as BHT-COOH in the urine (equal amounts of conjugated and unconjugated compound) and 10% as BHT in the faeces in 4 days. In humans, 2.8% of the dose was found in the urine as BHT-COOH (mainly conjugated) and no BHT could be detected in the faeces. On a comparative dose basis, it seems that BHT in plasma reaches a higher level in humans than in rats (Verhagen *et al.*, 1989).

2.1.2 Biotransformation

2.1.2.1 Mice

The oxidative metabolism of BHT by liver microsomes from 3 inbred mouse strains, NGP/N, A/J and MA/MyJ was compared. The strain order shown is the order of increasing susceptibility of these mice to BHT lung tumour promotion which correlates with their increasing ability to produce BHT-BuOH, by hydroxylation of BHT at one of the *tert*-butyl groups. Four weekly i.p. injections of BHT selectively induced the BHT oxidation pathway leading to formation of BHT-BuOH (Thompson *et al.*, 1989).

The metabolism of BHTOOH was examined to assess the role of reactive intermediates in mediating tumour promotion in mouse skin. Incubation of BHTOOH with either isolated neonatal mouse keratinocytes or a cell-free haematin system resulted in the generation of the BHT-phenoxyl radical. Only one non-radical metabolite of BHTOOH-BHT-quinol was detected in keratinocytes, while incubation of BHTOOH with haematin produced several metabolites: oxacyclopentenone, BHT-quinone, BHT, BHT-stilbene quinone, and BHT-quinone methide. In contrast to the action of BHTOOH, topical application of epidermal doses of BHT-quinol, BHT-quinone, BHT-stilbene quinone, as well as BHT itself to mouse skin, did not induce epidermal ornithine decarboxylase activity (Taffe *et al.*, 1989).

Co-administration of BHA (200 mg/kg bw) with a subtoxic dose of BHT (200 mg/kg bw) enhanced the lung toxicity of BHT in male mice. BHA co-administration significantly increased the radioactivity covalently bound to lung macromolecules at 4-8 h after [^{14}C]-BHT. The pretreatment also reduced the rate of *in vitro* metabolism of BHT in mouse liver supernatant. The co-administration of BHA and BHT caused a decrease in metabolism of BHT in the liver with the result that the lung was exposed to a larger amount of BHT (Yamamoto *et al.*, 1988).

2.1.2.2 Rats

Examination of the biliary metabolites from i.v. and i.p. doses of small amounts of ^{14}C-BHT, showed the presence of four principal metabolites. Some 34 to 53% of the ^{14}C-label in the bile was identified as 3-5-di- t-butyl-4-hydroxy-benzoic acid, which was probably present as the ester glucuronide. The other metabolites present were 3,5-di- *t*-butyl-4-hydroxybenzaldehyde, 3-5-di- *t*-butyl-4-hydroxybenzyl alcohol, and 1,2-bis (3,5-di- t-butyl-4-hydroxyphenyl) ethane (Ladomery *et al.*, 1967b).

Rats were given a single dose of ^{14}C-BHT and urine and bile collected for periods ranging from 48 to 96 h. Faeces were also collected during this same period. About 19% to 59% of the radioactivity appeared in the urine during this period, and 26% to 36% in the bile. The major metabolites in the urine were 3,5-di- *tert*-butyl-4-hydroxybenzoic acid, both free (9% of the dose), as well as glucuronide (15%) and S-(3,5-di- *tert*-butyl-4-hydroxybenzyl)-N-acetylcysteine. The ester glucuronide and mercaptic acid were also the major metabolites in rat bile. Free 3,5-di- *tert*-butyl-4-hydroxybenzoic acid was the major metabolite in faeces (Daniel *et al.*, 1968).

Male Wistar rats were dosed intraperitoneally with 200 mg/kg bw BHT-acid or 2,6-di- *tert*-butyl phenol (DBP). Faeces and urine were collected for 5 days after administration of BHT-acid and for 3 days after administration of DBP. Bile was collected from rats treated with BHT-acid for 24 h after administration. Following administration of BHT-acid, the metabolites DBP, 2,6-di- *tert*-butyl- *p*-benzoquinone (BBQ), 2,6-di- *tert*-butylhydroquinone (BHQ) glucuronide and BHT-acid glucuronide were identified in the faeces and bile. This suggested that BHT-acid, considered as a main metabolic end-product of BHT, was metabolized to the quinone and hydroquinone following its decarboxylation to form the di-substituted phenol, DBP. An alternative route for formation of the quinone, BBQ, was a homolysis reaction of the O-O bond of the hydroperoxide which is catalyzed by rat liver cytochrome P-450 (Yamamoto *et al.*, 1991).

Rabbits were given single or repeated doses of BHT in the range of 400-800 mg/kg bw. About 16% of the dose was excreted as ester glucuronide and 19% as ether glucuronide. Unconjugated phenol (8%), ethereal sulfate (8%) and a glycine conjugate (2%) were also excreted. Excretion of all detectable metabolites was essentially complete 3 to 4 days after administration of the compound and about 54% of the dose was accounted for as identified metabolites (Dacre, 1961).

The metabolism of butylated hydroxytoluene (BHT) orally administered to rabbits in single doses of 500 mg/kg bw was studied. The metabolites 2,6-di- *tert*-butyl-4-hydroxymethylphenol (BHT-alcohol), 3,5-di- *tert*-butyl-4-hydroxy-benzoic acid (BHT-acid) and 4,4'-ethylene- *bis*-(2,6-di-tert-butylphenol) were identified. The urinary metabolites of BHT comprised 37.5% as glucuronides, 16.7% as ethereal sulfates and 6.8% as free phenols; unchanged BHT was present only in the faeces (Akagi & Aoki, 1962a); 3,5-di- *tert*-butyl-4-hydroxybenzaldehyde (BHT-aldehyde) was also isolated from rabbit urine (Aoki, 1962). The main metabolic pathway was confirmed by administering BHT-alcohol to rabbits and isolating BHT-aldehyde, BHT-acid, the ethylene- *bis* derivative and unchanged BHT-alcohol in the urine (Akagi & Aoki, 1962b).

2.1.2.4 Ruminants

When [^{14}C]BHT was activated *in vitro* by the prostaglandin H synthase system in microsomes from ram seminal vesicles or by horseradish peroxidase, significant covalent binding to protein could be detected. BHT-quinone methide was detected at only minor concentrations, therefore an intermediate free radical was suggested as an active metabolite. Addition of BHA to the medium greatly increased the formation of BHT-quinone methide and covalent binding to proteins (Thompson *et al.*, 1986).

2.1.2.5 Humans

A group of 8 men each received 100 mg of BHT on two occasions separated by a 4-day interval. Urine was collected for 24 h after BHT administration. The metabolites were identified as BHT-COOH and benzoyl-glycine. In another study in which two adults were given 1.0 g of BHT, BHT-COOH and its ester glucuronide were the only major metabolites identified in urine (Holder *et al.*, 1970b).

2.1.2.6 Combined species

The metabolism of BHT was studied with liver and lung microsomes from rats and mice. Two main metabolic processes occurred, hydroxylation of alkyl substituents and oxidation of the aromatic 1-4 electron system. The former led to the 4-hydroxymethyl product (BHT-CH_2OH) and a primary alcohol resulting from hydroxylation of a *t*-butyl group (BHT- *t*BuOH). Additional metabolites were produced by oxidation of BHT-CH_2OH to the corresponding benzaldehyde and benzoic acid derivatives. Hydroxylation of BHT- tBuOH occurred at the benzylic methyl position, and the resulting diol was oxidized further to the hydroxybenzaldehyde derivative. Oxidation of the 1-4 system led to BHT-quinol (2,6-di- *t*-butyl-4-hydroxy-4-methyl-2,5-cyclohexadienone), BHT-quinone (2,6-di- *t*-butyl-4-benzoquinone), and BHT-quinone methide (2,6-di- *t*-butyl-4-methylene-2,5-cyclohexadienone) probably via the hydroperoxide (BHTOOH). Derivatives of the quinol and quinone with a hydroxylated *t*-butyl group were also formed. Quantitative data demonstrated that BHT-CH_2OH was the principal metabolite in rat liver and lung microsomes. The mouse produced large amounts of both BHT-CH_2OH and BHT- tBuOH in these tissues. The metabolite profile was similar in rat liver and lung. Mouse lung, however, produced more quinone relative to other metabolites than mouse liver (Thompson *et al.*, 1987).

The *in vitro* peroxidase-catalyzed covalent binding of BHT to microsomal protein and the formation of BHT-quinone methide was enhanced by addition of BHA. Several other phenolic compounds commonly used in food also enhanced the metabolic activation of BHT. Microsomes from lung, bladder, kidney medulla and small intestine of various animal species, including humans, were also able to support this interaction of BHA and BHT using either hydrogen peroxide or arachidonic acid as the substrate (Annex 1, reference 95).

Phenobarbital (PB) pretreatment of Sprague-Dawley rats and A/J mice had little or no effect on respective quinone methide formation from BHT or BHT-BuOH in pulmonary microsome preparations, but resulted in a 6-37 fold induction of this activity in hepatic microsomes from these species. PB administration had little effect on the two-step oxidation of BHT to QM-OH in pulmonary microsomes of the mouse, while in hepatic microsomes, PB pre-treatment resulted in a greater than 100-fold increase in this activity. This was found to be mainly due to a greater than 100-fold increase in the initial *tert*-butyl hydroxylation step in mouse liver microsomes. The enhancement was somewhat higher than for 7-pentoxyresorufin O-dealkylase activity, demonstrating that *tert*-butyl hydroxylation could serve as a specific marker for the enzyme. The results also showed that pulmonary microsomes from mice, but not rats, had a relatively high constitutive P-450 activity for *tert*-butyl hydroxylation of BHT, supporting the proposal that this metabolite was involved in BHT-induced pneumotoxicity. Two cytochrome P-450 inhibitors, SKF 525-A and metapyrone, inhibited the conversions of BHT to QM and QM-OH to a similar extent in PB-treated mouse liver microsomes; the terpenoid alcohol cedrol was found to selectively inhibit BHT conversion to QM-OH. This compound has been found previously to inhibit pneumotoxicity when administered to mice prior to treatment with BHT. In untreated mouse liver microsomes, BHT hydroxylation to BHT-MeOH showed the greatest activity, with oxidation to QM second and hydroxylation to BHT-BuOH last (Bolton & Thompson, 1991).

2.1.3 Effects on enzymes and other biochemical parameters

2.1.3.1 Mice

Mice (BALB/c strain) were maintained on a diet containing 7.5 g BHT/kg of feed. After 3 weeks on the test diet, there was an enhanced activity in plasma esterases which persisted throughout the experimental period of 20 weeks. Following electrophoretic separation of the esterases, the increased enzyme activity was shown to be located in two specific bands (Tyndall *et al.*, 1975).

Dietary administration of BHT to male Swiss Webster mice resulted in a marked increase in hepatic microsomal epoxide hydrolase and glutathione-S-transferase (Hammock & Ota, 1983).

2.1.3.2 Rats

Feeding experiments were carried out on 45 pairs of weanling male rats for 5 to 8 weeks with diets containing 0, 10 or 20% lard supplements to which 0.01, 1 or 5 g BHT/kg had been added. At 0.01 g/kg, no changes were observed in any of the serum constituents studied, while at 5 g/kg an increase in the serum cholesterol level was seen within 5 weeks. Female rats fed for 8 months a diet containing a 10% lard supplement with 1 g BHT/kg showed increased serum cholesterol levels, but no other significant changes. Diets containing 5 g BHT/kg in 10% and 20% lard supplements fed to female rats for the same period increased serum cholesterol, phospholipid and mucoprotein levels (Day *et al.*, 1959).

In further work with rats, it was found that increased output of urinary ascorbic acid accompanied liver enlargement induced by BHA or BHT in onset, degree and duration, being rapid but transient with BHA, and slower in onset but more prolonged with BHT (Gaunt *et al.*, 1965a). The simultaneous stimulation of processing enzyme activity, increase in urinary ascorbic acid output, and increase in relative liver weight brought about by BHT was unaffected by 14 days of dietary restriction, and all these changes except liver weight were reversible during 14 days' recovery on normal diet (Gaunt *et al.*, 1965b).

Rats given BHT by daily intubation showed increased activity of some liver microsomal enzymes. Stimulation of enzyme activity correlated with an increase in relative liver weight. The threshold dose for these changes in enzyme activity in female rats was below 25-75 mg BHT/kg bw/day. The storage of BHT in fat appeared to be influenced by the activity of the processing enzymes. In rats given 500 mg/kg bw/day, the level of BHT in fat attained values of 230 mg/kg in females and 162 mg/kg in males by the second day, by which time the relative liver weight and processing enzyme activities had become elevated. Thereafter, liver weight and enzyme activity continued to rise but the BHT content of fat fell to a plateau of about 100 mg/kg in both sexes (Gilbert & Golberg, 1965).

Groups of 12 SPF Carworth rats equally divided by sex were administered BHT dissolved in arachis oil daily for one week, at dose levels of 50, 100, 200 or 500 mg/kg bw. A group of 8 rats served as control. The animals were killed 24 h after the final dose, and histological and biochemical studies (glucose-6-phosphatase: and glucose-6-phosphate dehydrogenase) made on the livers of all animals. A histochemical assessment of the livers of test animals was also carried out. BHT caused an increase in liver weight in males at dose levels of 100 mg/kg bw and greater, and in females at 200 mg/kg bw and greater. BHT caused a decrease in glucose-6-phosphatase activity in females at dose levels greater than 100 mg/kg bw, and an increase in glucose-6-phosphate dehydrogenase in both males and females at the highest dose tested. In another study, rats were dosed according to the above schedule and then maintained for 14 to 28 days following the final dosing. By day 28, no biochemical changes were observed, and relative liver weights returned to normal by day 14 (Feuer *et al.*, 1965).

Rats fed diets containing BHT at levels of 100 to 5000 mg/kg for 12 days showed liver enlargement, as well as increased activity of liver microsomal biphenyl-4-hydroxylase, at all levels except the lowest level of 100 mg/kg. Enzyme activity was not significantly altered at 5000 mg/kg BHT fed for one day (Creaven *et al.*, 1966).

Rats (male and female Carworth Farm SPF) were given an oral dose of BHT equivalent to 500 mg/kg bw. Dosing was from 1 to 5 days, and rats varying in size from 100-400 g body weight were used. Microsomal preparations from the livers of treated rats were assayed for BHT oxidase, an enzyme that metabolizes BHT to the BHT alcohol (2,6-di- *tert*-butyl-4-methylphenol to 2,6-di- *tert*-butyl-4-hydroxy methylphenol). Treatment of female rats with BHT (500 mg/kg bw/day for 5 days) caused a six fold increase in the activity of the enzyme/gram of liver and a 35% increase in relative liver weight, both being prevented by actinomycin D. The induction was more pronounced in males than in females, and the induction of the enzyme, low in rats in the 100 g body-weight range, reached a maximum in rats in the 200 g body-weight range, and fell in larger animals (300-400 g range) (Gilbert & Golberg, 1967).

Groups of female Alderly Park SPF rats were maintained on diets containing 0%, 0.01%, 0.1%, 1% or 5% BHT for periods up to 28 days, and then on diets free of BHT for 56 days. Animals were killed in groups of four, 2 being used for enzyme assay (aminopyrene demethylase) and 2 for electron microscopy. The increase in enzyme activity was directly related to the dietary level of BHT. No detectable increase was observed at the lowest level over the 28-day feeding period. Following withdrawal of BHT from the diet, the enzyme level returned to normal in all test animals. The degree of endoplasmic reticulum proliferation was proportional to the amount of BHT in the diet and the duration of feeding at the 5% and 1% level. At the 0.1% level, there was a transient rise in smooth endoplasmic reticulum. No proliferation was observed at the 0.01% level. Following removal of BHT from the diet, there was a rapid disappearance of the proliferated smooth endoplasmic reticulum.

In a second study, groups of rats were fed diets containing 1% BHT for 10 days, and then for a second period of 10 days after an interval of 20 days on a normal diet. The animals were killed in groups of five at 10, 30, 40, 42 or 47 days. Livers were removed for aminopyrene demethylase assay and electron microscopy. Enzyme activity did not differ significantly following both 10-day periods of administration of BHT. Electron microscopy showed similar smooth endoplasmic reticulum response during both these periods (Botham *et al.*, 1970).

Microsomal preparations from livers of rats, dosed daily with 450 mg/kg bw BHT for up to 7 days, showed an increased capacity to incorporate labelled amino acids, when compared to preparations from controls. BHT also stimulated the *in vivo* incorporation of amino acids, mainly into the proteins of the endoplasmic reticulum (Nievel, 1969).

A group of 23 female SPF rats (Wistar strain) was administered 500 mg/kg BHT dissolved in rape-seed oil, for 11 days. The control group was administered rape-seed oil alone. Groups of 7 rats were killed following administration of the final dosing. The remaining rats were maintained without further exposure to BHT, and killed on days 28 or 63 of the study. Livers of the rats were examined for weight, DNA content and number of cell nuclei. Treatment with BHT resulted in enlargement of the liver, with a concomitant increase in its DNA content, and in the number and ploidy of its nuclei. The liver mass returned to normal within two weeks. However, the DNA content of the liver of BHT-treated animals remained elevated up to the time of termination of this study, and there was no reduction in the total number of nuclei or the degree of ploidy (Hermann *et al.*, 1971).

Two groups of 10 male and 10 female rats (Alderly Park, SPF Wistar strain) were dosed by stomach tube with 200 mg/kg bw/day of BHT dissolved in maize oil for 7 days. Four rats/sex dosed with an equivalent amount of maize oil served as controls. Urinary ascorbic acid excretion was measured in urine samples collected following 5 days on the test compound. The animals were killed 24 h after the final dose and the livers removed for biochemical assays (aminopyrine demethylase-AMPM, hexobarbitone oxidase-HO, cytochrome P-450, and glucose-6-phosphatase), and electron microscopy. Another group of treated rats was maintained for a 7-day recovery period, and a similar battery of liver studies was carried out. Administration of BHT resulted in an increase of urinary excretion of ascorbic acid which remained constant throughout the treatment period. Following cessation of BHT treatment there was a gradual return towards control values. There were significant sex differences in some of the biochemical responses to BHT, with the exception of the glucose-6-phosphatase activity. Female rats showed a marked increase in APDM and HO activity, which was not observed in male rats. Cytochrome P-450 levels were increased in both males and females. The biochemical parameters, with the exception of APDM activity in female rats, returned to normal following the 7-day recovery period. Electron microscopy showed

hepatic cells. No other morphological changes were detected (Burrows *et al.*, 1972).

BHT (500 mg/kg bw/day) was administered by gavage to groups of young Wistar male and female rats for 7 days and the animals were housed in metabolism cages. Control animals received corn oil vehicle only. They were then sacrificed and liver enzymes (aniline-4-hydroxylase, biphenyl-4-hydroxylase, ethyl morphine N-demethylase, and 4-methyl umbelliferone glucuronyl transferase) were assayed and the cytochrome P-450-CO interaction spectrum evaluated. Urinalysis using GC was conducted to assay for D-glucaric acid, D-glucuronic acid, l-gulonic acid, xylitol and L-ascorbic acid. Administration of BHT enhanced all the parameters measured with the exception of the hepatic microsomal protein content. BHT was a more potent inducer of xenobiotic metabolism in female rats (Lake *et al.*, 1976).

BHT in the diet of Sprague-Dawley rats resulted in a marked decrease in the NADPH-cytochrome P450 reductase activity of isolated liver microsomal preparations. This effect was not observed when BHT was added *in vitro* to liver microsomes (Rikans *et al.*, 1981).

Dietary BHT was also shown to affect the carboxylation process in the conversion of rat liver microsomal protein to prothrombin (Takahashi & Hiraga, 1981a).

Rats fed a diet containing 0.4% BHT showed an increase in GSH-S transferase activity in the liver, but not in lungs and kidneys. GSH-reductase levels were increased in liver and lungs (Partridge *et al.*, 1982).

BHT at 300-6000 mg/kg in the diet caused a dose-dependent increase in gamma-glutamyl transpeptidase in normal F344 male rats. However, cytosolic glutathione S-transferase was only enhanced at dietary concentrations of 3000 or 6000 mg/kg (Furukawa *et al.*, 1984).

Groups of 4 male F344 rats were pretreated with buthionine sulfoximine, a glutathione-depleting agent (900 mg/kg bw), and after 1 h given intraperitoneal injections of BHT (100, 250, 400, or 500 mg/kg bw). A dose-related elevation of serum GOT and GPT activities was observed. BHT or buthionine sulfoximine alone had no effect. In contrast, pretreatment with cysteine (100-200 mg/kg bw) inhibited the elevation of serum enzyme activities at a toxic dose of BHT (1000 mg/kg bw) (Nakagawa, 1987).

Supplementation of AAF-containing diets with 0.3% BHT, which affords protection against AAF hepatocarcinogenesis in high-fat fed Sprague-Dawley rats, protected and/or induced total hepatic nuclear envelope cytochrome P-450 content. Short-term feeding with AAF without BHT resulted in a marked loss of total hepatic nuclear envelope P-450 (Carubelli & McCay, 1987). Immunological studies showed that BHT enhanced the AAF-dependent induction of P-450c, but not P-450d. BHT by itself had no effect on these nuclear envelope enzymes (Friedman *et al.*, 1989).

Administration of 0.5% BHT in the diet of male Wistar rats for 2 weeks increased UDP-glucuronosyl transferase activity in liver microsomes for several substrates to 236-269% of controls. The amounts of UDP-glucuronosyl transferase protein and associated mRNA in liver microsomes were also increased, paralleling the increases in enzyme activity. In addition to induction of hepatic activity, BHT treatment resulted in increased activity in microsomes from the kidney and small intestine (Kashfi *et al.*, 1994).

2.1.3.3 Rabbits

Acute effects on electrolyte excretion, similar to those described for large doses of BHA were also obtained following administration of BHT at doses of 500-700 mg/kg bw. No such effects were observed at lower dosage levels (Denz & Llaurado, 1957).

2.1.3.4 Monkeys

Groups of 2-4 juvenile rhesus monkeys *(Macaca mulatta)* were fed BHT dissolved in corn oil at dose levels of 0, 50, or 500 mg/kg bw for 4 weeks. Blood samples were taken prior to treatment and then at weekly intervals from the control and test animals in the high-dose group, and from test animals in the low-dose group at the end of the 4-week period, for determination of total plasma cholesterol, lipid phosphorus and triglyceride. Liver biopsies were taken from the test animals in the high group at two weeks. At the end of the test period all animals were fasted for 24 h and sacrificed, and liver and blood samples obtained. Liver samples were analyzed for succinic dehydrogenase and susceptibility to peroxidation. Extracted liver lipids were analyzed for total cholesterol, lipid phosphorus and triglycerides. Total cholesterol levels in plasma and liver were significantly lowered. Lipid phosphorus levels in the plasma were increased at the high-dose level, as were cholesterol:lipid phosphorus ratios in the plasma and liver. The susceptibility of liver lipids to oxidation was reduced in the high-dose group (Branen *et al.*, 1973).

2.2 Toxicological studies

2.2.1 Acute toxicity studies

The results of acute toxicity studies with BHT are summarized in Table 1.

Acute oral, intraperitoneal (mice) and eye irritation (rabbits) and skin irritation (rats) were measured for 7 breakdown products of BHT. All compounds tested were less toxic than the parent compound (Conning *et al.*, 1969).

Table 1. Acute toxicity studies with BHT

Animal	Route	LD_{50} (mg/kg bw)	Approximate lethal dose (mg/kg bw)	Reference
Rat	oral	> 1700-1970	-	Deichmann et al., 1955
Cat	oral	-	940-2100	Deichmann et al., 1955
Rabbit	oral	-	2100-3200	Deichmann et al., 1955
Guinea-pig	oral	-	10 700	Deichmann et al., 1955
Rat	oral	2450		Karplyuk, 1959
Mouse	oral	2000		Karplyuk, 1959

As shown in Table 2, the LD_{50} (i.p.) for BHT showed considerable differences for strains of inbred and non-inbred male mice. In all cases death occurred 4 to 6 days after administration of BHT, and was accompanied by massive edema and haemorrhage in the lung (Kawano *et al.*, 1981).

Table 2. Variation in LD_{50} with strains of mice (Kawano et al., 1981)

DBA/2N (inbred)	138
BALB/cNnN (inbred)	1739
C57BL/6N (inbred)	917
ICR-JCL (non-inbred)	1243

2.2.2 Short-term toxicity studies

2.2.2.1 Mice

In order to estimate the MTD for BHT in mice, groups of 5/sex $B6C3F_1$ mice, 6-week old, were fed diets containing 0, 3100, 6200, 12 500, 25 000 or 50 000 mg BHT/kg of feed for 7 weeks. Each animal was weighed twice weekly. Gross necropsy was performed on all animals. One female mouse in the 25 000 mg/kg group, and 1 male and 4 female mice in the 50 000 mg/kg group died before the end of the study.

Body-weight decrements, mostly dose-related, were noted in all treatment groups compared with controls. Histopathologic examination of male mice receiving 25 000 mg/kg revealed a very small amount of centrilobular cytoplasmic vacuolization of hepatocytes which was not observed in females receiving 12 500 mg/kg (high-dose females were not examined) (NCI, 1979).

A similar study was conducted to establish the dose levels for a subsequent carcinogenicity study. Groups of $B6C3F_1$ mice (10/sex) received BHT in the diet at concentrations of 0.25%, 0.5%, 1%, 2%, or 4% for 10 weeks. Twenty mice/sex were used for the control group. Both male and female mice receiving the highest dose of BHT experienced a retardation of body-weight gain which exceeded 10% of control values. In addition, histopathological examination of mice in the 4% group, revealed marked starvation atrophy of the spleen, heart and kidneys. None of these changes were noted in mice at the next lower dose level (2% diet) or any of the other groups. The MTD was considered to be 2% diet (Inai *et al.*, 1988).

Groups of male and female C_3H mice (17-39 mice/group), 6-10 weeks old, were maintained for 10 months on a semi-synthetic diet containing 0.05 or 0.5% BHT. Control groups were maintained on BHT-free semi-synthetic diet or commercial lab chow. At the end of the test period, the liver and lungs were excised and inspected grossly for proliferative lesions. Of the proliferative lesions considered to be clearly identifiable as tumours, approximately 50% were examined microscopically. Mice maintained on diets containing BHT had lower body weights than controls. Male mice fed BHT showed an increase in liver tumours, compared to controls. Histologically, the tumours were identified as hepatocellular adenomas. No increase was observed in female mice. The reported incidence of liver tumours in male C_3H mice was 38% (10/26) in the 0.5% BHT group, 58% (15/26) in the 0.05% BHT group, 5% (2/37) in the control semi-synthetic diet group, and 18% (7/38) in the control lab chow group.

In a study in which C_3H mice were maintained on diets containing 0.5% BHT for one month followed by lab chow for 10 months, or control diet (BHT-free) for one month followed by lab chow for 10 months, the incidence of liver tumours in the two groups of male mice were 9% (3/35) and 17% (5/29), respectively. Dietary BHT did not result in an increased incidence of lung tumours in either male or female mice.

In another study in which male BALB/c mice were maintained for one year on a BHT-free diet, or diets containing 0.05% BHT or 0.5% BHT, the incidence of liver tumours were 13% (4/30), 14% (6/43), and 7% (2/28) for the respective groups (Lindenschmidt *et al.*, 1986).

It has previously been demonstrated that the incidence of spontaneously-occurring hepatic tumours in C_3H mice is modified by sex, population density, level of dietary protein, and caloric intake. Historical dam were not available for a 10-month study. The incidence of hepatic tumours in a 12-month study in this strain ranged from 6-13% for females, and 41-68% for males (Peraino *et al.*, 1973). Thus, the reported incidence of hepatocellular tumours was not significantly different from other controls of similar age in studies with the same inbred strain.

2.2.2.2 Rats

Feeding experiments conducted for 20 or 90 days indicated that rats did not find food containing respectively 0.5 or 1% BHT palatable. However, the animals ingested food so treated more readily if these concentrations were attained gradually. Paired feeding experiments with groups of 5 or 10 rats for 25 days demonstrated that diets containing 0.8 and 1% BHT reduced the daily intake of food below control values. A level of 1% in the diet retarded weight gain (Deichmann, 1955).

BHT (0.3%) in the diet of pregnant rats that had been kept for 5 weeks on a diet deficient in vitamin E produced no toxic symptoms, while 1.6% caused drastic loss of weight and fetal death (Ames *et al.*, 1956).

BHT was fed to rats (12/group) for 7 weeks at a level of 0.1% BHT in diets containing 20% or 10% lard supplement. With the 20% lard supplement diet, significant reduction of the initial growth rate and mature weight of male rats was observed. No effect was noted in female or male rats receiving the 10% lard supplement diet. A paired feeding experiment showed that this inhibition of growth was a direct toxic effect of BHT and could not be explained by a reduction in the palatability of the diet. At this level BHT produced a significant increase in the weight of the liver, both absolute and relative to body weight. Rats under increased stress showed significant loss of hair from the top of the head. The toxic effect of BHT was greater if the fat load in the diet was increased. Anophthalmia occurred in 10% of the litters (Brown *et al.*, 1959).

BHT administered to rats at 250 mg/kg bw/day for 68 to 82 days caused fatty infiltration of the liver and reduction in body-weight gain (Karplyuk, 1959).

Groups of 6 weanling rats (3/sex) were fed diets containing a 20% lard supplement and BHT at levels of 0, 0.1, 0.2, 0.3, 0.4 or 0.5% for 6 weeks. BHT reduced the growth rate, especially in males, the effect becoming significant at 0.3% BHT. It also increased the absolute liver weight and the ratio of liver weight to body weight in both sexes, the latter effect becoming significant at 0.2% BHT. BHT increased the ratio of left adrenal weight to body weight in male rats but had no consistent effect in females. There were no histological changes in the adrenal attributable to treatment. All dietary levels of BHT increased the serum cholesterol and the concentration of cholesterol was directly proportional to the BHT level. There was also a significant increase in the concentration of adrenal cholesterol. BHT produced no significant changes in the concentration of total or percentage esterified liver cholesterol, total liver lipid or concentration of total polyunsaturated fatty acids in the liver (Johnson & Hewgill, 1961).

Rats fed diets supplemented with 20% lard, and containing 0, 0.2,

serum cholesterol that was directly related to the level of dietary BHT. BHT increased the relative weight of the male adrenal and also caused a significantly greater decrease in growth rate of male as compared to the female. Increased liver weight in test animals was paralleled by increased absolute lipid content of the liver (Johnson & Hewgill, 1961).

In another study, rats were maintained on diets containing 0.5% dietary BHT in the presence or absence of a 20% lard supplement. BHT increased the basic metabolic rate, the concentration of body cholesterol and the rate of synthesis of body and liver cholesterol, and reduced the total fatty acid content of the body, irrespective of the presence or absence of dietary lard. In the animals fed BHT without lard, BHT increased the rate of synthesis and turnover of body and liver fatty acids and reduced the growth rate. These effect occurred to a greater extent in animals fed BHT with lard (Johnson & Holdsworth, 1968).

Groups of 8 young rats were fed diets containing 19.9% casein and 0, 0.02 or 0.2% BHT for 8 weeks. The experiment was repeated with 16.6% casein in the diet of further groups for 4 weeks and again with 9.6% casein (and no added choline) for 7 weeks. In all three instances BHT caused stimulation of growth and improved protein efficiency. The nitrogen content of the liver was, however, greatly reduced in BHT-treated animals, except when the level of BHT was reduced to 0.02%. Recovery of hepatic protein after fasting (details not given) was also impaired in rats on 0.2% BHT. Liver lipid content was increased at 0.2%, but not at 0.02% BHT. A dietary level of 0.2% BHT also increased the adrenal weight and ascorbic acid content, although if recalculated on the basis of weight of gland, there was no significant difference. The increase in adrenal ascorbic acid was interpreted as indicating a stress imposed on the organism by BHT (Sporn & Schöbesch, 1961).

Groups of 20 male and 20 female rats fed 1% BHT in the diet for 10 weeks showed recovery both in liver to body weight ratios and in morphological appearance of the liver cells within a few weeks after restoring the animals to a normal diet (Goater *et al.*, 1964).

Groups of 48 weanling rats (24/sex) were given diets containing 0 or 0.1% BHT for periods of up to 16 weeks. Measurements of growth rate, food consumption, weight and micropathological examination of organs at autopsy revealed no difference between treated and control groups. However, increase in relative liver weight and in the weight of the adrenals was produced without histopathological evidence of damage. Biochemical measurements and histochemical assessments of liver glucose phosphatase and glucose 6-phosphate dehydrogenase activities revealed no difference from the control group (Gaunt *et al.*, 1965a).

Groups of rats (16/sex) were fed diets containing 20% fat and BHT levels of 0, 0, 0.03, 0.1 or 0.3% BHT for 10 weeks. No definite effect on body weight was observed at any level in females and there was only a slight depression in males at the 0.3% level. There was no significant effect on blood cholesterol level in either sex after feeding BHT at any of the levels. Four males at the 0.3% and two at the 0.1% level died during the experiment. Two deaths occurred among females at 0.3%. Only one male rat died in both control groups (Frawley *et al.*, 1965b).

In order to determine the MTD for BHT in rats, groups of F344 rats (5/sex) were given diets containing 0, 6200, 12 500, 25 000 or 50 000 mg BHT/kg diet for 7 weeks. Body weights were determined twice weekly. Gross necropsy was performed on all animals in the study. All of the male and female rats in the 50 000 mg/kg group died before the end of the study. With the exception of one male in the 12 500 mg/kg group, all of the animals in the other treatment groups and control groups survived to the end of the study. Body weights at week 7 of the study showed a dose-related decrement, with animals in the 25000 mg/kg group weighing only 38% to 44% of control values. At dietary levels of 12 500 mg/kg, there was a slight increase in haematopoiesis in both sexes (NCI, 1979).

2.2.2.3 Dogs

Mild to moderately severe diarrhoea was induced in a group of 4 dogs fed BHT at doses of 1.4-4.7 g/kg bw every 2 to 4 days for 4 weeks. Postmortem examination revealed no significant gross pathological changes. No signs of intoxication and no gross or histopathological changes were observed in dogs fed doses of 0.17-0.94 g/kg bw, 5 days/week for a 12-month period (Deichmann *et al.*, 1955).

2.2.2.4 Monkeys

Groups of 3 infant or juvenile monkeys *(Macaca mulatta)* received BHT at doses of 0, 50 or 500 mg/kg bw/day for 4 weeks. Blood analysis (complete cell count, serum sodium and potassium, bilirubin, cholesterol and GOT) was carried out weekly, as was a complete urinalysis. Liver biopsies were taken from the juvenile monkey at 2 weeks, following a 24-h fast. At the end of the test period, all animals were fasted 24 h and sacrificed. Tissues from all major organs were prepared for light and electron microscopy. Liver tissue was also analyzed for protein, RNA and cytochrome P-450. Microsomal preparations prepared from the livers were used to measure nitroanisole demethylase and glucose-6-phosphatase activity. Urine and blood values of test and control animals were similar. Histological evaluation of all organs other than the liver from either infant or juvenile monkeys did not indicate any compound-related changes. Test animals receiving BHT showed hepatocytomegaly and enlargement of hepatic cell nuclei. The hepatocytes of treated animals showed moderate proliferation of the endoplasmic reticulum. Lipid droplets were also prominent in cytoplasm of these hepatic cells. There was fragmentation of the nucleolus in 15% of the hepatic cells in the test animals in the high-dose group. DNA, RNA and cytochrome P-450 levels in the liver of test and control animals were similar. BHT-treated juveniles showed an increase in nitroanisole demethylase activity which increased with time. The enzyme activity was unaffected in infant monkeys. Glucose-6-phosphatase activity declined in juvenile monkeys but was unchanged in infant monkeys (Allen & Engblom, 1972).

2.2.3 Long-term toxicity/carcinogenicity studies

2.2.3.1 Mice

Groups of 60 FAF male mice were maintained on semi-synthetic diets containing 0, 0.25 or 0.5% BHT. The mean life-span of the test animals was significantly greater than controls, being 17.0 ± 5.0 and 20.9 ± 4.7 months respectively for the 0.25% and 0.5% BHT, as compared to 14.5 ± 4.6 months for controls (Harman, 1968).

A group of 18, 8-week old male BALB/C mice fed BHT at a level of 0.75% for a period of 12 months, developed marked hyperplasia of the hepatic bile ducts with an associated subacute cholangitis (Clapp *et al.*, 1973).

Eleven mice (BALB/C strain) were maintained on a diet containing 0.75% BHT for a period of 16 months. The incidence of lung tumours in the test group was 63.6%, compared with 24% in controls (Clapp *et al.*, 1974). However, a repeat of this study using a larger group of test animals, showed that BHT had no effect on the incidence of

Groups of 48 mice (CFI strain) equally divided by sex were maintained on diets containing 1000 mg/kg BHT. At week 4, one group was then fed a diet containing 2500 mg/kg BHT, and at week 8, another group was fed a diet containing 5000 mg/kg BHT. The animals were maintained on these diets until 100 weeks of age. There was no statistically significant reduction in survival of animals on the BHT diet, although survival was poorer in males at the high-dose level during the last quarter of the study. Animals dying or sacrificed during the course of the study showed greater centrilobular cytomegaly and karyomegaly than controls. Bile duct hyperplasia was only observed in 3/141 test animals. There was no significant difference in the incidence of malignant tumours in the high-dose group and control. However, there was an increased incidence of lung neoplasia in treated mice (75%, 74%, 53% and 47% in the 5000, 2500, 1000 mg/kg and control groups, respectively). There were no morphological features to distinguish the lung tumours in treated mice from those in controls. There was also an apparent increase in benign ovarian tumours in BHT-treated female mice, since none were observed in control animals (Brooks *et al.*, 1976).

BHT was administered in the diet at levels of 0, 3000, or 6000 mg/kg to groups of 20 (control) or 50 (treated) male and female $B6C3F_1$ mice for 107108 weeks. The mice were observed twice daily for signs of ill-health. Physical examinations were performed each month and body weights were recorded at least once a month. Gross and microscopic examination of 28 major organs, tissues and gross lesions including the liver, thyroid and forestomach, was performed on all animals at the end of the study and all animals dying on test where possible. Peripheral blood smears were made for all animals where possible. The body weights of treated male and female mice were lower than the control mice throughout the study [numerical data not provided]. The magnitude of the body-weight decrements was dose-related. Administration of BHT in the diet resulted in similar or improved survival in the treated groups compared with controls. At termination, survival in male mice was 60%, 86% and 92% and in females 85%, 82% and 90%, for the control, low-and high-dose groups, respectively. There was a marked dose-related increase in the incidence of hepatocytomegaly and non-neoplastic lesions of the liver (peliosis, hepatocellular degeneration/necrosis and cytoplasmic vacuolation) in males but not in females. The incidence of hepatocellular adenoma or carcinoma was not significantly increased in either treated male or female mice, although there was a small increase in the incidence of combined adenomas and carcinomas in the treated females (1/20, 4/46 and 5/49 for the control, low- and high-dose females, respectively) which was not statistically significant. The historical control incidence for hepatocellular neoplasms was not provided. The incidence of alveolar/bronchiolar carcinomas or adenomas in female mice was significantly higher than controls (5%) in the low dose (35%), but not the high dose (14%). The historical control incidence was 4.7% for alveolar/bronchiolar adenomas or carcinomas. Chronic ingestion of BHT in the diet was related to a significant reduction in the incidence of sarcomas of multiple organs in female mice. Four adenomas of the eye/lacrimal gland were observed in high-dose males (8%) and in 2 low-dose females but not in corresponding controls. The historical incidence of this tumour in male mice was 1.2%. Since the lacrimal gland was only examined microscopically in animals with grossly apparent lesions, the report states that the lacrimal gland tumours could not be clearly related to BHT administration (NCI, 1979).

Groups of $B6C3F_1$ mice (100/sex), were fed diets containing 0, 200, 1000 or 5000 mg/kg BHT for 96 weeks, followed by a basal diet for 8 weeks. At the end of the test period the surviving animals were killed. A complete autopsy was carried out, and the principal organs and tissues were examined microscopically. Mice that died during the course of the study were also autopsied. In addition, terminal blood samples were collected for haematological examination and serum clinical biochemistry. Urine samples were also examined. During the course of the study, food consumption was similar for test and control groups. Body weights of females in the 1000 and 5000 mg/kg groups were lower than controls, as was the body weight of males in the 5000 mg/kg group. There were minor changes in the absolute weight of some organs in the high-dose groups (salivary glands, heart and kidney). In males, the serum GOT and GPT levels in the 5000 mg/kg group were higher than controls. No other compound-related effects were observed in the haematological, serum and urine analysis. Neoplastic lesions were reported in both test and control animals. The tumours that occurred with greatest frequency were adenomas of the lungs, hyperplastic nodules and hepatocellular carcinomas of the liver and malignant lymphomas. However, there was no statistically significant difference between the BHT-treated and control groups for the incidence of any type of tumour (Shirai *et al.*, 1982).

BHT was administered in the diet at concentrations of 0, 1% or 2% to groups of $B6C3F_1$ mice (50/sex) for 104 weeks. After the treatment period, all the surviving mice were given basal diet for an additional 16 weeks. Treated animals underwent a 16-week recovery period prior to pathological examination. Mean body weights of the treated male and female mice were lower than those of controls. The body-weight decrements were dose-related in both sexes and were more marked in female mice. Treatment with BHT was found to improve survival in a dose-related manner in both males and females. At the end of treatment (104 weeks), the percent survival for male/female mice was 40%/58% (control), 64%/81% (1% diet) and 74%/89% (2% diet). In male mice administered BHT, there was a statistically significant increase in the incidence of either a hepatocellular adenoma (19%, 38% and 53% in control, low- and high-dose groups, respectively) or a focus of hepatocellular alteration, showing a clear dose-response relationship. No increases in the number of female mice with hepatocellular adenomas or foci of altered hepatocytes were noted. The incidence of male mice with other tumours and the incidence of female mice with any tumour were not significantly increased as a consequence of BHT administration. There was a dose-related tendency to lower incidence of lymphoma and leukemia in both males and females (Inai *et al.*, 1988).

2.2.3.2 Rats

Groups of rats (15/sex) given diets containing 1% lard and 0.2, 0.5 or 0.8% BHT for 24 months showed no specific signs of intoxication, and micropathological studies were negative. For the group given a diet containing 0.5% BHT, the BHT was dissolved in lard and then heated for 30 minutes at 150°C before incorporation in the diet. There were no effects on weight gain or blood constituents and micropathological studies of the main organs were negative. The feeding of 0.8% BHT was followed in both male and female rats by a subnormal weight gain and by an increase in the weight of the brain and liver and some other organs in relation to body weight. Micropathological studies were negative in this group also. BHT had no specific effect on the number of erythrocytes and leucocytes, or on the concentration of haemoglobin in the peripheral blood. A number of rats of both sexes died during this experiment, but the fatalities were not treatment-related. Micropathological studies supported this observation. At 0.5%, BHT had no effect on the rat reproductive cycle, the histology of the spleen, kidney, liver and skin, or on the weight of the heart, spleen or kidney. There was no significant increase in

coconut oil for a period of two years (Deichmann *et al.*, 1955).

Groups of JCL strain rats (20/sex/group), reared under a barrier system and 4 weeks of age at the start of the study, received BHT at 0, 0.005, 0.062 or 0.32% in the diet. Of each group of 40, 15 received compound for a "lifetime", 10 for 24 months, and 5 each for 3, 6 or 12 months. At the interim and final kills, liver, kidney, heart, spleen, thyroid and caecum weight were determined as were haematology, serum biochemistry, urinalysis, and histological investigation of the tissues. At 24 months, heart, liver, kidney, spleen, pituitary, thyroid, adrenal, testes, prostate and brain were weighed, haematological and biochemical measurements conducted, and histopathology done. There was an increase in liver weight, serum cholesterol, serum K^+ and histological changes in liver and kidney at the 0.32% dietary level. There was no change in quantity of food intake, body-weight gain, mortality during feeding or mean life span and no finding suspicious of tumour induction. There was no indication of a dose-related trend in tumour prevalence in either 24-month or "lifetime" groups. The tumours found were said to be typical of those described in aged rats. It is to be noted that the number of surviving rats was small and that tumour data included both lifetime groups and animals dead or sacrificed moribund during the 6, 12 and 24-month feeding. The available data do not list the number of each of the individual tissues examined, although the number of rats is listed. The data show a tendency for a decreased number of tumours per rat at higher BHT levels (Hiraga, 1978).

Groups of Fischer 344 rats (50/sex) received 3000 mg/kg or 6000 mg/ kg BHT in the diet for 105 weeks. The compound was mixed with autoclaved lab meal containing 4% fat. A control group of 20 animals/sex received lab meal only. The animals were observed twice daily for signs of ill-health. Physical examinations were performed each month and body weights were recorded at least once a month. Gross and microscopic examination of 28 major organs, tissues and gross lesions including the liver, kidney, thyroid and forestomach, were performed on all animals at the end of the study and all animals dying on test where possible. Peripheral blood smears were made for all animals where possible. There was a dose-related decrease in body weights of treated male and female rats throughout the study. There was no significant effect of BHT on mortality and no difference in the incidence of various neoplasms between treated and control groups. The incidence of adenomas of the pituitary was significantly reduced in female rats with BHT administration. The incidence of focal alveolar histiocytosis was elevated in treated male and female rats in a dose-related manner compared with controls. The effect was more pronounced in females than in males (NCI, 1979).

Groups of 7-week old Wistar rats (57/sex/group) were maintained on diets containing 0.25 or 1% of BHT for 104 weeks. Control groups consisted of 36 rats/sex. At the end of the test period, the surviving animals were killed and a complete autopsy was carried out and the weights of liver, spleen and kidneys were taken. The principal organs and tissues were examined microscopically. Terminal blood samples were collected for haematological examination and serum clinical biochemistry. Survival in test groups was between 40% and 68%. A significant increase in the mortality of the high-dose males was noted after week 96 of the study. Food intake was similar for test and control animals, but body-weight gain was significantly reduced in high-dose males up to week 60 and in high-dose females for most of the study. Increases in the mean absolute and relative liver weights were observed in all treated animals, and decreases in the absolute and relative spleen weights were observed in the treated females. Dose-related changes in serum triglyceride (reduction) and GGT (increase) in treated males and in total blood cholesterol (increase) in treated females were noted. No significant morphological changes were observed in the liver which were attributable to BHT treatment. A variety of tumours were noted on histopathological examination at the end of the study, with no dose-related response in either type of tumour or total number as compared to controls. The incidence of hyperplastic nodules in the liver and of pancreatic carcinomas in female rats and of pituitary adenomas and adeno-carcinomas in both sexes of test animals was higher than in controls. However, with the exception of the incidence of pituitary adenomas in the low-dose females, these differences were not significantly different from controls. Since this effect was not dose-related, it was concluded that BHT, under the conditions of this test. was not carcinogenic (Shibata *et al.*, 1979; Hirose *et al.*, 1981).

Groups of 60, 40, 40, or 60 Wistar rats of each sex (F_0 generation) were fed BHT in the diet at doses of 0, 25, 100, or 500 mg/kg bw/day, respectively. The F_0 rats were mated after 13 weeks of dosing. The F_1 groups consisted of 100, 80, 80, and 100 F_1 rats, respectively, of each sex from the offspring from each group. Because of an adverse effect on the kidney in the parents, the concentration of BHT in the highest dose group was lowered to 250 mg/kg bw/day in the F_1 generation. The study was terminated when rats in the F_1 generation were 144 weeks of age. Parameters studied were food consumption (weekly), body weight, appearance, and mortality. Autopsy and complete histopathological examinations were performed on all animals dying during the study, or sacrificed *in extremis* or at termination.

All animals consuming BHT experienced a dose-related increase in survival. In both sexes differences ($p < 0.001$) in longevity were seen. The average body weights of the F_1 pups at birth in the mid- and high-dose groups were slightly lower than those in the control group. Body weights of all dosed animals from weaning through the entire experiment were lower than those of control animals. In the low-, mid-, and high-dose groups, the reductions in body weight were for males/females 7%/5%, 11%/10%, and 21%/16%. Food intake was comparable for all groups. Clinical appearance and behaviour were reported to be normal for all animals. The high-dose males voided a slightly reddish urine. Haematological parameters were reported to be unchanged by BHT treatment, but no data were given. Serum triglycerides were reduced in both sexes and cholesterol was somewhat elevated in females only.

Histological studies indicated an increase in hepatocellular carcinomas in male rats and an increase in hepatocellular adenomas in both male and female rats. Most liver tumours were found during terminal sacrifice at 141-144 weeks. One hepatocellular carcinoma was found in a control male at 117 weeks and one in a high-dose male at 132 weeks. The remainder of the carcinomas occurred at terminal sacrifice. The first adenoma was noted in a high-dose male at 115 weeks. Tables 3 and 4 summarize the data on mortality and the appearance of adenomas and carcinomas of the liver. Data on mortality and tumour incidence in different groups were analyzed using the procedure of Peto *et al.* (1980). The dose-related increases in the number of hepatocellular adenomas were statistically significant (at $p < 0.05$) in male F_1 rats, when all groups were tested for heterogenicity or analysis of trend. The increase in hepatocellular adenomas and carcinomas in treated female F_1 rats was statistically significant only for adenomas (at $p < 0.05$) in the analysis for trend. Reports on the spontaneous incidence of hepatocellular neoplasms in Wistar rats from the laboratory performing this study, as well as

3% (Solleveld *et al.*, 1984; Deerberg *et al.*, 1980; Olsen *et al.*, 1984). The median life-span for animals in these studies ranged from 28-36 months for males and 28-33 months for females. Other sites reported to have a slight but not statistically significant increase in neoplastic lesions were as follows: thyroid, pancreas, ovary, uterus, thymus, reticuloendothelium system, and mammary gland.

Non-neoplastic lesions occurred incidentally and showed no relationship to BHT treatment, with the exception of lesions of the liver, which showed a dose-related increased incidence of bile duct proliferation and cysts in males, and focal cellular enlargement in females. At the highest dose (250 mg/kg bw/day), there was no adverse effect on the kidney (Olsen *et al.*, 1986).

A long-term study was initiated in order to investigate the role of hepatic changes in the development of hepatocellular carcinomas in rats following *in utero*/lifetime exposure to BHT. The dosing regimen of the study and strain of rat used were similar to those in the two-generation study conducted by Olsen *et al.* (1986). Groups of 6 male and 48 female Wistar rats, aged 13 weeks and 9 weeks, respectively, were fed BHT in the diet at doses of 0, 25, 100 or 500 mg/kg bw/day for 3 weeks prior to mating (13 weeks in Olsen study). The rats were then mated on a 1:8 ratio for up to 21 days. When pregnancy had been established by abdominal palpation, dams were removed to individual cages. On day 20 of gestation, 5 pregnant rats were sacrificed for assessment of body and liver weights and liver histopathology. The pups were delivered by Caesarian section and retained for assessment of a number of parameters. The remaining females (20 dams in the control and high-dose groups; 24 dams in the low- and mid-dose groups) were allowed to deliver normally. On day 6 *post-partum*, litters were either culled or augmented to comprise 8 pups, at the same time maximizing the number of robust males in each litter. At weaning (3 weeks), 4 pups from each of 5 litters per group were selected randomly, maximizing the numbers of males, for assessment of a number of parameters. The male pups from the remaining litters (approximately 60/group) were selected to continue in the study and were placed in one of 4 groups corresponding to the diets fed to their parents, with the exception that the high dose was reduced to 250 mg/kg bw/day as in the Olsen study. Interim kills were conducted at 1, 6, 11, or 16 months. The study was terminated 22 months after the F_1 male rats were placed on test diets. All animals were observed daily throughout the study for clinical or behavioural signs of toxicity. F_0 females were palpated from pregnancy to weaning and F_1 males were palpated from 15 months to termination.

Body weights were recorded approximately every 2 weeks and food consumption monitored every few days. Animals dying during the study or at scheduled sacrifices were subjected to gross necropsy. At each of the scheduled sacrifices the following parameters were measured in all groups of F_1 fetuses and weanlings and F_1 male rats: body and liver weights; hepatic enzyme activities for glucose-6-phosphatase, epoxide hydrolase, glutathione-S-transferase, ethoxyresorufin O-deethylase and pentoxyresorufin O-depentylase; hepatic content of total cytochrome P-450, total glutathione, total microsomal and cytosolic protein; and histopathological changes in the liver. In addition, immunochemical staining of liver sections for cytochrome P-450 1A and 2B and epoxide hydrolase was performed in the control and high-dose groups at all scheduled sacrifices. Cellular proliferation in the liver was measured in 5 animals per dose group at 4 weeks and all subsequent sacrifices using pulse labelling techniques with bromodeoxyuridine (only control and high-dose results were reported). At the 11, 16 and 22-month sacrifices, a number of parameters in addition to those listed above were monitored: histopathological changes in the adrenal, kidney and thyroid; assessment of distribution of hepatic glucose-6-phosphatase and gamma-glutamyl transferase by histochemical staining; and serum thyroxine concentration (presented for 16 and 22 months only).

In the first 5 weeks of BHT administration, a reduction in body-weight gain was noted in the high-dose males. This trend started prior to BHT administration and continued after BHT administration was initiated. Body-weight gain in all other treatment groups was similar to that in controls. No treatment-related effects on the health of the animals were noted. During pregnancy and lactation, there was no difference in food consumption between treated and control female rats, and body weights of the dams were similar at weaning. At the sacrifice on day 20 of gestation, both absolute and relative liver weights of the dams were increased in a dose-related manner, statistically significant at the high dose. The body weights of the females, both including and excluding their litters, were similar in all groups. Histopathological examination of the liver revealed mild enlargement of centrilobular hepatocytes and eosinophilia in 4/5 high-dose (500 mg/kg bw/day) animals, and 1/5 low-dose (25 mg/kg bw/day) animals, consistent with induction of mixed function oxidase activity. A decrease in the mitotic index of hepatocytes from dams receiving 100 and 500 mg/kg bw/day was noted; the significance of this result was unclear.

Table 3. Mortality (and combined adenomas and carcinomas of the liver) in F_1 rats (Olsen et al., 1986)

BHT (mg/kg bw/day)	Effective number of rats	Number of deaths during weeks[a]							
		0-90	91-104	105-113	114-118	119-126	127-132	133-140	141-144	Total
MALES										
0	100	20	10	13	(1)/8	11	10	(1)/12	16	(2)/100
25	80	8	11	6	3	(1)/13	11	8	20	(1)/80
100	90	8	12	3	2	10	7	(2)/11	(4)/27	(6)/80
250	99	7	7	6	(1)/4	(2)/8	(2)/13	(1)/10	(20)/44	(26)/99
FEMALES										
0	100	16	15	18	(2)/8	11	7	8	17	(2)/100
25	79	10	9	4	6	13	(1)/10	(1)/8	(1)/19	(3)/79
100	80	5	17	5	5	(1)/7	(1)/9	(1)/11	(3)/21	(6)/80
250	99	9	5	11	12	8	5	(2)/10	(12)/39	(14)/99

[a] Figures in parenthesis are combined adenomas and carcinomas occurring during that period.

Table 4. Incidence of hepatocellular nodular hyperplasia, adenomas, and carcinomas (Olsen et al., 1986)

BHT (mg/kg bw/day)	Effective No. of rats	Nodular hyperplasia	Adenomas	Carcinomas
MALES				
0	100	2	1	1
25	80	0	1	0
100	90	2	5	1
250	99	2	18[a]	8[b]
FEMALES				
0	100	2	2	0
25	79	0	3	0

250	99	5	12[c]	2[d]

[a] Over-all test for heterogeneity, p< 0.001, chi-square 18.17, 3 df.
Test lot trend, p< 0.001, chi-square = 17.97, 1 df.

[b] Over-all test for heterogeneity, p < 0,05. chi-square 11.12, 3 df.
Test for trend, p< 0.01, chi-square = 9.40. 1 df.

[c] Over-all test for heterogeneity, not significant, chi-square = 5.20, 3 df.
Test for trend, p< 0.05, chi-square = 4.99, 1 df

[d] Over-all test for heterogeneity, not significant, chi-square = 2.87, 3 df.
Test for trend, not significant, chi-square = 2.59, 1 df.

Reproductive parameters were as follows: mating index was in the range 54-65%; gestation index, 93-96%; viability index (birth to day 6), 93-96%. No effect of treatment was evident in these parameters. The absolute numbers of resorptions/dam were also similar in treated and control groups. There was a slight decrease in the numbers of pups/litter in the low- and high-dose groups, but a dose-related trend was not evident. Body weights of the pups from the high-dose group were significantly lower than controls at birth (10%), and at days 6 (12%) and 21 (21%) of lactation. Mortality of the pups remaining after culling, to day 21 of lactation was 2%, 8%, 12% and 15%, in order of ascending dose. Since the culling of pups at day 6 was non-random, these data could not be used for an unbiased evaluation of reproductive function in BHT-treated animals.

Body weights of the F_1 males which continued in the study were lower in the high-dose group, compared with controls, throughout the 22-month treatment period. During the first year of the study, the difference was in the range of 10-20%. Lower body weights which differed from controls by about 5% were also noted in the mid-dose animals during the first half of the treatment period. No adverse reactions to treatment or effects on food consumption were noted. Subjective assessment of the coats of rats indicated that there was less age-related deterioration in the 100 and 250 mg/kg bw/day groups than in controls. At the scheduled sacrifices, consistent dose-related increases in relative, but not absolute liver weights were observed, which were statistically significant at the high dose.

A dose-related incidence of enlargement and eosinophilia of the centrilobular hepatocytes was observed consistently at the scheduled sacrifices, starting at 6 months. This was indicative of proliferation of the SER, consistent with an induction of mixed function oxidases. Immunohistochemical staining of liver sections from control and high-dose rats revealed a marked increase in hepatocellular content and distribution of cytochrome P-450 2B with BHT treatment which persisted throughout the study. No such alteration was seen in the staining patterns for cytochrome P-450 1A or epoxide hydrolase. Histochemical staining revealed a marked induction of gamma-glutamyl trans-peptidase activity in the periportal hepatocytes of nearly all of the high-dose rats, starting at 11 months of treatment. This effect was noted to a lesser extent in the mid-dose group. The distribution of glucose-6-phosphatase activity in the treated rats was normal. There was no evidence of treatment-related bile duct hyperplasia or inflammatory cell infiltrate in the portal tract. Some rats with altered hepatocellular foci (AHF) were noted in treated groups at 11 months and in all groups at 16 and 22 months. There was no treatment relationship in the incidence of specific types of foci. At 22 months, there was a higher incidence of eosinophilic and basophilic foci in the high-dose group. Histochemical staining of liver sections revealed a small number of high-dose animals with glucose-6-phosphatase-deficient AHF which was statistically significant. No treatment-related increase was noted in the incidence of AHF staining positive for GGT At 22 months, there was also a significant increase in the number of rats with hepatic nodules in the high-dose group (6/19 animals compared with none in the other groups).

No increases in the rate of hepatocellular proliferation were detected as a result of BHT administration at any point in the study commencing from 4 weeks post-weaning. It is of interest to note that Clayson *et al.* (1993) observed an increase in hepatocellular proliferation between 2 and 4 days after initiation of treatment of male Wistar rats with 0.5% dietary BHT. Such a transient increase would be difficult to detect with the widely spaced sampling times used in this study.

A number of hepatic enzyme activities and other parameters related to xenobiotic metabolism were altered as a result of BHT treatment. Total cytochrome P-450 content was increased by 30-60% in the high-dose animals starting at 21 days of age. Dose-related increases were noted in epoxide hydrolase, glutathione-S-transferase and pentoxyresorufin O-depentylase (PROD) activities, starting at 21 days of age, which were statistically significant in the mid- and high-dose groups. The increases in PROD activity were very large, 10-25 fold in the mid-dose, and 20-80 fold in the high-dose groups. Modest dose-related increases in ethoxyresomfin O-deethylase activities were noted which were not statistically different from controls. No effects on hepatic glutathione levels or glucose 6-phosphatase activity were noted throughout the study.

Histopathological examination of the kidneys revealed a reduction in severity of chronic progressive nephropathy which affected the rats in all groups from 11 months. No effects on the adrenal were noted, although in a nearly identical, but invalidated study conducted in the same laboratory (Robens Institute, 1989) cytomegaly of cells of the zona fasciculata was observed in the mid- and high-dose groups at weaning and at 4 weeks post-weaning, but not at subsequent time points. In the present study, histopathology of the adrenal was conducted starting at 11 months post-weaning. Evidence of thyroid hyper-activity, characterized by reduction of follicular size, absence or reduction of colloid, irregularities in the follicular outline, hyperaemia and increase in the number of follicular cells was noted starting at 11 months in both the mid-dose group (mild changes affecting 75-82% of the rats) and the high-dose group (marked changes affecting 100% of the rats). This effect was probably secondary to the effects of BHT on the liver. Serum thyroxine levels in treated rats did not differ from controls.

The demonstrated effects on hepatic enzyme induction and consequent thyroid hyperactivity in the mid- and high-dose groups together with the tumour data from the Olsen study suggested a NOEL of 25 mg/kg bw/day (Price, 1994).

BHT was added to the diet of male F344 rats for periods of up to 110 weeks. In the first experiment, a group of 36 male F344 rats received basal NIH-07 diet only, while groups of 21 rats received diets containing 300, 1000, 3000 or 6000 mg/kg BHT. Four animals were randomly selected from each group for measurement of altered hepatocellular foci at 12, 36, 48 or 76 weeks.

The remaining animals were sacrificed at 76 weeks. In a second experiment, groups of 27 male F344 rats were fed basal diet, or diets containing 12 000 mg/kg BHT or 12 000 mg/kg BHA for 110 weeks, at which time all surviving animals were sacrificed. In both experiments, body weights were recorded every four weeks. Gross necropsy was performed on all animals. The livers were weighed and submitted for

All the rats in experiment 1 survived the entire treatment period (76 weeks). In experiment 2, the survival of rats in control and treated groups started to decline after 84 weeks, but no treatment-related trend was evident. At the end of the treatment periods, rats fed 3000, 6000 or 12000 mg/kg BHT had significantly lower body weights compared with respective controls. The body-weight decrement for these three groups was approximately 10%. Rats fed 6000 mg/kg BHT for 76 weeks had significantly increased absolute and relative liver weights compared with controls. In rats fed 12000 mg/kg BHT for 110 weeks, the absolute liver weights were significantly decreased and relative liver weights were comparable to controls. The density of iron storage deficient hepatocellular foci in the one affected rat per group was slightly but not significantly increased in BHT-treated animals at 48 and 76 weeks; the incidence and size of foci were slightly decreased at 110 weeks in rats receiving 12000 mg/kg BHT. No hepatocellular carcinomas were detected in any of the groups. Hepatocellular adenomas were detected in all groups, including controls, with no treatment-related trend in incidence. There was also no treatment-related effect of BHT on the incidence of grossly observable tumours in specific organs (Williams *et al.*, 1990a).

2.2.4 Reproductive toxicity studies

2.2.4.1 Mice

Diets containing 0.1 or 0.5% BHT together with two dietary levels of lard (10 or 20%) were given to mice. The 05% level of BHT produced slight but significant reduction in mean pup weight and total litter weight at 12 days of age. The 0.1% level of BHT had no such effect. Out of 7754 mice born throughout the reproductive life span of the mothers, none showed anophthalmia, although 12 out of the 144 mothers were selected from an established anophthalmic strain (Johnson, 1965).

The chronic ingestion of 05% BHT by pregnant mice and their offspring resulted in a variety of behavioural changes. Compared to controls, BHA-treated pregnant mice showed increased exploration, decreased sleeping, decreased self-grooming, slower learning, and a decreased orientation reflex. BHT-treated offspring showed decreased sleeping, increased social and isolation-induced aggression, and a severe deficit in learning (Stokes & Scudder, 1974).

A 3-generation study was performed in mice for the evaluation of reproductive, developmental, and behavioural effects. Groups of 10 Crj:CD-1 mice/sex received BHT in the diet at concentrations of 0, 0.015%, 0.045%, 0.135%, or 0.405% (equivalent to 0, 20, 70, 200 or 610 mg/kg bw/day) starting at 5 weeks of age. At 9 weeks of age, the mice were mated on a 1:1 basis for a period of 5 days. The pups resulting from these matings were weaned at 4 weeks of age and 10 mice/sex/group were randomly selected to continue in the study. Males and females were mated at 9 weeks as in the previous generation.

Reproductive parameters measured for each of the F_1 and F_2 groups were: number of litters and pups, litter size and weight, sex ratio, pup weights on lactation days 0, 4, 7, 14, and 21, and survival to day 21. Neurobehavioural parameters measured at various times during the lactation period were: surface righting, negative geotaxis, cliff avoidance, swimming behaviour and olfactory orientation. Administration of BHT in the diet to mice adversely affected only one of the measured reproductive parameters. Body-weight gain was consistently reduced from day 7 to day 21 of lactation in the F_1 high-dose pups, but no body weight differences were noted in the F_2 pups compared with controls. In the F_2 males, lower scores were assigned to the treated groups for the 180° turn in the open field trial but this effect was not apparent in the F_2 female pups. Consequently, no toxicologically significant effects of treatment were apparent with BHT at the dietary concentrations tested in this study (Tanaka *et al.*, 1993).

2.2.4.2 Rats

Weanling rats (16/sex) were fed a diet containing 20% lard and 0, 300, 1000 or 3000 mg/kg BHT and mated at 100 days of age (79 days on test). Ten days after weaning of the first litter, the animals were again mated to produce a second litter. The offspring (16 females and 8 males) were mated at 100 days of age. Numerous function and clinical tests including serum cholesterols and lipids were performed on the parents and the first filial generation up to 28 weeks and gross and microscopical examination at 42 weeks. At the 3000 mg/kg dietary level, 10-20% reduction in growth rate of parents and offspring was observed. A 20% elevation of serum cholesterol levels was observed after 28 weeks, but no cholesterol elevation after 10 weeks. A 10-20% increase in relative liver weight was also observed upon killing after 42 weeks on diet. All other observations at 3000 mg/kg and all observations at 1000 mg/kg and 300 mg/kg were comparable with control. All criteria of reproduction were normal. No teratogenic effects were detected (Frawley *et al.*, 1965b).

Similar results to those obtained with the parental and first filial generations were also obtained with the second filial generation. Examination of two litters obtained from the latter at 100 days of age revealed no effects except a reduction of mean body weight at the 3000 mg/kg level. The offspring were examined for litter size, mean body weight, occurrence of stillbirth, survival rate and gross and microscopic pathology (Kennedy *et al.*, 1966).

Breeding pairs of Sprague-Dawley rats (200-220 g) received Purina chow supplemented with 0.125%, 0.25% or 0.5% BHT *ad libitum* beginning from the week before mating and continuing in females through lactation and weaning of the pups. Growth rates and survival were adversely affected. Pre-weaning pups born of mothers at the highest dose level weighed significantly less than controls at ages 7, 14 and 21 days. The total number of pups dying on study, born of dams receiving 0.25 or 0.5% BHT, was significantly higher than controls. Behavioural tests were conducted, consisting of righting reflex, pivoting, cliff avoidance, startle response, swimming, open field, running, wheel activity, roto-rod, active avoidance, position discrimination and passive avoidance. For pre-weaning testing, no differences were noted at the low- or mid-dose groups. At the high-dose level, there was significant increase in surface righting time, delayed forelimb swimming development and a trend to less activity in open field tests. In post-weaning tests, males in the 0.25% BHT group showed an effect on passive avoidance, with more partial re-entries into compartments where shocked. For all other tests, there were no statistical differences, suggesting that BHT had no effect on basic motor coordination, active avoidance acquisition, or extinction performance (Brunner *et al.*, 1978).

Groups of 46 rats, 6-week old (Wistar outbred, SPF) were fed diets containing 0, or 0.5 to 0.9% BHT so that the dietary intake of BHT was equivalent to 500 mg/kg bw/day during the course of the study. At week 19, the F_0 generation was mated. Twenty-four hours after birth of F_1 rats, the size of the litters was reduced to 8, and half of the litters were cross-fostered. The body weight of parents and offspring and the developmental events of offspring were monitored during the course of the study, as well as the reproductive performance of the F_0 rats. Auditory and visual function and locomotive coordination tests were carried out on tile F_1

histological examination made of the brains. Body weights and weight gain of test animals were reduced when compared to controls, and this persisted during gestation. The duration of pregnancy, average body weight, and litter size were similar for test and control animals. The average body weight and weight gain of the F_1 offspring were significantly reduced in pups nursed by dosed mothers. Pups exposed *in utero* to BHT also showed a relatively slower development than controls when fostered with non-dosed mothers. Pups exposed to BHT *in utero* and/or mothers milk showed alterations in the behavioural patterns examined as well as higher incidence in average number of dead cells in the brain (Meyer & Hansen, 1980).

Detailed comments were submitted by the Chemical Manufacturers Association (CMA) (1983) on studies of the effect of BHT on reproduction and teratogenicity. The major comments were concerned with the studies of Brunner *et al.* (1978) and Vorhees *et al.* (1981) previously reviewed by JECFA in 1980 (Annex 1, reference 54) as well as the study by Meyer & Hansen (1980).

In the case of the Brunner *et al.* and Vorhees *et al.* studies, it was concluded that the study showed normal pup survival and development in pups raised by dams on diets containing 0.125% BHT. Normal post-weaning development was observed in pups raised by dams on diets containing 0.25% BHT, although increased post-weaning mortality occurred in pups raised by dams on the 0.25% and 0.5% diet; developmental delays occurred in pups in the 0.5% group. In the case of the Meyer and Hansen (1980) study, developmental delays were seen in rats raised by dams on diets containing 0.5% BHT. At the 0.25% and 0.5% level, the effect may be due either to toxic effects of BHT on the dam, or direct toxicity during lactation. A number of questions were also raised about the design of the Brunner and Vorhees study. These are: (i) the pup selection, in which all litters of fewer than 8 live pups were discarded; and (ii) the excess mortality was reported in terms of pup count rather than affected litters. The data from this study have been audited by the U.S. FDA (1983). It was concluded that the raw data support the authors' observations of increased mortality in the mid-dose and high-dose BHT offspring. However, excess mortality occurred in a limited number of litters. For example, in the 0.5% group, of the 60 deaths reported in 19 litters, 49 of the deaths occurred in 5 litters; in the 0.25% group, of the 42 deaths, 21 occurred in 2 litters, and at the 0.125% level, of the 12 deaths, 11 occurred in 1 litter. It was also noted that in the high dose-group, there was an increased number of litters with 8 pups or less, and no litters larger than 12 pups. In the other dose groups, the litter size was comparable to controls.

In the case of the Meyer & Hansen (1980) study, the CMA comments noted that the level of BHT used in the study caused toxicity in the dams, which appeared to affect the pups directly or indirectly. Reports of teratogenicity studies and/or 1-generation reproductive toxicity studies in several strains of mice and rats as well as a 3-generation reproductive toxicity study in rats were also submitted in the comments to support a "no effect" level of 0.1% BHT in the diet.

Groups of 60, 40, 40, or 60 Wistar rats of each sex, 7 weeks of age, were fed a semi-synthetic diet containing BHT so that the dietary intake was equivalent to 0, 25, 100, or 500 mg/kg bw/day, respectively. After 13 weeks on the test diet the rats were mated.

Food consumption was similar in all groups. Male and female rats in the high-dose group showed a significant decrease in body weight which persisted throughout the study. Gestation rate was similar for all test groups. The litter size and number of males per litter were significantly lower in the 500 mg/kg bw/day group than in the controls. Viability was similar in test groups and in the control group during the lactation period. The average birth weight of the pups in the 500 and 100 mg/kg bw/day groups was slightly lower than in the controls. During the lactation period, BHT caused a significantly lower dose-related body-weight gain (5%, 7%, and 41% lower body weight for the 25, 100, and 500 mg/kg bw/day groups, respectively, as compared to controls) (Olsen *et al.*, 1986).

A study was initiated to determine the maximum dietary dose of BHT tolerated by female rats exposed prior to and through pregnancy, and by pups similarly exposed *in utero* and until weaning. Groups of 3 male and 16 female Wistar rats were administered BHT in the diet corresponding to 0, 500, 750, or 1000 mg/kg bw/day for 3 weeks before mating. At least 8 females per group were dosed during the pregnancy, and until weaning (21 days after the delivery). After mating, the males and the remaining females were autopsied. No effect of treatment was seen on blood-clotting times in these animals. Food consumption of treated females was considerably higher than controls from the fourth week of the study onwards. No significant effect was seen on body weight although a dose-related trend to reduction was apparent. No effects were seen on general health except for fur discoloration in treated animals.

Successful mating occurred less frequently in rats pretreated with 1000 mg/kg bw/day of BHT than in the other groups. No major differences were observed between the groups of pregnant females. The weight gain in rats treated with the two highest doses appeared to be inhibited in the last week of the pregnancy. There was no significant difference between litter number or litter weight between pups born of control or treated animals, although a dose-related trend towards reduction in litter size was seen. No evidence of teratogenic effects of BHT was provided.

Litter sizes were standardized to 8 pups if possible. At weaning, the dams treated with 1000 mg/kg bw/day of BHT had lower body weights and very little body fat was observed at autopsy. Pups from the dams treated with the lowest BHT dose were markedly stunted in their growth, but appeared healthy. Pups from dams treated with the two highest doses were severely stunted, showed poor fur condition, and were less active. It was noted that in BHT-treated animals, where the litter size was less than 8, the average pup weight was generally considerably greater. This implies that the reduced weight gains in litters of normal size were associated with poor milk production rather than BHT toxicity. Pups from two litters from each dose group were maintained on control diet for 4 weeks after weaning. Pups born to dams receiving BHT-containing diets remained of lower body weight than control pups. Pups from the two highest dose groups continued to show poor condition. Treatment with BHT caused a marked increase in liver weight in all dams. The liver weights were almost 10% of the body weights, the maximum degree of enlargement possible in rats. The relative liver weights of pups from BHT treated dams were not different from controls (Robens Institute, 1989).

2.2.4.3 Chickens

When BHT was fed at a level of 0.125% for 34 weeks to a group of 10 pullets, no differences in fertility, hatchability of eggs or health of chicks in comparison with a similar control group were found. The eggs of the antioxidant-treated birds contained more carotenoids and vitamin A than those of the controls (Shellenberger *et al.*, 1957).

2.2.4.4 Monkeys

A group of 6 adult female rhesus monkeys were maintained on a

equivalent to 50 mg BHT/kg bw/day and 50 mg BHA/kg bw/day. Another group of 6 adult female rhesus monkeys were used as controls. The monkeys were fed the diet for one year prior to breeding and then for an additional year, including a 165-day gestation period. Haematologic studies including haemoglobin, haematocrit, total and differential WBC, cholesterol, Na^+, K^+, total protein, serum GPT, and GOT, were carried out at monthly intervals. Body weights were taken at monthly intervals. Records of menstrual cycles were maintained through the test period.

After one year the females were bred to rhesus males not receiving test diets. During pregnancy complete blood counts were done on days 40, 80, 120 and 160 of gestation and on days 30 and 60 post-partum. A total of 5 infants were born to the experiment monkeys and 6 to the control monkeys. Haematological evaluations were made on infants of the test and control monkeys at days 1, 5, 15, 30 and 60, and observations of the infants were continued through two years of age. Two experimental and 2 control infants, 3 months of age, were removed from their mothers for 1 month of psychological home cage observations.

No clinical abnormalities were observed in parent or offspring during the period of study. The gestation of test animals was free of complications and normal infants were delivered. Adult females continued to have normal infants. Infants born during the exposure period remained healthy, with the exception of one infant that died from unrelated causes. Home cage observations at the third month of life did not reveal any behavioural abnormalities (Allen, 1976).

2.2.5 Special studies on teratogenicity

In a study on the embryotoxicity of BHT, 3 dosing schedules were employed: single doses (1000 mg/kg bw) on a specific day of gestation, repeated daily doses (750 mg/kg bw) from the time of mating throughout pregnancy, and daily doses (250-500 mg/kg bw for mice and 500 and 700 mg/kg bw for rats) during a 7- to 10-week period before mating, continuing through mating and gestation up to the time the animals were killed. No significant embryotoxic effects were observed on examination of the skeletal and soft tissues of the fully developed fetuses as well as by other criteria. Reproduction and postnatal development were also unaffected (Clegg, 1965).

2.2.6 Special studies on genotoxicity

The results of genotoxicity studies with BHT are summarized in Table 5.

At a concentration as low as 10 µg/ml (optimal 50-100/µg/ml), BHT exerted a strong inhibitory effect on cell-to-cell dye transfer (lucifer yellow transfer) in cultures of SV-40-transformed Djungarian hamster fibroblasts. The effect was reversible. BHT shared this effect with a series of well known tumour promoters (Budunova *et al.*, 1989).

An extensive database of genotoxicity studies for BHT, including those documented above, were reviewed in a paper by Bombard *et al.* (1992). The authors concluded that the majority of evidence indicated a lack of potential for BHT to induce point mutations, chromosomal aberrations, or to interact with or damage DNA, and that BHT does not represent a genotoxic risk to humans.

The ability of BHT and its metabolites to induce cleavage of supercoiled plasmid DNA was studied in pUC18 by agarose gel electrophoresis. Dose-related cleavage of DNA was noted with BHT-quinone in the range 10^{-6}-10^{-3}M, and to a lesser extent using higher doses of BHT-aldehyde and BHT-peroxyquinone. BHT, BHT- quinone methide and other BHT metabolites had no effect on the plasmid DNA. Free radical scavengers were effective against the effects of BHT-quinone and -peroxyquinone, but not against BHT-aldehyde. Formation of superoxide radical as measured by a reduction in cytochrome c, was noted only with BHT-quinone (Nagai *et al.*, 1993).

The addition of BHT (5 to 20 µg/plate) caused a two-fold increase in the mutagenic potency of aflatoxin B_1 using *Salmonella typhimurium* strains TA98 and TA100, with and without activation (Shelef & Chin, 1980).

The addition of 50-250 µg of BHT/plate inhibited 3,2'-dimethyl-4-aminobiphenyl-induced mutagenicity in *Salmonella typhimurium* strains TA98 and TA100 in the presence of rat liver S-9 fraction (Reddy *et al.*, 1983a).

Table 5. Results of genotoxicity assays with BHT

Test System	Test Object	Concentration of BHT	Results	Reference
Point Mutation				
Ames test[1]	S. typhimurium TA1535, TA1537 TA1538	0.015-0.6%	Negative	Brusick 1975
Ames test[1]	S. typhimurium TA97, TA102 TA104, TA100		Negative	Hageman et al. 1988
Ames test[1]	S. typhimurium TA98, TA100 TA1535, TA1537 TA1538	100-10 000 µg/plate	Negative	Williams et al. 1990b
Ames test[1]	S. typhimurium TA98, TA100	100-1000 µg/plate	Negative	Yoshida 1990
Ames test[1]	S. typhimurium TA 98	10 µg/plate	Negative	Detringer et al. 1993
Host-mediated assay	ICR Swiss mouse/ S. typhimurium G46, TA1530	30-1400 mg/kg - acute 30-500 mg/kg - subacute	Negative	SRI 1972
Mammalian cell gene mutation	rat liver epithelial cell (line 18), HGPRT locus	60-90 µg/ml	Negative	Williams et al. 1990b

Table 5 (cont'd)

Test System	Test Object	Concentration of BHT	Results	Reference
Sex-linked recessive lethal	Drosophila melanogaster	2.0×10^{-6} µg	Negative[2]	Prasad & Kamra 1974
Sex-linked	Drosophila	5% diet	Negative	Brusick 1975

Clastogenic effects and chromosomal aberrations

Chromosomal aberration assay	human WI-38 (embryonic lung cells)	2.5-250 µg/ml	Positive[2]	SRI 1972
Sex chromosome loss	Drosophila melanogaster	2.0×10^{-6}	Negative[2]	Prasad & Kamra 1974
Micronucleus assay	rat bone marrow	30, 90, 1400 mg/kg (acute and subacute)	Negative	SRI 1972
Dominant lethal assay	Sprague-Dawley rat	30, 900, 1400 mg/kg bw (acute) 30, 250, 500 mg/kg bw/day (subacute)	Negative Positive	Brusick 1975
Dominant lethal assay	male Sprague-Dawley rats male mice	50, 150, 500 mg/kg bw/day 1% diet	Positive[2] Negative	Sheu et al. 1986

Table 5 (cont'd)

Test System	Test Object	Concentration of BHT	Results	Reference
Heritable translocation assay	male mice	1% diet	Negative	Sheu et al. 1986

DNA interactions

Mitotic recombination	Saccharomyces cerevisiae D4	0.6-2.4%	Negative	Brusick 1975
Mitotic recombination - Host-mediated	Saccharomyces cerevisiae D3/ICR Swiss mouse	30, 900, 1400 mg/kg bw (acute 30, 250, 500 mg/kg bw/day (subacute)	Negative Negative	SRI 1972
Sister chromatid exchange[1]	CHO cells	1 - 1000 µg/ml	Negative	Williams et al. 1984
DNA excision repair synthesis	UV-irradiated human lymphocytes	?	Positive	Daugherty 1978
DNA repair test	hepatocyte primary culture	0.01 - 10 µg/ml	Negative	Williams et al. 1990b

[1] Both with and without rat liver S9 metabolic activation.
[2] A discussion of this result is contained in Bombard et al. (1992).

The addition of 100-250/µg of BHT/plate was shown to inhibit 3,2'-dimethyl-4-aminobiphenyl-induced mutagenicity in *Salmonella typhimurium* strains TA98 and TA100 Mutagenicity was further inhibited by use of S-9 preparations from rats fed dietary BHT (0.6%) as compared to S-9 preparation from rats fed BHT-free diets (Reddy *et al.*, 1983b).

BHT was a moderately effective inhibitor of benzidine-induced mutagenicity in *Salmonella typhimurium* strain TA98, activated with a hamster liver S-9 fraction (Josephy *et al.*, 1985).

BHT (0.11-11 /µM) protected against DNA damage induced in rat hepatocytes by AAF or N-hydroxy AAF as shown by a marked reduction of unscheduled DNA synthesis. BHT also inhibited AAF-induced DNA damage in human hepatocytes. In addition, rats pre-treated with 0.5% BHT in the diet for 10 days provided hepatocytes which exhibited less unscheduled DNA synthesis than did hepatocytes from control rats when these cells were exposed to either AAF or N-hydroxy AAF (Chipman & Davies, 1988).

2.2.7 Special studies on hepatotoxicity

2.2.7.1 Mice

Groups of male ddY mice treated perorally with BHT (200-800 mg/kg bw) in combination with an inhibitor of GSH synthesis, buthionine sulfoximine (BSO, 1 h before and 2 h after BHT, 4 mmol/kg bw/dose, i.p.) developed hepatotoxicity characterized by an increase in SGPT activity and centrilobular necrosis of hepatocytes. The hepatotoxic response was both time- and dose-dependent. BHT (up to 800 mg/kg bw) alone produced no evidence of liver injury. Drug metabolism inhibitors such as SKF-525A, piperonyl butoxide, and carbon disulfide prevented the hepatotoxic effect of BHT given in combination with BSO while inducers of drug metabolism such as phenobarbital tended to increase hepatic injury. The results suggested that BHT was activated by a cytochrome-P-450-dependent metabolic reaction and that the hepatotoxic effect was caused by inadequate rates of detoxification of the reactive metabolite in mice depleted of hepatic GSH by BSO administration. Based on studies with structural BHT analogues, the authors suggested that a BHT-quinone methide may play a role in the hepatotoxicity in mice (Mizutani *et al.*, 1987).

2.2.7.2 Rats

Female (albino Wistar) rats, initial body weight 120-130 g, were maintained on a diet containing 0 or 0.4% BHT, for 80 weeks. After one week on the test diet, significant increases were observed in liver weight, microsomal protein, cytochrome P-450, cytochrome b5, NADPH-cytochrome c reductase, biphenyl-4-hydroxylase and ethyl morphine N-demethylase but not aniline-4-hydroxylase. Total liver protein, succinic dehydrogenase and glucose-6-phosphatase were slightly decreased. There was little change in this pattern during the period of the study. Rats removed from the BHT test diet at the end of the test period and maintained on BHT free diet for 18 days, showed a return to normal for many liver parameters except for cytochrome b5, cytochrome c reductase and ethylmorphine N-demethylase. Histological changes at the end of 80 weeks feeding consisted of centrilobular cell enlargement, which was reversible following 18 days on a BHT-free diet. The only ultrastructural change was a proliferation of smooth endoplasmic reticulum (Gray *et al.*, 1972).

Groups of female rats (80-100 g) received 0.4% BHT with corn oil mixed in ground lab chow and were sacrificed at intervals of 1, 8, 16, 32, or 80 weeks, and compared with controls. Samples of liver were taken for biochemical, histochemical, and morphological studies. To examine for reversibility of hepatic changes, control diet was administered for 18 days to a group of 4 rats following 80 weeks of BHT administration. After one week on BHT, there was a marked liver enlargement with relative liver weight increased up to 35% and with an increase in drug metabolizing activities and NADP cytochrome c reductase activity. After 18 days of removal from the 80-week

effect was therefore reversible. Histologically, after BHT treatment the liver was characterized by enlarged centrilobular hepatocytes, with a heterogenous appearance of this zone. Ultrastructurally, there was a proliferation of smooth endoplasmic reticulum. The authors noted that although the evidence of liver injury was equivocal, there were two features that were also seen with many hepatotoxins and hepatocarcinogens: depression of glucose-6-phosphatase activity and cell enlargement. However, there were no lysosomal changes characteristic of cytologic injury, and effects were reversible (Crampton *et al.*, 1977).

Groups of 8 male Wistar rats were given diets containing 0, 0.1, 0.25, 0.5, or 0.75% BHT for 30 days. BHT did not induce cellular proliferation in the liver, urinary bladder or thyroid after 30 days as measured by the [^{3}H]thymidine labelling index or mitotic index. In a second experiment, groups of 8 rats were treated with 0.5% dietary BHT for 2, 4, 8, 10, or 14 days. This treatment led to a time-limited increase in liver cell [^{3}H]thymidine-labelling index that subsided to control values within 8 days. This increase in [^{3}H]thymidine labelling in the liver was accompanied by an unexpectedly large increase in the mitotic index (Briggs *et al.*, 1989).

Groups of female Sprague-Dawley rats were given 700 mg BHT/kg bw and selected hepatic biochemical effects were determined after 4 and 21 h. Ornithine decarboxylase (ODC) activity and cytochrome P-450 content were increased 190 and 30% respectively. No effect was seen on hepatic glutathione content or serum alanine aminotransferase activity. Indication of hepatic DNA damage was obtained as measured by an increased alkaline DNA elution. No effects on these parameters could be detected when the BHT dose was 140 mg/kg bw. It was concluded that BHT in high doses may have a DNA damaging effect (Kitchin & Brown, 1987).

BHT was administered to male Wistar rats by savage at doses of 0, 25, 250 or 500 mg/kg bw/day for 7 days (5 rats/group), or 28 days (10/group) and also at daily doses of 1000 or 1250 mg BHT/kg bw/day (5 rats/group) for up to 4 days (sublethal doses). The sublethal doses induced centrilobular necrosis within 48 h, whereas administration of 250 or 500 mg/kg bw/day BHT for 7 or 28 days caused dose-related hepatomegaly and, at the highest dose level, induced progressive periportal hepatocyte necrosis. The periportal lesions were associated with proliferation of bile ducts, persistent fibrous and inflammatory cell reactions, hepatocyte hyperplasia and hepatocellular and nuclear hypertrophy. Evidence of mild cell damage was also obtained at 250 mg/kg bw/day, while there was no evidence that BHT caused liver damage at 25 mg/kg bw/day. Biochemical changes consisted of dose-related induction of epoxide hydrolase, dose-related changes in the ratio of cytochrome P-450 isoenzymes and depression of glucose-6-phosphatase. Measurement of BHT demonstrated a dose-related accumulation in fat but not in the liver (Powell *et al.*, 1986).

The acute effects of a single oral administration of 500 mg/kg bw BHT to rats were investigated in combination with phenobarbitone (PB), a microsomal enzyme inducing agent, and buthionine sulfoximine (BSO), a glutathione-depleting agent. Groups of 10 male Sprague-Dawley rats received BHT in corn oil by gavage, BHT with 3 days prior administration of 80 mg/kg bw/day PB in saline i.p., BHT with 1 h prior administration of 900 mg/kg bw BSO in saline i.p., or corn oil/saline alone. Thirty-six hours after administration of BHT, the animals were sacrificed and blood was collected for assay of serum enzyme activities (ALT, AST, alkaline phosphatase, lactate dehydrogenase), albumin, APTT and clotting factors II, VII, X and IX. Samples of liver (from each of 4 lobes), lung and kidney were processed for histopathological examination which included immunochemical staining of liver lot visualization of cytochrome P-450 1A and 2B distribution. Liver homogenates were also assayed for reduced glutathione concentration, microsomal cytochrome P-450, ethoxyresorufin- *O*-deethylase (EROD) activity, ethoxycoumarin- *O*-deethylase (ECOD) activity, epoxide hydrolase activity and malondialdehyde.

The results showed that a single oral dose of 500 mg/kg bw BHT was below the threshold for acute hepatotoxicity. BHT administration had no effect on any of the serum parameters, with the exception of a slight reduction in the levels of clotting factor IX. There was no evidence that BHT induced hepato-cellular necrosis, and hepatic malondialdehyde concentration, an indicator of lipid peroxidation, was reduced from controls. A single dose of BHT was also associated with an increase in mitotic activity of hepatocytes in 8/10 animals, increased hepatic activity of ECOD without affecting microsomal protein content or EROD activity, and a marked increase in hepatic epoxide hydrolase activity. The last was postulated to be related to BHT-induced inhibition of phylloquinone epoxide reductase activity. There was no effect on hepatic GSH levels, no clear treatment-related change in immunochemical staining of hepatocytes for the cytochrome P-450 1A or 2B isoenzyme families and no histopathological changes in the lung or kidney. Prior administration of PB or BSO resulted in unequivocal liver damage (hepatocyte degeneration or coagulative necrosis), mostly in the centrilobular areas, in about half the rats, without affecting serum parameters which are commonly used indices for tissue damage (ALT, AST, lactate dehydrogenase). Since neither of these treatments duplicated the periportal cell damage observed with repeated administration of BHT, the results could not be used as a model for investigation of alterations in enzyme profiles induced by repeated administration of BHT (Powell & Connolly 1991).

2.2.8 Special studies on nephrotoxicity

A single large dose of BHT (1000 mg/kg bw) in male F344 rats produced some renal damage, as measured by reduced accumulation of p-aminohippuric acid in renal slices, proteinuria and enzymuria, in addition to hepatic damage. Administration of phenobarbital (80 mg/kg bw, i.p., daily for 4 days) prior to BHT treatment of male rats produced renal damage accompanied by slight tubular necrosis and more pronounced biochemical changes. Female rats were less susceptible to BHT-induced renal and hepatic damage than male rats (Nakagawa & Tayama. 1988).

The nephrocalcinogenic effect of BHT was studied in groups of 10-20 female Wistar rats (5 weeks old) fed 1% BHT for 13-48 days in semi purified diets using sodium caseinate or lactalbumin as the only protein source. BHT induced nephropathy in female rats irrespective of the diet used. Pronounced nephrocalcinosis was only found in rats fed the sodium caseinate diet. Thus a connection between the development of nephropathy and nephrocalcinosis after BHT was not established (Meyer *et al.*, 1989).

Groups of 10 male ddY mice received 0, 1.35%, 1.75%, 2.28%, 2.96%, 3.85% or 5.00% BHT in a purified diet, (equal to 0, 1570, 1980, 2630, 3370, 4980 or 5470 mg/kg bw/day, respectively) for 30 days. Terminal body weights in all groups of treated mice were lower than those of controls, the difference ranging from 15% at the lowest dose to 30% at the highest dose, statistically significant at the highest three doses. Absolute and relative kidney weights exhibited dose-related decreases and increases, respectively, which were related to reduced body-weight gain. Results from gross pathology of the kidney showed 7/10 of the high-dose animals with "misshapen kidney" compared with none in any of the other treated or control groups. Histopathology of the kidney revealed a dramatic dose-related increase

and 10 out of 10 mice/group) as indicated by a number of tubular lesions (distal and proximal tubular degeneration, distal tubular necrosis, distal tubular regeneration, tubular dilatation and cysts). No pathological changes were noted in the liver of these same mice which could be related to treatment. The ED_{50} for toxic nephrosis in the tidy male mouse following 30 days of administration of BHT in the diet was calculated to be 2300 mg/kg bw/day (Takahashi, 1992).

2.2.9 Special studies on pulmonary toxicity

Young male Swiss Webster mice were injected i.p. with BHT at dose levels ranging from 63 to 500 mg/kg bw BHT. The animals were killed 1, 3 or 5 days after BHT administration. Histopathological changes were well-developed 3 days after administration of 500 mg/kg bw, and consisted of a proliferation of many alveolar cells, formation of giant cells and macrophage proliferation. These changes were accompanied by an increase in lung weight and total amounts of DNA and RNA. The changes were dose dependent, the smaller effective dose being 250 mg/kg bw (Saheb & Witschi, 1975).

Sixty male Swiss mice were given i.p. injections of 400 mg/kg bw BHT dissolved in corn oil. Six experimental animals and 6 controls were sacrificed daily for 9 days. Two hours before sacrifice, each animal received 2 µCi/g of tritiated thymidine. No animals died during the study and none showed signs of respiratory distress. Two days after dosing, cellular lesions were noticed in the type I alveolar epithelium. Abnormal giant type II cells were observed in mitosis and many had an accumulation of tritiated thymidine. Labelled endothelial cells were seen after day 6 in small vessels and capillaries, and there was an increase in fibroblastic cells in the interstitium and capillaries. There was an increase in thymidine-labelled pulmonary cells from days 2 through 5, after which labelling dropped off and approached control levels by day 9. Levels of lung thymidine kinase activity rose sharply on days 1-4 after dosing and then dropped off rapidly (Adamson *et al.*, 1977).

Groups of NMRI mice (25-35 g) and Wistar rats (160-320 g) received BHT as a single dose of 500 mg/kg bw dissolved in soya bean oil, either i.p. or by gavage. Four days later, radiolabelled ^{14}C thymidine was given. After 90 minutes, the animals were sacrificed, lungs removed and DNA levels were measured. In mice, DNA synthesis was equally increased in males and females by oral or i.p. administration. Although lung weight was increased, the concentration of DNA was not affected. No effect was seen in male rats and only a slight increase in females (Larsen & Tarding, 1978).

Groups of 16-24 Swiss male mice (25-30 g) received a single i.p. injection of BHT in corn oil (63, 215, or 500 mg/kg bw) or corn oil only (Tocopherol stripped 0.5 ml). Three days later the mice were sacrificed. After BHT treatment, wet lung weights were increased to 120% of control, as were dry lung weights. There were significant increases in DNA content and level of non-protein sulfhydryl (133-156% of control). Superoxide dismutase and other oxidative enzyme levels were increased. The authors concluded that BHT apparently increased inflammatory and reparative-proliferative processes of the lung (Omaye *et al.*, 1977).

Following acute exposure to BHT, the initial sequence of events involved infiltration of type I (squamous) epithelial cells followed by multifocal necrosis and destruction of the blood barrier. A detailed discussion of the sequence of tissue changes and repair mechanisms was given. It was stated that the susceptibility of the squamous epithelium to injury was similar to that seen after oxygen exposure, radiation exposure, and treatment with blood-borne bleomycin, but the recovery pattern was quite different. BHT was thought to cause cell lysis and death as a result of interaction with the cell membrane (BIBRA, 1977).

The increase in lung weight and increase in thymidine incorporation into lung DNA observed in mice following BHT injection was inhibited by treatment with cedar terpenes. No increase in lung weight was observed in animals treated with BHT alone if they were less than 3-week old. This may result from the inability of infant mice to metabolize BHT (Malkinson, 1979).

In a study of lung toxicity of BHT analogues in mice, it was established that the structural feature essential for toxic activity is a phenolic ring structure having a methyl group at the 4-position and *ortho*-alkyl group(s) which can result in a moderate hindering effect of the hydroxyl group (Mizutani *et al.*, 1982).

In another study, the toxic potency of BHT in mice was decreased by deuteration of the 4-methyl group, suggesting that lung damage following administration of BHT was caused by the metabolite 2,6-di- *tert*-butyl-4-methylene-2,5-cyclohexadienone (Mizutani *et al.*, 1983).

Male mice given a single dose of BHT showed ultrastructural changes of the lung, which were characterized by selective destruction of type I epithelial cells, which were replaced by type II cuboidal cells. These changes were accompanied by a marked decrease in the number of peroxisomes, as well as catalase activity (Hirai *et al.*, 1983).

Subcutaneous injections of BHA significantly enhanced the lung/body weight ratio of mice given intraperitoneal injections of subthreshold doses of BHT (Thompson *et al.*, 1986).

The ability of BHA to modify BHT-induced changes in lung weight was studied in male CD-1 mice. BHA alone had no effect on lung weight up to a dose of 500 mg/kg bw (s.c.). When injected 30 minutes prior to sub-threshold doses of BHT (0-250 mg/kg bw, J.p.), BHA significantly enhanced lung weight in a dose-dependent manner. The ability of BHA to enhance BHT-induced changes in lung weight was dependent on both the time and the route of administration of BHA relative to BHT (Thompson & Trush, 1988a).

In experiments with mouse lung slices, BHA enhanced the covalent binding of BHT to protein. Subcutaneous administration of either BHA (250 mg/kg bw) or diethyl maleate (DEM, 1 ml/kg bw) to male CD-1 mice produced a similar enhancement of BHT-induced lung toxicity. In contrast to DEM, the administration of BHA (250 or 1500 mg/kg bw) did not decrease mouse lung glutathione levels. *In vitro* results suggested that BHA facilitates the activation of BHT in the lung as a result of increased formation of hydrogen peroxide and subsequent peroxidase-dependent formation of BHT-quinone methide (Thompson & Trush, 1988b).

BHT administration lowered cytosolic Ca^{++} -activated neutral protease (calpain) activity in the lungs of male and female A/J mice. The altered proteolytic activity occurred earlier (day 1) and at a close lower than that which caused observable lung toxicity as assessed by the lung weight/body weight ratio (day 4) (Blumenthal & Malkinson, 1987).

A range of doses from 10-200 mg/kg bw of BHT or BHT-BuOH, a metabolite of BHT, were administered i.p. to groups of 2-3 inbred, C57BL/6J mice. BHT-BuOH had a 4- to 20-fold greater potency than BHT in increasing the relative lung weight, decreasing lung cytosolic Ca^{++}-dependent protease activity, and causing pulmonary histopathology. Nature of damage (type I cell death) and regenerative

with the two compounds. BHT-BuOH also caused damage to liver, kidney or heart. The authors suggested that BHT-BuOH formation may be an essential step in the conversion of BHT to the ultimate pneumotoxin, which might be the corresponding BHT-BuOH-quinone methide (Maikinson *et al.*, 1989).

The synthetic corticosteroid methylprednisolone (MP; 30 mg/kg bw, s.c. given twice daily for 3 days) partially protected male C57BL/6N mice from the pulmonary toxicity of BHT when administered 0, 24 or 48 h after BHT treatment (Okine *et al.*, 1986).

The activity of a metabolite of BHT hydroxylated on one *tert*-butyl group, (BHT-BuOH) in inducing pneumotoxicity was investigated in the mouse on the basis that pneumotoxicity had been observed following administration of BHT in all inbred strains of mice tested, but not in rats, and BHT-BuOH was a major product of mouse liver and lung microsomes and formed only in traces in rat microsomes. Lung damage was assessed by determining lung/body weight ratios, Ca^{2+}-dependent protease (calpain) activity and by histopathological examinations of the lungs. The liver, heart and kidneys were investigated for histopathological changes resulting from i.p. injection of BHT-BuOH. BHT-BuOH induced nearly a doubling of lung/body weight ratios 4 days after an i.p. injection of 50 mg/kg bw. By comparison, doses of 200 mg/kg bw BHT or greater were required to produce consistent increases in this parameter. Other BHT metabolites, DBQ, BHT-MeOH, BHT-OOH and BHT-OH, had no effect at i.p. doses of 200 mg/kg bw. Pulmonary calpain activity was significantly decreased in mice which received 50 mg/kg bw BHT-BuOH, reaching a maximal loss at 3-4 days after administration. This effect was similar in time course and extent to that induced by 400 mg/kg bw BHT. A dose of 10 mg/kg bw BHT-BuOH also resulted in a significant decrease in calpain activity which was less marked than the higher dose. Alveolar deterioration and compensatory Type II cell hyperplasia, inflammatory response and bronchiolar cell hyperplasia were observed in response to i.p. doses of 50 mg/kg bw BHT-BuOH. Less extensive effects were noted with doses of 10 mg/kg bw and these effects were considered comparable to those induced with doses of 400 mg/kg bw BHT. On the basis of the qualitative similarity in the effects of BHT and BHT-BuOH on the mouse lung, the higher potency of the metabolite, and the species correlation of formation of BHT-BuOH and pneumotoxicity, the authors concluded that BHT-BuOH was an intermediate in the biotransformation of BHT to a toxic metabolite in the mouse (Malkinson *et al.*, 1989).

A quinone methide metabolite of BHT which is formed subsequent to hydroxylation of the *tert*-butyl side group (QM-OH) was investigated as the metabolite responsible for pulmonary toxicity in mice. QM-OH was more strongly electrophilic than BHT-quinone methide as indicated by a reaction time with GSH which was 6 times faster. Liver and lung microsomes from both rats and mice produced quinone methide from BHT, but only microsomes from the mouse produced QM-OH readily from BHT. Microsomes from rat liver produced traces of QM-OH and lung microsomes produced none. These results were used to reconcile previous results linking pulmonary toxicity in the mouse to quinone methide formation with species specificity of this effect. The authors postulated that the organ specificity of BHT toxicity in mice was due to lower concentrations of GSH in the lung compared with the liver for inactivation of toxic metabolites (Bolton *et al.*, 1990).

The time course for repair of BHT-induced lung injury was investigated in four strains of mice with i.p. LD_{50}s ranging from 350-1700 mg/kg bw. The four strains of mice tested developed similar levels of injury at equivalent doses and no correlation could be made between lung injury and lethality (Kehrer & DiGiovanni, 1990).

A number of compounds which modify the activity of specific cytochrome P-450 isoenzymes were used to identify the isoenzymes involved in bioactivation of some compounds, including BHT, to pulmonary toxins. Pretreatment of mice with O,O,S-trimethylphosphorodithioate, bromophos, *p*-xylene, ß-naphthoflavone or pyrazole all produced a reduction of pneumotoxicity in MF1 outbred mice induced by a single i.p. dose of 400 mg/ kg bw BHT as indicated by a lowering of lung/body weight ratios measured 3 days after administration of BHT. The first three agents greatly reduced lethality of BHT in mice as indicated by an increase in LD^{50} values. The prevention of lung toxicity by these agents was proportional to the reduction in lethality of BHT. These three agents also markedly inhibited pentoxyresomfin (PROD) activity, an action attributed to CYP 2B1 in rat lung microsomes. ß-Naphthofiavone exerted a less marked effect on these LD^{50} values and PROD activity, and pyrazole-induced PROD activity. The authors concluded that since the three agents which prevented the pneumotoxicity of BHT in mice also inhibited PROD activity in rat lung microsomes, CYP 2B1 was the most likely candidate for the bioactivation of BHT in mouse lung. However, since BHT is not toxic to the rat lung, results obtained with rat lung microsomes would not necessarily be relevant to the mouse. In addition, the authors did not mention that pretreatment of mice with pyrazole, while reducing the lung/body weight ratio increase induced by BHT, also induced PROD activity in rat lung microsomes (Verschoyle *et al.*, 1993).

The ability of isolated Clara (non-ciliated bronchiolar epithelial) cells from mouse lung to metabolize BHT to the putative toxic quinone methide QM-OH was investigated as well as comparison of the toxic effects of BHT and BHT-BuOH on these cells. These cells contain most of the monooxygenase activity of the lung, mainly as CYP 2B1/2B2. Analysis of quantitative metabolite data revealed that hydroxylation of BHT occurred 5 times more readily at a *tert*-butyl group, producing BHT-BuOH, than at the 4-methyl position to produce BHT-MeOH in mouse Clara cells. The data also suggested that QM-OH was more readily produced from BHT-BuOH than was BHT-QM from BHT. BHT-BuOH more effectively reduced the viability of Clara cells in culture than did BHT. Concentrations of 5 and 10 µM BHT-BuOH reduced viability in a comparable manner to 75 and 100 µM BHT. Inhibition of cytochrome P-450 with SKF 525-A reduced damage to Clara cells induced by both BHT and BHT-BuOH. The authors concluded that BHT-BuOH is an intermediate in P-450-catalyzed oxidation of BHT to a cytotoxic species which they propose is QM-OH (Bolton *et al.*, 1993).

Groups of 20 male Swiss albino mice received BHT in olive oil or olive oil alone by a single i.p. injection at doses of 0, 200, 400 or 800 mg/kg bw. Five animals from each group were sacrificed at 24 h, 48 h or 7 days after exposure. Lavage fluid was collected from the lungs and assayed for total protein content and lactate dehydrogenase (LDH) activity. Cells in the sediment were counted. Histopathological examination of the lungs was performed. A time- and dose-dependent increase in the number of cells in the bronchoalveolar lavage fluid and in the total protein content and LDH activity was noted at 48 h and 7 days. The severity of histopathological lesions, described as congestion of capillaries and small blood vessels, and increased cellularity and diffuse thickening of alveolar septa, was also increased in a time- and dose-dependent manner (Waseem & Kaw, 1994).

2.2.10 Special studies on haemorrhagic effects

2.2.10.1 Mice

See Combined species, section 2.2.10.3

2.2.10.2 Rats

Groups of male Sprague-Dawley rats (6 weeks of age) received BHT in a semi-synthetic diet at concentrations ranging from 0.6% to 1.4%

0.7% or greater. Spontaneous massive bleeding to the pleural and peritoneal cavities, or as external haemorrhage, was observed in all dead or dying animals. The prothrombin index was decreased as the daily dose of BHT was increased. Mild diarrhoea was noted after 4 days. Rough hair coat, and redness of urine was noted. Death was due to haemorrhage and was classified by the authors as a secondary type of toxicity, probably due to a decrease in prothrombin concentration. According to the authors, the effect seemed to depend on strain of rats and dietary concentration (Takahashi & Hiraga, 1978a).

Groups of 10 male Sprague-Dawley CLEA rats were fed diets containing 0.6, 0.7, 0.8, 1.0, 1.2, or 1.4% BHT for 40 days. A dose-related effect on mortality (21 death/50 rats) was observed with rats given 0.7% or more BHT during the period from 9 to 37 days. Spontaneous massive haemorrhages were observed in these animals. The prothrombin index of survivors was decreased, which was dependent on the BHT dose. At the lowest level, the decrease was approximately 65% (Takahashi & Hiraga, 1978b).

Male Sprague-Dawley CLEA rats were maintained on diets containing levels of 85, 170, 330, 650, 1300, 2500, or 5000 mg/kg BHT for 1 to 4 weeks. A significant decrease in the prothrombin index was observed at week 1 in all groups fed BHT at levels of 170 mg/kg or higher. However, when the rats were maintained on the test diets for 4 weeks, a significant decrease in the prothrombin index was observed only in the 5000 mg/kg group. This was the only group which showed an increase in relative liver weights compared to those of the control group. In another study, haemorrhagic death, haemorrhage, and a decrease in prothrombin index in male Sprague-Dawley rats caused by 1.2% BHT were prevented by the simultaneous addition of 0.68 mole phylloquinone/kg bw/day. Phylloquinone oxide also prevented hypoprothrombinemia due to BHT (Takahashi & Hiraga, 1979).

Male Sprague-Dawley rats were fed diets containing 0 or 1.2% BHT for one week. BHT-treated rats showed haemorrhages in most organs. There was a significantly increased leakage of Evans Blue into the epididymis. In addition, inhibition of ADP-induced platelet aggregation and decreased platelet factor 3 availability were observed. Plasma prothrombin factors were decreased, but fibrinolytic activity was unchanged (Takahashi & Hiraga, 1981b).

Male albino rats (CRL COB CD(SD) BR) given 3 consecutive daily doses of 380, 760, or 1520 mg BHT/kg bw/day showed no evidence of haemorrhage. However, BHT produced a dose-dependent increase in prothrombin time, with no effect on prothrombin time seen in the 380 mg/kg bw/day group (Krasavage, 1984).

Male rats receiving 0.25% dietary BHT for 2 weeks showed decreased concentrations of vitamin K in the liver and increased faecal excretion of vitamin K (Suzuki *et al.*, 1983).

Dietary BHT at a level of 1.2% was shown to affect platelet morphology (distribution width), and to cause changes in the fatty acid composition of the platelet lipids (Takahashi & Hiraga, 1984).

Groups of 4-5 male Sprague-Dawley rats (5-6 weeks old) were fed a diet containing 1.2% BHT for 1-7 days, and blood coagulation factors II(prothrombin), VII, VIII, IX and X, and platelet aggregation were measured. The average dose of BHT was about 1000 mg/kg bw/day. The plasma concentrations of factors II, VII, IX and X were significantly reduced in a time-dependent fashion when BHT was administered for 2-7 days and haemorrhages in epididymis were found in rats given BHT for 4-7 days. On the contrary, thrombin-induced and calcium-required aggregation of washed platelets was unchanged throughout the experiment. These results suggest that factors II, VII, IX and X rapidly decrease immediately after the administration of BHT, but hypoaggregability of platelets may be a secondary effect caused by bleeding (Takahashi, 1986).

Groups of 4-10 male Sprague-Dawley rats (5-6 weeks old) were given single oral doses of 800 mg BHT/kg bw and 0.5-72 h later, plasma concentrations of blood coagulation factors II (prothrombin), VII, IX and X and hepatic levels of BHT and BHT-quinone methide were determined. Levels of the coagulation factors were reduced 36-60 h after BHT treatment, but by 72 h some recovery had occurred. Hepatic levels of BHT reached maxima at 3 h (a major peak) and 24 h after BHT dosing and BHT-quinone methide reached maxima at 6 and 24 h (a major peak). When BHT was given in doses of 200, 400 or 800 mg/kg bw, factors II, VII and X decreased after 48 h only in rats given the highest dose, but factor IX was more susceptible to BHT and showed a dose-dependent decrease. Neither pretreatment with phenobarbital for 3 days nor the feeding of 1% cysteine in the diet throughout the experiment prevented the decrease in vitamin-K-dependent factors at 800 mg/kg bw. In contrast, pretreatment with cobaltous chloride or SKF 525A partially prevented the decrease in the blood coagulation factors. The results indicate that the anticoagulant effect may require the metabolic activation of BHT (Takahashi, 1987).

The diets used in the above mentioned studies, and in previous studies from the same laboratory contained no added vitamin K, and the animals apparently were marginally vitamin K deficient (Faber, 1990).

BHT was less efficient than synthetic retinoids in elevating the prothrombin times and causing haemorrhagic deaths in male Sprague-Dawley rats maintained on a diet devoid of vitamin K (McCarthy *et al.*, 1989).

The effects of BHT on platelet aggregation were examined. *In vitro* experiments indicated that BHT, at concentrations greater than about 10^{-5}M, inhibited both ADP- and collagen-induced platelet aggregations, but not those induced by arachidonic acid. BHT-quinone methide also inhibited ADP- and collagen-induced aggregations to a lesser extent. In another experiment, male Sprague-Dawley rats were fed a diet containing 1.2% BHT (650-740 mg/kg bw/day) for 4 or 7 days. The treated animals showed marked decreases in body-weight gain compared with controls. Haemorrhage was detected in epididymal adipose tissues of all animals receiving BHT in the diet. Platelet aggregation capacity induced by ADP or collagen in platelet-rich plasma collected from these rats was considered to be normal. Although aggregation induced by 3.9 mM arachidonic acid was markedly inhibited in platelets from the rats fed 1.2% BHT for 7 days, platelet aggregation induced by the optimal concentration of arachidonate (2.0 mM) was normal. These results suggested a difference in plasma or platelet properties between the BHT-treated and control rats. The authors concluded that BHT-induced haemorrhage in rats was not due to a direct effect of BHT on platelet aggregation. The differences between *in vitro* and *in vivo* results were attributed to the low plasma concentrations of BHT or BHT-quinone methide which were present *in vivo* (Takahashi, 1991).

This study reported on the effect of BHT on vitamin K-dependent clotting factors in rats receiving vitamin K-sufficient and vitamin K-supplemented diets. Groups of 5-6 male Wistar rats received diets with BHT added to give a nominal intake of: (1) 0, 3000 mg/kg bw/day or 3000 mg/kg bw/day plus 250 mg vitamin K_3/kg of feed for 7, 14 or 21 days; (2) the same regimen for 7 or 14 days with a 250 mg/kg Vitamin K control group added; (3) 0, 12.5, 125 or 600 mg/kg bw/day or 600 mg/kg bw/day plus 3 mg/kg vitamin K_3 for 28 days. The basic diet (SDS Ltd., Witham, Essex, UK) was found to contain a minimum of

the recommended intake of this vitamin. In the first experiment, prothrombin time (PT) was measured as well as specific vitamin K-dependent factor deficiencies. The results showed that 3000 mg/kg bw/day BHT had no effect on PT, but specifically decreased the levels of vitamin K-dependent clotting factors II, VII, X and IX in rats receiving a diet containing adequate vitamin K. In experiments 2 and 3, a Thrombotest optimized for rodents was used in addition to different assay methods for PT and APTT. Serum fibrinogen levels were measured in experiment 2 only. Thrombotest time, PT and APTT were significantly increased from controls within 7 days at a close of 3000 mg/kg bw/day. Vitamin K^3 supplementation at 250 mg/kg in the diet prevented the BHT-induced increases in Thrombotest time and APTT and reduced the effects of BHT on PT. BHT administration had no effect on serum fibrinogen concentrations. Thrombotest time, but not PT or APTT, was significantly increased from controls after administration of 600 mg BHT/kg bw/day for 28 days. The effect was prevented by concurrent dietary supplementation with 3 mg vitamin K_3/kg of feed. This study confirmed an antagonistic effect of BHT on vitamin K in rats, which resulted in a reduction in blood-clotting factors even when the diets contained adequate vitamin K. The authors pointed out that this was a high-dose phenomenon with a threshold and a steep dose-response curve (Cottrell *et al.*, 1994).

2.2.10.3 Combined species

A number of strains of rats (Sprague-Dawley, Wistar, Donryu and Fischer), mice OCR, ddY, DBA/c, C_3H/HBe, BALB/CaAn and C57BL/6), New Zealand White-Sat rabbits, beagle dogs, and Japanese quail were fed diets containing BHT (1.2% in the diet for rats and mice; 1% for quail; 170 or 700 mg/kg bw/day for rabbits; and 173,400, or 760 mg/kg bw/day for dogs) for a period of 14-17 days. Haemorrbagic deaths occurred among male rats of all strains and female rats of the Fischer strain. Female rats of the Donryu and Sprague-Dawley strains showed no obvious haemorrhaging. No haemorrhagic effects were noted in quails, rabbits or dogs (Takahashi *et al.*, 1980).

Administration of 0.5%, 1.0% or 2.0% BHT in a purified diet (equal to 660, 1390 or 2860 mg/kg bw/day) for 21 days resulted in a dose-related increase in the mortality due to massive haemorrhage of the lungs in male ddY mice housed in wire-bottomed cages. The surviving animals exhibited a dose-related increase in both absolute and relative lung and liver weights at termination. Haemorrhagic deaths and increased absolute lung and liver weights were not observed in ddY male mice fed diets containing 1.35% 5.0% BHT for 30 days and housed in cages with soft-wood chip bedding, or in Hartley guinea-pigs fed 0.125% - 2.0% BHT in the diet for 14-17 days. The prothrombin times were significantly increased in all groups of ddY mice receiving BHT in the diet at levels of 1.0% or higher (with the exception of the 2.0% wire-caged group). The effect was of a similar magnitude (approximately 25%) in treated groups housed on soft-wood bedding, regardless of the dose. Results for kaolin-activated partial thromboplastin time were similar. Effects on coagulation in guinea-pigs were equivocal. The authors suggested that the coagulation defect in mice in the absence of a dose-response relationship might be due to minor damage to hepatobiliary function and/or fatty liver and the haemorrhages in the lungs to injury to that organ. The haemorrhage and coagulation defect would consequently not have the same cause as that observed in rats. BHT was detected in the livers of mice and guinea-pigs in dose-related concentrations (0.2-4/µg/g tissue). BHT-quinone methide was not detected in these species although it has been detected in the livers of rats (7-40 µg/g tissue) in a study by Takahashi *et al.* (1980) (Takahashi, 1992).

2.2.11 Special studies on effects on the thyroid

Male MOL/WIST SPF rats, outbred strain (approximately 200 g) were used for the study. BHT was added to a semi-synthetic diet in which the iodine content was controlled at about 12/µg/100 g (nutritional requirement for the rat is 15 µg/100 g). In one study, rats were fed 0, 500 or 5000 mg BHT/kg of feed for 8, 26 or 90 days, and the uptake of ^{125}I by the thyroid was determined. The presence of BHT in the diet resulted in a marked increase in the uptake of ^{125}I at all time periods studied. When rats were fed BHT in diets containing varying amounts of iodine (12, 150 or 300/µg/100 g) for 30 days, there was a significant increase in thyroid weight in BHT-treated animals when compared to controls. BHT in the diet of rats increased liver and thyroid weights at 5000 mg/kg of the diet, but only thyroid weight at 500 mg/kg. BHT did not change levels of T_3 and T_4 in the blood. The biological half-life of thyroxine was increased after 13 days on a BHT diet but returned to normal after 75 days. Electron microscopy of the thyroid glands of rats exposed to 5000 mg/kg BHT for 28 days showed an increase in the number of follicle cells (Sondergaard & Olsen, 1982).

2.2.12 Special studies on effects on the immune system

In vitro, BHT (50 /µg/culture) suppressed the plaque-forming cell response of mouse spleen cell cultures as measured by the method of Mishell & Dulton (Archer *et al.*, 1978).

Addition of cyclic GMP (cGMP added as the dibutyl or 8-bromo form) to BHT suppressed Mishell-Dulton cultures and effected a reversal of the BHT suppression of antibody production (Wess & Archer, 1982).

2.2.13 Special studies on potentiation or inhibition of cancer

Male Strain A mice were injected i.p. with 500 mg/kg bw urethan, then one week later received repeated injections (1/week for 8 weeks) of either 300 mg/kg bw BHT, or 500 mg/kg bw BHA, or 1000 mg/kg bw Vitamin E all dissolved in corn oil. At the termination of the study, only BHT was shown to produce a significant increase in tumour yield. Although the number of tumours produced by BHA treatment was greater than usual, it was not statistically significant. A/J mice treated with 3-methylcholanthrene or dimethylnitrosamine, followed by treatment with BHT (i.p.), resulted in an increase in tumour yield (Witschi *et al.*, 1981).

Groups of Charles River rats (20/sex) were fed diets (males 24 weeks, females 36 weeks) containing 6600 mg BHT/kg of feed and/or carcinogen (N-2-fluorenylacetamide or N-hydroxy-N-2-fluorenyl-acetamide) in the molar ratio of 30:1, then continued on control diets for another 12 weeks. The N-2-fluorenylacetamide alone resulted in hepatomas in 70% of the male rats, and mammary adenocarcinoma in 20% of the females. With N-hydroxy-N-2-fluorenylacetamide, 60% of the males had hepatomas and 70% of the females had mammary adenocarcinoma. BHT reduced the incidence of hepatomas in males to 20% when the carcinogen was N-2-fluorenylacetamide, and to 15% when N-hydroxyl-N-2-fluorenylacetamide was the test compound. Similar results were obtained with Fischer strain rats. Liver and oesophageal tumour production with diethylnitrosamines at 55 mg/litre in drinking-water for 24 weeks was not affected by BHT (Ulland *et al.*, 1973).

Groups of 20 male F344 rats were given a single intragastric administration of 100 mg/kg bw MNNG or 750 mg/kg bw EHEN, 2 s.c. injections of 0.5 mg/kg bw MBN or 4 s.c. injections of 40 mg/kg bw DMH. At the same time, the rats received 0.1% DBN for 4 weeks, followed by 0.1% DHPN for 2 weeks in drinking-water for a total carcinogen exposure period of 6 weeks. Three days after completion of these treatments, the rats received 0 or 0.7% BHT in the diet for 36

basal diet alone. Final body weights of both BHT-treated groups were significantly lower than those of respective controls (by 7% compared with carcinogen-treated control and by 13% compared with basal diet control) and this was reflected in higher relative kidney weights. Relative liver weights were increased by about 60-75%. Dietary BHT following carcinogen treatment eliminated the appearance of colon carcinomas and reduced the incidence and multiplicity of some preneoplastic and neoplastic lesions of the kidney. BHT administration increased the incidence of hyperplasia, adenomas and carcinomas of the thyroid gland and had no effect on tongue, oesophagus, forestomach, glandular stomach, duodenum, small intestine, liver, lung or urinary bladder (Hirose *et al.*, 1993).

2.2.13.1 Bladder

Male F344 rats were treated with 0.01 or 0.05% N-butyl-N (4-hydroxy-butyl) nitrosamine (BBN) in drinking-water for 4 weeks, then fed diets containing 0 or 1% BHT for 32 weeks. BHT in the diet was associated with a significant increase in the incidence of cancer and papilloma of the bladder of rats treated with 005%, but not 0.01% BBN (Imaida *et al.*, 1983).

Rats were administered 200 mg/kg N-2-fluorenylacetamide (FAA) in the diet alone or with 6000 mg/kg BHT for 25 weeks. No bladder neoplasms resulted from feeding FAA alone, but the combination of FAA and BHT resulted in 17/41 papillomas and 3/41 carcinomas in the bladder (Williams *et al.*, 1983).

Four dietary levels of BHT (300, 1000, 3000, or 6000 mg/kg) were simultaneously fed with 200 mg/kg FAA for 25 weeks. FAA feeding alone produced no neoplasms, but when combined with BHT at 3000 or 6000 mg/kg, the incidence of bladder tumours were 18% and 44%, respectively. The incidence of bladder tumours in the 300 and 1000 mg/kg BHT groups was low and not significantly different from the incidence with FAA alone (see also effects on liver) (Maeura & Williams, 1984).

Male F344 rats were given injections of methylnitrosourea (MNU) twice a week for 4 weeks, and then a basal diet containing 1% BHT for 32 weeks. BHT significantly increased the incidence of papilloma and papillary or nodular hyperplasia of the urinary bladder, and the incidence of adenoma (but not adenocarcinoma) of the thyroid (Imaida *et al.*, 1984).

Groups of 20 male F344 rats (6-week old) were pretreated with 0.05% N-butyl-N-(4-hydroxybutyl)nitrosamine in the drinking-water for 2 weeks and thereafter given diets containing 0, 0.25, 0.5, or 1% BHT. On day 22 of the experiment, the lower section of the left ureter of each rat was ligated. Animals were killed at week 24 of the experiment. BHT increased dose-dependently the incidence and number of preneoplastic lesions, papillary or nodular hyperplasia of the urinary bladder. The incidence of bladder lesions was increased particularly at 1% BHT (Fukushima *et al.*, 1987a).

Groups of 20 male F344 rats (6-week old) were given 0.05% N,N-dibutylnitrosamine in their drinking-water for 16 weeks, and simultaneously administered 0 or 0.7% BHT in the diet. The simultaneous administration of BHT led to increased incidence in liver lesions. The incidence of transitional cell carcinomas or papillary or nodular hyperplasia of the urinary bladder and papillomas or carcinomas of oesophagus was not altered. A decrease in hyperplastic nodules in the forestomach was observed (Imaida *et al.*, 1988).

Groups of 20 male F344 rats (6-week old) were pretreated with 0.05% N-butyl-N-(4-hydroxybutyl)nitrosamine in the drinking-water for 4 weeks and thereafter maintained on diets containing 0, 0.4% BHA + 0.4% BHT + 0.4% TBHQ, or 0.8% BHT. The study was terminated after 36 weeks. An increase in urinary crystals and incidence and density of papillary or nodular hyperplasia of urinary bladder epithelium was observed in all groups fed BHT-containing diets. The incidence of papillomas and carcinomas of the bladder was not increased and no proliferative changes were seen in renal pelvis. Hepatocyte hypertrophy was induced in the group administered 0.8% BHT (Hagiwara *et al.*, 1989).

Ten male F344 rats (6-week old) were given a diet containing 1% BHT with 7 mg/kg vitamin K. A decrease in body weight was observed. DNA synthesis in the urinary bladder epithelium was increased after 4 weeks while no morphological changes were seen after 8 weeks using light microscopy. Using electron microscopy, morphologic surface alterations such as formation of pleomorphic or short, uniform microvilli and ropy or leafy microridges were seen (Shibata *et al.*, 1989).

A study was performed to investigate early proliferation-related responses of the renal pelvic epithelium in response to bladder tumour promoters. Groups of 10 male F344 rats received 0 or 1% BHT. At week 4, the DNA-labelling index of the renal pelvic epithelial cells was determined from 1000 cells in 5 rats/group. At week 8, kidney sections were prepared for SEM examination. Body weights of the treated animals were significantly lower than for controls, 18% at 4 weeks and 25% at 8 weeks (Shibata *et al.*, 1989). The mean DNA labelling index in the renal pelvic epithelium after 4 weeks treatment was slightly higher than in controls, but without statistical significance. No cell surface alterations were observed by SEM after 8 weeks of treatment (Shibata *et al.*, 1991).

Groups of 20 F344 rats, 4 or 54 weeks of age, were injected s.c. with 50 mg/kg bw of 3,2'-dimethyl-4-aminobiophenyl (DMAB), a multi-organ carcinogen, once a week for 10 weeks. At the same time, the animals received 1% BHT in the diet for 11 weeks. The study was terminated 55 weeks after initiation of treatment. Combined treatment with DMAB and BHT resulted in the development of urinary bladder papillomas and carcinomas in more than 95% of both young and old rats.

The induction of liver foci and pancreatic acinar cell foci by DMAB was inhibited by concurrent treatment with BHT. Increased formation of DMAB-DNA adducts, detected by immunohistochemical staining was demonstrated in urinary bladder epithelial cells from the BHT-treated rats. BHT treatment suppressed the formation of these adducts in the liver and had no effect on formation of adducts in the colon, pancreas or prostate. The authors suggested that these effects of BHT were related to its ability to induce a number of drug-metabolizing enzymes, thus altering the quantity of active metabolites in a particular tissue (Shirai *et al.*, 1991).

Simultaneous feeding for 76 weeks of 6000 mg/kg BHT with 50 mg/kg AAF, a minimally effective carcinogenic dose of this compound in F344 rats, resulted in an increase in the incidence and multiplicity of urinary bladder neoplasms, including carcinomas. Feeding of both 3000 and 6000 mg/kg BHT in conjunction with AAF resulted in an increase in the incidence of nodular hyperplasia of the urinary bladder, and a positive trend in the incidence of this lesion was noted down to dietary levels of 300 mg/kg BHT. In the same study, dietary administration of 100 - 6000 mg/kg BHT resulted in a dose-related decrease in induction of altered hepatic foci (iron-storage deficient and GGT-positive) and a decrease in the multiplicity of AAF-induced adenomas and carcinomas of the liver and the incidence of carcinomas (Williams *et al.*, 1991).

2.2.13.2 GI tract

Mice

Groups of male BALB/c mice treated intrarectally with methyl-nitrosurea, and then maintained on diets containing BHT, showed a marked increase in the incidence and multiplicity of GI tract tumours when compared to treated mice maintained on BHT-free diets. In another study, BALB/c mice were injected with dimethylhydrazine (6 weekly injections) and then maintained on control (BHT-free) diets or on diets containing 0.05% or 0.5% BHT. The colon tumour incidence were 10%, 0%, and 32%, in the respective groups (Lindenschmidt *et al.*, 1986).

Rats

The observed increase in tumour-specific antigen activity in the colon chromatin of rats treated with 1,2-dimethylhydrazine was eliminated by simultaneous treatment with BHT (Gabryelak *et al.*, 1981).

Male F344 rats were treated with a single dose of N-methyl-N'-nitro-N-nitrosoguanidine, and then maintained on diets containing no BHT, 1.0% BHT, 5% NaCl, or 5% NaCl + 1.0% BHT for 51 weeks. The incidence of squamous cell carcinomas of the forestomach were 11%, 16%, 3%, and 53% in the respective groups (Shirai *et al.*, 1984).

When rats were given 0.5% BHT in the diet for 36 weeks following 4 injections (1 per week) of 1,2-dimethylhydrazine, BHT did not affect the number of rats with colon tumours, but the number of tumours per rat occurring in the distal colon was significantly decreased (Shirai *et al.*, 1985).

Wistar rats fed 1.0% BHT in the diet during treatment with N-methyl-N'-nitro-N-nitrosoguanidine (administered in drinking-water at a concentration of 1.0 mg/ml) for 25 weeks, and then maintained on the test diet for another 14 weeks, showed a significant reduction in the incidence of gastric cancer, when compared to rats receiving BHT-free diets (82% versus 37%) (Tatsuta *et al.*, 1983).

Seven-week old male Wistar rats (20/group) were given N-methyl-N'-nitro-N-nitrosoguanidine in the drinking-water (100 mg/litre) for 8 weeks, and were also fed a diet supplemented with 10% sodium chloride. Thereafter, they were maintained on a diet containing 1% BHT for 32 weeks. A carcinogen control group was fed the basal diet without BHT supplementation. The experiment was terminated 40 weeks after the beginning of administration of MNNG. BHT did not increase the incidence of tumours in the glandular stomach or in the forestomach (Takahashi *et al.*, 1986).

Groups of 21 male F344 rats were given 0.5 g/litre N,N-dibutyl-nitrosamine in drinking-water for 4 weeks and then treated with a basal diet containing 1% BHT with 7 mg/kg vitamin K for 32 weeks. BHT enhanced oesophageal carcinogenesis (papillomas: 16/21 versus 3/21; carcinomas 9/21 versus 0/21) but did not enhance forestomach carcinogenesis. BHT induced an increased incidence of papillary or nodular hyperplasia and papilloma in the bladder, while no statistically significant increase was seen in liver lesions (Fukushima *et al.*, 1987b).

Groups of 5 male F344 rats were given diets containing 0 or 0.7% BHT for 4 weeks. Histological examination of the forestomach showed that BHT did not induce hyperplasia in the forestomach epithelium (Hirose *et al.*, 1987).

When male Fischer 344 rats were fed diets containing 0, 0.5% or 1.0% BHT for 5 or 6 months immediately following initiation with 2 or 4 s.c. injections of DMH (40 mg/kg bw), a significantly higher incidence of colon tumours (5-month study) and a significantly increased incidence of small intestinal tumours (duodenum, jejunum, and ileum) were seen in the BHT-treated animals than in the animals fed a BHT-free control diet. Administration of N-nitroso-N-methylurea (NMU; 90 mg/kg bw given orally) produced stomach and colon tumours; 0.5% BHT in the diet did not affect tumour incidence. It was concluded that dietary BHT may enhance development of gastrointestinal tumours produced by DMH, but not by NMU, provided exposure to BHT occurs after exposure to the carcinogen (Lindenschmidt *et al.*, 1987).

Hamsters

Male Syrian golden hamsters were given a diet containing 1% BHT. Induction of hyperplasia and neoplastic lesions of the forestomach were examined histopathologically and autoradiographically at weeks 1, 2, 3, 4, and 16. Mild hyperplasia occurred slightly more often in hamsters fed the BHT diet than in the control group. BHT induced no severe hyperplasia or papillomatous lesions. No significant increase in the labelling index was observed at any time during the experiment (Hirose *et al.*, 1986).

2.2.13.3 Liver

Groups of 93 rats (22-day old) received 0 or 0.5% BHT diets for 407 days following 18 days of administration of AAF (0.02%). Prolonged feeding of BHT diet after AAF produced a significant increase of liver tumours (Peraino *et al.*, 1977).

Rats were administered 200 mg/kg AAF in the diet, alone or with 6000 mg/kg BHT for 25 weeks. AAF alone induced a 100% incidence of liver neoplasms. Simultaneous administration of BHT resulted in a decreased frequency of benign neoplasms, neoplastic nodules and malignant neoplasms, and hepatocellular carcinomas (Williams *et al.*, 1983).

BHT at concentrations of 300, 1000, 3000, or 6000 mg/kg was fed simultaneously with 200 mg/kg AAF for 6, 12, 18, or 25 weeks. BHT produced a reduction in the incidence of tumours in a dose-dependent manner (100% incidence in the absence of BHT to 56% at 6000 mg/kg BHT) (see also effects on the bladder) (Maeura *et al.*, 1984).

Rats were fed 200 mg/kg AAF for 8 weeks, then diets containing BHT at levels of 300, 1000, 3000, or 6000 mg/kg for up to 22 weeks. The area of altered hepatocellular foci, identified by iron exclusion and gamma-glutamyl transferase (GGT) activity, that was induced by feeding the AAF, showed increased development at the highest level of BHT (the number of foci, the area occupied by GGT-positive preneoplastic and neoplastic lesions, and the neoplasm incidence were increased). These parameters were unaffected at the lower BHT levels (Maeura & Williams, 1984).

Rats were given a single i.p. injection of 200 mg/kg bw of diethylnitrosamine, and then maintained on a diet containing 1% BHT for 6 weeks. At week 3 the rats were subjected to partial hepatectomy. The number of gamma-glutamyl transpeptidase positive foci in the liver of BHT-fed rats was significantly decreased when compared to controls (Imaida *et al.*, 1983).

BHT was compared to phenobarbital (PB) and DDT with respect to its effect on liver carcinogenesis in male Wistar rats using an initiation-selection-promotion protocol. The rats were initiated with a single dose of diethylnitrosamine (DEN; 200 mg/kg bw). Two weeks later, selection was carried out by feeding AAF for 2 weeks and giving a necrogenic dose of carbon tetrachloride after 1 week. After another week the rats were maintained on a diet with the promoters, or BHT at a level of 0.5%. Groups of 8-10 animals were examined after 3, 6, 14,

the frequency of GGT-positive lesions in the liver at week 14, but in contrast to PB and DDT, BHT did not enhance the development of hepatocellular carcinomas at week 22. It was suggested that BHT was not a promoter of liver carcinomas in male Wistar rats when given after initiation (Préat *et al.*, 1986).

Initiation of liver carcinogenesis with a single dose of diethylnitrosamine (DEN), and selection with AAF combined with a proliferative stimulus (CCl_4 administration), was followed by a treatment with PB or BHT (0.5% in the diet) for periods up to 22 weeks. Control animals received no treatment after the initiation and selection procedure. An increase in the amount of 2N nuclei was found in the putative preneoplastic lesions of animals that received initiation and selection (I-S) and 3 weeks basal diet. When the diet was supplemented with PB (after I-S), the increase in diploid nuclei started earlier. At the time carcinomas appeared (22 weeks PB treatment) a decrease in the frequency of 2N nuclei was found. BHT-treated animals which develop no carcinoma within the considered time span showed a clear increased amount of 2N nuclei in the precancerous lesions only after 14 weeks treatment (Baesen *et al.*, 1988).

Dietary administration of 1% BHT for 26 weeks was commenced during or immediately after 2 weekly i.p. injections of azaserine (30 mg/kg bw) to male Wistar rats. Administration of BHT after azaserine enhanced the frequency of GST-A positive focal pancreatic acinar lesions, while GST-P positive hepatocellular lesions were significantly reduced. When BHT was given together with azaserine, no effect was seen in the liver, while the frequency of preneoplastic lesions in the pancreas was significantly reduced (Thornton *et al.*, 1989).

A study was conducted to establish whether the modulating effect of dietary fats and BHT on AAF-induced hepatocarcinogenesis was related to the levels of cytochrome P-450 in the nuclear envelope (NE). Treatment of weanling rats with 0.3% dietary BHT for 2 weeks was followed by up to 16 weeks of treatment with 0.05% AAF in the diet. Prior treatment with BHT protected against the AAF-induced reduction in the NE cytochrome P-450 content of liver cells for 9 weeks in rats fed a diet with a high saturated fat content, compared with 3 weeks in rats fed a diet with a high polyunsaturated fat content. The magnitude of this effect of BHT on cytochrome P-450 content was apparently correlated with its tumour reduction effect in rats fed AAF in high-fat diets. The results from rats fed low-fat diets, in which BHT apparently does not exert a significant effect on AAF-induced carcinogenesis, were different from those in rats fed the high-dose diets. In rats fed low-fat diets, AAF did not reduce cytochrome P-450 content until 9 weeks of feeding, at which point prior treatment with BHT resulted in a partial reversal of this effect. In the first 3 weeks when AAF-treated rats had cytochrome P-450 content similar to control animals, prior treatment with BHT resulted in its induction in the AAF-treated animals (Carubelli & McCay 1989).

BHT at concentrations of 1-14 mM in the presence of a rat liver microsomal preparation reduced the binding of AAF to calf thymus DNA to 20% of control values. When N-hydroxylated 2-AAF, a cytochrome P-450 activated oxidation product of AAF was used, BHT (0.1-8.0 mM) reduced its binding to DNA to only 80% of control levels. In rat hepatocyte cultures, BHT concentrations of 0.01-0.10 mM resulted in a reduction of AAF binding to DNA to about 85% of control levels. These results showed that mechanisms in addition to those demonstrated *in vivo* (i.e. induction of detoxifying enzymes) may be involved in the anticarcinogenic effects of BHT (Richer *et al.*, 1989).

Male $B6C3F_1$ mice were injected i.p. with 100 or 200 µmol/kg bw of diethylnitrosamine (DEN) once a week for 10 weeks. After a recovery interval of 4 weeks, the mice were fed diets containing 5000 mg/kg BHT or 500 mg/kg phenobarbital (positive control) for 24 weeks. At the end of this time, DEN alone induced a dose-related incidence of altered hepatic foci and hepatocellular adenomas. Treatment with BHT following DEN administration had no effect on the incidence or multiplicity of these lesions, whereas phenobarbital administration potentiated the effects of DEN 2 to 3 fold (Tokumo *et al.*, 1991).

Dietary administration of 50 mg/kg AAF was found to induce a 100% incidence of liver neoplasms after 76 weeks. Concurrent administration of BHT at levels of 100 - 6000 mg/kg of feed inhibited the induction of altered hepatic foci and reduced the multiplicity of hepatocellular adenomas and carcinomas and the incidence of carcinomas (Williams *et al.*, 1991).

Concurrent administration of BHT in the diet at 5, 25 or 125 mg/kg of feed for 42 weeks with gavage administration of aflatoxin B_1 (5 µg/kg bw, 3 times a week) for the final 40 weeks resulted in a reduction in the density of hepatic foci staining positive for the placental form of glutathione S-transferase at the highest dose of BHT (Iatropoulos *et al.*, 1994).

2.2.13.4 Lung

The tumorigenic potency of a single i.p. injection of 1000 mg/kg bw of urethane to male Swiss-Webster mice was significantly increased if followed by repeated weekly injections with 250 mg/kg bw BHT. The number of animals/group ranged from 9 to 22 and the animals were treated for 9 to 13 weeks. Only tumours on the lung surface itself were counted. About 90% of the animals treated with urethane alone developed lung tumours. There was a significant increase in the number of tumours/mouse after 11 or more weeks of treatment with BHT. Animals treated with BHT alone did not develop lung tumours. A/J strain mice were also given the same treatment with 10 weekly injections of BHT The number of lung tumours/mouse significantly increased in those receiving BHT in addition to urethane in comparison with those receiving urethane alone. With both strains of mice, repeated injection of BHT without prior urethane treatment did not result in an increased number of animals with lung tumours or tumours/mouse as compared to controls dosed with corn oil. With both mouse strains, there were fewer lung tumours in the animals given BHT as compared to the corn oil controls. In contrast to the above results, injection of animals with BHT for 0-7 days before urethane injection did not increase the number of animals with tumours or number of tumours/mouse (Witschi & Cote. 1976).

Groups of Swiss mice were given 50, 250, or 1000 mg/kg bw urethane or 0.9% NaCl. After 7 days, half the urethane-treated animals and half the controls received 300 mg/kg bw BHT i.p., the remaining animals receiving corn oil alone. The animals received 13 injections/week. The number of tumours/lung found 14-24 weeks after the initial urethane doses was significantly increased in the BHT-treated animals.

In another study, when the interval between injection of the urethane and the first treatment with BHT was delayed for 6 weeks, BHT treatment produced more tumours. When the number of BHT injections commencing 1 week after urethane treatment was reduced from 13 to 4, the same significant increase in tumour yield was observed as in the 13-dose study. However, 1 or 2 doses of BHT had no significant effect. When the mice were pretreated with 13 injections of BHT, and then treated with urethane 1 week later, there was no enhancement of tumour yield. Simultaneous administration of BHT and urethane resulted in

mouse strains (C57BL, C3H and BALB/C) which have a low naturally occurring incidence of lung adenoma were treated with urethane and then with multiple injections of BHT, the BHT treatment did not significantly increase tumour incidence or average numbers of lung tumours (Witschi & Lock, 1979).

Male A/J mice were injected i.p. with a single dose of urethane and then fed 0.75% of either BHT, BHA, or ethoxyquin in the diet, once a week or continuously for 8 weeks. Lung tumour yield was scored 4 months after the urethane treatment. Dietary BHT, but not BHA or ethoxyquin, under both test conditions, enhanced lung tumour formation.

Mice were fed diets containing BHA or BHT for 2 weeks prior to urethane treatment, and then maintained on conventional laboratory diets for 4 months. The BHT diet had no effect on tumour yield, but the BHA treatment significantly decreased the average number of tumours (Witschi, 1981).

A/J mice were given a single dose of BHT i.p. (400 mg/kg bw), sufficient to cause acute lung damage and produce cell proliferation in the lung for 6 to 7 days. Urethane was administered continuously by implanted mini pumps during this period. Continuous presence of urethane during the period of cell division did not result in an enhanced number of the tumours. When urethane-injected mice were dosed i.p. with SKF525A (2-diethylaminoethyl-2-,2-di-phenylvalerate hydrochloride) and BHT (SKF inhibits lung cell division normally seen following BHT administration), or BHT alone, both treatment gave a very significant increase in lung tumour yield compared to urethane-treated controls. Repeated pulmonary cell division brought about by other treatment e.g., 95-100% oxygen, were also shown not to enhance tumour development (Witschi & Kehrer, 1982).

BHT was shown to enhance the lung tumour incidence in mice treated with doses of urethane greater than 50 mg/kg bw. At lower doses of urethane (subcarcinogenic doses) BHT did not enhance tumour development. In another study, it was shown that following treatment of mice with urethane, a two-week exposure to 0.75% BHT in the diet was sufficient to enhance tumour development, and that 0.1% BHT was an effective enhancer when fed for 8 weeks.

BHT, administered within 24 h post-treatment and fed for 8 weeks, enhanced tumour development in mice treated once with 3-methylcholanthrene, benzo(a)pyrene, or N-nitrosodimethylamine.

When mice were injected weekly with BHT, there was a rapid increase in cell proliferation, and in both the cumulative labelling index (incorporation of ^{3}H-thymidine) and the number of labelled type II cells. These effects were smaller after each injection, and by the fifth injection, no increase was observed (Witschi & Morse, 1985).

A single i.p. injection of BHT (200 mg/kg bw) 6 h before a single urethane injection (1000 mg/kg bw) had varying effects on lung tumorigenesis in mice of different strains and ages. Strains exhibiting both high (A/J, SWR/J) and low (BALB/cByJ, 129/J, C57BL/6J) susceptibility to urethane tumorigenesis were tested. BHT treatment decreased tumour multiplicity by an average of 32% in adult A/J mice but acted as a cocarcinogen by increasing tumour number 48% in adult SWR/J mice, 240% in adult C57BL/6J mice, 655% in adult 129/J mice, and 38% in 14-day old A/J mice. The numbers of both alveolar type 2 cell-derived and bronchiolar Clara cell-derived lung adenomas were similarly affected by these BHT treatments. BHT pre-treatment had no effect on adenoma multiplicity in either young or adult BALB/cByJ mice. Multiplicity in young BALB cByJ mice was also unaffected by chronic BHT administration (6 injections/week) following urethane, while multiplicities increased several-fold with such treatment in adult mice of this strain (Malkinson & Thaete, 1986).

A/J mice given 1000 mg/kg bw urethane followed by 400 mg/kg bw BHT by injection, developed 40% more lung tumours than mice treated with urethane alone. In mice treated with 3-methylcholanthrene, repeated injections of BHT (300 mg/kg bw) increased tumour multiplicity by a much larger factor (500-800). Pretreatment of mice with BHT reduced the number of tumours produced by methylcholanthrene. The enhancing effect of BHT on lung tumour development was not due to the production of diffuse alveolar cell hyperplasia (Witschi, 1986).

Lung tumour promotion by BHT and 3 of its metabolites was compared in the inbred mouse strain MA/MyJ. MA/MyJ mice were given a single injection of urethane (50 mg/kg bw) followed by 6 weekly i.p. injections of 50 or 200 mg/kg bw BHT, BHT-BuOH, 2,6-di- tert-butyl-4-hydroxymethyl phenol (BHT-MeOH) or 2,6-di- tert-butyl-1,4-benzo-quinone (DBQ). The only metabolite that enhanced lung tumour formation was BHT-BuOH, and it was effective at one-fourth the effective dose of BHT. The study implicates BHT-BuOH formation as an important step in the chain of events leading to promotion of lung tumours (Thompson *et al.*, 1989).

The susceptibility of different strains of mouse to the lung tumour-promoting effects of BHT was correlated with ability of hepatic microsomal preparations from each strain to produce a metabolite of BHT, BHT-BuOH, which is hydroxylated on one *tert*-butyl group. No correlation existed between tumour promotion and microsomal production of BHT-MeOH (hydroxylated on the methyl group) or DBQ (the quinone metabolite). In the MA/MyJ strain, which was found to be the most sensitive to promotion of urethane-induced lung tumours by BHT, 6 weekly i.p. injections of 50 mg/kg bw of the BHT metabolite, BHT-BuOH, resulted in a similar promotional effect to 200 mg/kg bw BHT, while 200 mg/kg bw of the BHT metabolites, BHT-MeOH or DBQ, had no promotional effect. The authors cited other evidence which implicated the *tert*-butyl hydroxylation pathway in lung-tumour promotion by BHT: preferential *in vitro* formation of this metabolite relative to other metabolic products of BHT was correlated with species (rat versus mouse) and strain susceptibility to lung tumour promotion by BHT; the repeat-dose administration regimen of BHT associated with lung tumour formation was also associated with induction of hydroxylation on the *tert*-butyl group; and this pathway is a major route of metabolism in the mouse lung (Thompson *et al.*, 1989).

Evidence was cited to show that the genes which regulate sensitivity to the lung tumour-promoting effects of BHT are distinct from the *pas* (pulmonary adenoma susceptibility) genes which predispose some inbred strains of mice to the development of lung tumours. It was suggested that strain differences in response to the effects of BHT are mediated through genes which regulate the ability to metabolize BHT along specific pathways (Malkinson, 1991).

2.2.13.5 Mammary gland

Groups of female Sprague-Dawley rats were treated with 7,12-dimethylbenz[a]anthracene (DMBA) or nitrosomethylurea (NMU), and then fed diets containing 0 or 0.3% BHT for 30 weeks. Rats treated with DMBA and maintained on the control diet developed 100% tumour incidence (mammary gland) by week 27, whereas those maintained on the BHT supplemented diet had an incidence of 54% by the end of the study. Dietary BHT had no effect on the incidence of tumours induced by NMU treatment (King *et al.*, 1981).

Female rats were fed diets containing 0, 0.25, or 0.5% BHT. The test diets were administered either (a) 2 weeks before until 1 week after DMBA administration or (b) 1 week after DMBA administration to

dose of 8 mg. BHT was an effective inhibitor of mammary carcinogenesis when administered during either of these time frames (20% inhibition by regime (a) and 50% by regime (b)) (McCormick *et al.*, 1984).

Dietary BHT was shown to decrease the incidence of mammary tumours induced in female Sprague-Dawley rats by DMBA but had no effect on animals treated with MNU (King *et al.*, 1981).

The inhibitory effect of BHT was strongly influenced by the dose of initiating carcinogen and the type of diet in which BHT was fed. Administration of BHT in the AIN-76A diet, showed a markedly different effect from BHT in the NIH-07 diet. In the AIN-76A diet, 6000 mg/kg BHT had no effect on the incidence of mammary tumours induced by 15 mg DMBA, whereas a similar level of BHT in the NIH-07 diet resulted in a 40% inhibition of tumour development (Cohen *et al.*, 1984).

A dose-related inhibition of DMBA-induced mammary tumorigenesis in female Sprague-Dawley rats was seen after long-term exposure to dietary BHT. BHT was given from 14 days before carcinogen administration to termination at 210 days. In animals fed the cereal-based NIH-07 diet and receiving a low dose (5 mg/rat) of DMBA, there was a significant overall inhibitory trend in tumour incidence observed among those receiving 300, 1000, 3000, or 6000 mg BHT/kg of feed. Maximal inhibition was approximately 50% at the highest concentration of BHT. The inhibitory effect of BHT on mammary tumour incidence was less pronounced when BHT was administered to rats initiated with a high carcinogen dose. At 15 mg DMBA/rat, maximal inhibition was only 20% at the highest concentration of BHT. Similar results were obtained when BHT was fed in the casein-based AIN-76A diet. The inhibition seen in this study was less pronounced than that seen in an earlier study using short-term exposure to BHT (Cohen *et al.*, 1986).

Retinyl acetate (RA) and BHT had additive effects in inhibiting mammary carcinogenesis in female Sprague-Dawley rats. Chronic exposure to RA plus BHT induced a high incidence of hepatic fibrosis and bile duct hyperplasia; these changes were not observed in controls and were seen in low incidence in animals exposed to RA only or BHT only (McCormick *et al.*, 1986).

The effect of dietary administration of BHT on the formation of DNA adducts in the mammary gland by DMBA was investigated in female Sprague-Dawley rats using ^{32}P post-labelling techniques. Diets containing 0.4% or 0.8% BHT were fed to 39-day old rats for 2 weeks followed by oral administration of 32 mg/kg bw DMBA. BHT treatment resulted in a 42% or 36% reduction in the formation of all types of DMBA-derived DNA adducts 22 h later compared with DMBA-treated controls. Dietary BHT selectively inhibited the formation of adducts derived from the *anti* diastereomer of DMBA as opposed to the *syn* diastereomer. Dietary concentrations of BHT within the range used in this study have been reported to significantly inhibit the initiation of DMBA-induced carcinogenesis (Singletary & Nelshoppen, 1991).

In this study, the effects of BHT and its metabolites BHT-MeOH and DBQ (BHT-quinone) on DMBA-induced rat mammary carcinogenesis and the *in vivo* formation of rat mammary DMBA-DNA adducts were tested. The selection of these metabolites was based on the observation that they were the major metabolites detected following incubation of BHT with rat liver microsomes and that no metabolites were detected following inhibition with rat mammary microsomes. Each of the compounds were administered 2 weeks before and 1 week after oral administration of 33 mg/kg bw DMBA. The i.p. administration of 200 mg/kg bw BHT and DBQ (but not BHT-MeOH) resulted in 39% and 25% inhibition of mammary tumour formation, respectively. There was a good quantitative correlation between inhibition of mammary tumorigenesis by BHT and DBQ and the inhibition of DMBA-DNA adduct formation. Doses of 100 and 200 mg/kg bw BHT inhibited DMBA-DNA binding to a similar degree. BHT and DBQ differed in their selectivity of inhibition of specific adducts. A decrease in the formation of the *anti*-dihydrodiolepoxide adduct of DMBA to deoxyguanosine was most closely correlated with the abilities of BHT and DBQ to inhibit mammary tumorigenesis (Singletary *et al.*, 1992).

2.2.13.6 Pancreas

Male LEW inbred rats were given an injection of 30 mg azaserine once a week for 3 weeks, and then maintained on diets containing 0 or 0.45% BHT for 4 months. BHT treatment reduced the number of acidophilic foci per pancreas by 32%, but was without effect on focal size. BHT had no effect on the occurrence of basophilic foci (Roebuck *et al.*, 1984).

2.2.13.7 Skin

BHT had no tumour-initiating activity when tested in a two-stage mouse skin carcinogenesis model using 12-O-tetradecanoyl phorbol-13-acetate (TPA) as a promoter. BHT was applied twice weekly for 5 weeks at a total dose of 100 mg (Sato *et al*, 1987a).

The hydroperoxide metabolite of BHT, BHTOOH (2,6-di- *tert*-butyl-4-hydroperoxyl-2,5-cyclohexadienone), was an effective inducer of epidermal ODC activity in SENCAR mice. Maximal induction of ODC activity was observed 12 h after a single application of BHTOOH. Papilloma and carcinoma formation was observed when BHTOOH was applied twice weekly for 50 weeks to mice previously initiated with DMBA. Doses of 2, 8, and 20 µmol BHTOOH gave maximal papilloma responses. Progression of papillomas to carcinomas occurred after 60 weeks. The data suggest that BHTOOH, unlike BHT, is an effective tumour promoter in mouse skin. No papillomas or carcinomas were observed in uninitiated mice treated with BHTOOH only (Taffe & Kensler, 1988).

2.2.14 Special studies on other effects

Young adult BALB/c mice of both sexes were maintained on diets containing 0.75% BHT for 1 month, and then irradiated with 525-750 R of X-ray. Radiation protection was observed at all doses below that which produced 100% lethality (Clapp & Satterfield, 1975).

Hybrid ($C3H_1F_1$ male mice, 10-12 weeks of age, were maintained on diets containing 0 or 0.75% BHT, for a period of 30 days, and then injected i.p. with alkylating materials. There was a marked reduction in the 30-day mortality in mice fed BHT Males were protected against ethyl methane-sulphonate, n-propyl or isopropyl methanesulphonate, ethyl dibromide, diethylnitrosamine and cyclophosphamide, but not against methyl methane-sulphonate, N-methyl-N'-nitro-N-nitroso-guaridine or dipropylnitrosamine (Cumming & Walton, 1973).

Rats dosed with ^{14}C-aflatoxin B_1 and fed BHT (0.5% in the diet) showed an enhanced excretion of water-soluble metabolites of ^{14}C-aflatoxin B_1 in the urine and faeces. In addition, BHT pretreatment was shown to decrease the amount of ^{14}C bound to hepatic nuclear DNA (Fukayama & Hsieh, 1985).

2.3 Observations in humans

Double-blind, placebo-controlled challenge tests with a 1:1 mixture of BHT and BHA (50 mg) were carried out in 44 cases of chronic urticaria, 91 cases of atopic dermatitis, and 123 cases of contact dermatitis. No positive reactions were seen (Hannuksela & Lahti, 1986).

Support bandages containing BHT were found to induce allergic contact dermatitis in two leg ulcer patients. The lesions showed improvement when use of the bandages in question was discontinued. Both patients gave a positive response to skin patch testing with BHT (Dissanayake & Powell, 1989).

A study was conducted to evaluate the sensitizing risk of BHT based on patch testing of 1336 eczema patients and estimates of exposure derived from a data base on chemical products. During a 2-year period in which the patients were tested, BHT produced no positive reactions. It was concluded that these results, together with the frequent use of BHT in industrial settings and consumer products, suggested that concentrations of BHT encountered in normal use would not induce allergic contact dermatitis (Flyvholm & Menné, 1990).

Two patients with chronic idiopathic urticaria were subjected to double-blind, placebo-controlled, oral challenges with a series of food additives. During testing, BHT and BHA were identified as causative agents. Avoidance of foods containing BHT and BHA resulted in long-term reduction in severity and frequency of urticarial episodes (Goodman *et al.*, 1990).

Using platelets from healthy volunteers, the basis for the inhibitory effects of BHT on thrombin-induced platelet activation was investigated. Concentrations of 20-300 µM BHT in incubation mixtures of platelets resulted in a dose-dependent activation of protein kinase C. The authors indicated that this action desensitizes platelets against subsequent phospholipase C activation associated with platelet activation by the physiological agonists thrombin and collagen (Ruzzene *et al.*, 1991).

BHT concentrations of 100 µg/ml were cytotoxic to human peripheral lymphocytes in culture. Concentrations up to 60 µg/ml had no effect on tritiated thymidine uptake in phytohaemagglutinin-stimulated lymphocytes, although a dose-related synergistic inhibition with cortisol or prednisolone was noted. The mixed lymphocyte reaction (MLR) was suppressed by concentrations of 50 µg/ml BHT. There was a linear relationship between the stimulation ratio for lymphocytes from each pair of subjects and the extent of inhibition of the MLR by 50 µg/ml BHT. The concentrations of BHT used in lymphocyte cultures in this study were not considered by the authors to be relevant to plasma concentrations achieved with dietary exposures (Klein & Bruser, 1992).

3. COMMENTS

The effects of long-term BHT administration have been adequately documented in a number of rodent studies, in only one of these studies (Olsen *et al.*, 1986) conducted in the Wistar rat, was a hepatocarcinogenic effect evident. This study differed from those conducted previously in that the rats were exposed to BHT *in utero*, during the lactation period, and for a further 40 weeks after the standard 2-year exposure period. The dose levels employed were 25, 100 or 500 mg/kg bw/day. It was necessary to reduce the highest dose from 500 mg/kg bw/day in the reproduction segment to 250 mg/kg bw/day in the long-term feeding portion of the study. A statistically significant increase in the survival-adjusted incidence of hepatocellular neoplasms was observed in both male and female rats at the highest dose tested. The majority of these tumours were not malignant; however, the incidence of hepatocarcinomas was significantly higher in male rats in the high-dose group than in untreated males. The tumours were detected very late in the study, in most cases when the animals were killed following 141-144 weeks of treatment. The NOEL was 25 mg/kg bw/day, based on effects on litter size, sex ratio, and pup body-weight gain during the lactation period in the reproduction segment of the study.

The Committee was aware that the above study had been reviewed by the International Agency for Research on Cancer (IARC, 1986), and concluded that it was difficult to draw conclusions about the observed incidence of liver lesions in the treated groups because of the large differences in survival between treatment and control groups. The carcinogenicity of BHT to humans could not be evaluated.

The protocol employed in the new study (Price, 1994), the purpose of which was to investigate the hepatic changes in male Wistar rats that occur following *in utero* and lifetime exposure to BHT for up to 22 months, was almost identical to that of the Olsen *et al.*, 1986 study. In the new study, hepatomegaly was observed in the F_0 dams receiving the highest dose (500 mg/kg bw/day), while toxicologically significant liver enlargement was not observed in any F_1 dose group up to 250 mg/kg bw/day, the highest dose tested. The body weights of the pups from the highest dose group were significantly lower than those of control pups throughout the lactation period, and mortality was increased in the treated pups in a dose-related manner between days 6 and 21 of lactation. In the F^1 males, BHT administration resulted in a persistent, marked induction of cytochrome P-450 2B and gamma-glutamyl transpeptidase activity in the centrilobular and periportal hepatocytes in the highest dose group throughout the study, commencing at a very early stage (21 days of age); gamma-glutamyl transpeptidase activity was also increased, but to a lesser extent, at the middle dose (cytochrome P-450 2B activity was not determined). Total cytochrome P450 content and epoxide hydrolase, ethoxyresorufin *O*-deethylase, and glutathione *S*-transferase activities were also consistently elevated in a dose-related manner in the mid- and highest dose groups. In a separate study, uridine diphosphate (UDP)-glucuronosyl transferase activity was induced in the liver of male Wistar rats receiving BHT at a dose level of 5 g/kg (equivalent to 500 mg/kg bw/day). Consistent enlargement of the centrilobular hepatocytes was evident starting at 6 months in rats receiving the highest dose of 250 mg/kg bw/day, indicative of proliferation of the smooth endoplasmic reticulum consistent with the induction of mixed-function oxidases. However, histopathological examination failed to reveal any signs of hepatocellular necrosis in this group. In addition, no evidence of hepatoxicity as indicated by a decrease in glucose 6-phosphatase activity and intracellular glutathione content in the treated groups, was seen. There was no indication of sustained hepatocellular proliferation during the study. A small, but significant, number of altered hepatic foci deficient in glucose-6-phosphatase were found in the highest dose group (250 mg/kg bw/day) at 22 months. In this same group, lesions described as hepatic nodules were detected in 6/19 animals as compared with none in the lower-dose groups and controls. Evidence of thyroid enlargement with follicular hyperplasia, in the absence of elevated serum thyroxine levels, was noted in the groups receiving 100 and 250 mg/kg bw/day. There was evidence in the mid- and highest dose groups of an early, transient effect on the adrenal cortex before sexual maturation of the F_1 males, which took the form of cytomegaly of the cells of the zona fasciculata.

BHT has been shown to induce hepatocellular necrosis and proliferation in male Wistar rats at doses higher than those used in either of these long-term studies and which exceeded the maximum tolerated dose. Sublethal oral doses of 1000 or 1250 mg/kg bw/day for 4 days induced hepatocellular necrosis in the centrliobular region within 48 hours. When a lower dose of BHT (500 mg/kg bw/day) was administered for periods of 1 to 4 weeks, allowing for enzyme induction to occur, bile duct proliferation, hepatocellular hyperplasia, and persistent fibrous and inflammatory cell reactions were observed in the periportal region. The shift in the localization

involvement of inducible hepatic drug-metabolizing enzymes in the production of reactive metabolites.

4. EVALUATION

In view of the probable involvement of hepatic enzyme induction in the development of the hepatocellular damage associated with repeated doses of BHT, the Committee concluded that, in this case, enzyme induction was the most sensitive index of effects on the liver. A well-defined threshold was demonstrated at 100 mg/kg bw/day in the long-term study reviewed for the first time at this meeting, giving a NOEL of 25 mg/kg bw/day. Effects observed in the reproduction segments of the *in utero*/lifetime exposure studies were also taken into account in the derivation of this NOEL. The Committee used a safety factor of 100 to allocate an ADI of 0-0.3 mg/kg bw for BHT.

5. REFERENCES

ADAMSON, I.Y.R., BOWDEN, D.H., COTE, M.G. & WITSHI, H. (1977). Lung injury induced by butylated hydroxytoluene: cytodynamic and biochemical studies in mice. *Lab. Invest.*, 36: 26-32.

AKAGI, M. & AOKI, I. (1962a). *Chem. Pharm. Bull.* (Tokyo), 10: 101.

AKAGI, M. & AOKI, I. (1962b). Studies on food additives. VIII metabolism of alpha-hydroxy-2,6-di- tert-butyl-p-cresol. Isolation of metabolites. *Chem. Pharm. Bull.* (Tokyo), 10: 200-204.

ALLEN, J.R. (1976). Long-term antioxidant exposure effects on female primates. *Arch. Environ. Health*, 31: 47-50.

ALLEN, I.R. & ENGBLOM, J.F. (1972). Ultrastructural and biochemical changes in the liver of monkeys given butylated hydroxytoluene and butylated hydroxyanisole. *Food Cosmet. Toxicol.*, 10: 769-779.

AMES, SR., LUDWIG, M.I, SWANSON, W.J. & HARRIS, P.L. (1956). *Proc. Soc. Exp. Biol.* (N.Y.), 93: 39.

AOKI, I. (1962). *Chem. Pharm. Bull.* (Tokyo), 10: 105.

ARCHER, D.L., SMITH, B.G. & BUKOVIC-WESS, J.A. (1978). Use of an *in vitro* antibody-producing system for recognizing potentially immunosuppressive compounds, *Int. Arch. Allergy Appl. Immunol.*, 56: 90-93.

BIBRA. (1977). British Industrial Biological Research Association. BHT and lung tumours. *BIBRA information Bulletin*, 17(2): 88.

BLUMENTHAL, E.J., & MALKINSON, A.M. (1987). Changes in pulmonary calpain activity following treatment of mice with butylated hydroxytoluene. *Arch. Biochem. Biophys.*, 256(1): 19-28.

BOLTON, J.L. & THOMPSON, J.A. (1991). Oxidation of butylated hydroxytoluene to toxic metabolites. Factors influencing hydroxylation and quinone methide formation by hepatic and pulmonary microsomes. *Drug Metab. Dispos.*, 19(2): 467-472.

BOLTON, J.L., SEVESTRE, H., IBE, B.O. & THOMPSON, J.A. (1990). Formation and reactivity of alternative quinone methides from buylated hydroxytoluene: Possible explanation for species-specific pneumotoxicity. *Chem. Res. Toxicol.*, 3: 65-70.

BOLTON, J.L., THOMPSON, J.A., ALLENTOFF, A.J., MILEY, F.B. & MALINSON, A.M. (1993). Metabolic activation of butylated hydroxytoluene by mouse bronchiolar Clara cells. *Toxicol. Appl. Pharmacol.*, 123: 43-49.

BOMHARD, E.M., BREMMER, J.N. & HERBOLD, B.A. (1992). Review of the mutagenicity/genotoxicity of butylated hydroxytoluene. *Mutat. Res.*, 277: 187-200.

BOTHAM, C.M., CONNING, D.M., HAYES, J., LITCHFIELD, M.H. & McELLIGOTT, T.F. (1970). Effects of butylated hydroxytoluene on the enzyme activity and ultrastructure of rat hepatocytes. *Food Cosmet. Toxicol.*, 8: 1-8.

BRANEN, A.L., RICHARDSON, T., GOEL, M.C. & ALLEN, J. R. (1973). Lipid and enzyme changes in the blood and liver of monkeys given butylated hydroxytoluene and butylated hydroxyanisole. *Food Cosmet. Toxicol.*, 11: 797-806.

BRIGGS, D., LOK, E., NERA, E.A., KARPINSKI, K. & CLAYSON, D.B. (1989). Short-term effects of butylated hydroxytoluene on the Wistar rat liver, urinary bladder and thyroid gland. *Cancer Letters*, 46: 31-36.

BROOKS, T.M., HUNT, P.F., THORPE, E. & WALKER, A.I.T. (1976). Effects of prolonged exposure of mice to butylated hydroxytoluene. Unpublished report from Shell Research, Ltd., Tunstell Lab., Sittingbourne, Kent, U.K submitted to the World Health Organization by the authors.

BROWN, W.D., JOHNSON, A.R. & O'HALLORAN, M.W. (1959). *Aust. J. Exp. Biol. Med. Sci.*, 37: 533.

BRUNNER, R., VORHEES, C. & BUTCHER, K. (1978). Psychotoxicity of selected food additives and related compounds. Report prepared under FDA contract 223-75-2030.

BRUSICK, D. (1975). Mutagenic evaluation of compound FDA 71-25: butylated hydroxytoluene (IONOL). Unpublished report from Litton Biometrics, Inc., Kensington, Md., U.S. submitted to the World Health Organization by the U.S. Food and Drug Administration.

BUDUNOVA, I.V., MITTELMAN, L.A. & BELITSKY, G.A. (1989). Identification of tumor promoters by their inhibitory effect on intercellular transfer of lucifer yellow. *Cell. Biol. Toxicol.*, 5(1): 77-89.

BURROWS, C.M., CUMMING, D.M., GARTLAND, C.J. & LITCHFIELD, M.H. (1972). Topanol(R) 354 (BHT and BHA): Comparison of biochemical and ultrastructural effects on rat hepatocytes. Unpublished report (HO/IH/P49) from ICI Special Centre, Toxicology Bureau, submitted to the World Health Organization by the Imperial Chemical Industries Ltd., Alderley Park, Cheshire, U.K.

CARUBELLI, R. & McCAY, P.B. (1987). Dietary butylated hydroxytoluene protects cytochrome P-450 in hepatic nuclear membranes of rats fed 2-acetylaminofluorene. *Nutr. Cancer*, 10: 145-148.

CARUBELLI, R. & McCAY, P.B. (1989). Hepatic nuclear envelope cytochrome P-450 in rats fed 2-acetylaminofluorene. Effect of dietary fats and butylated hydroxytoluene. *Cancer Lett.*, 47: 83-89.

CHIPMAN, J.K, & DAVIES, J.E. (1988). Reduction of 2-acetylamino-fluorene-induced unscheduled DNA synthesis in human and rat hepatocytes by butylated hydroxytoluene. *Mutat. Res.*, 207(3-4): 193-198.

CLAPP, N.K. & SATTERFIELD, L.C. (1975). Modification of radiation lethality by previous treatment with butylated hydroxytoluene. *Radiation Res.*, 64: 388-392.

CLAPP, N.K., TYNDALL, R.L. & CUMMING, R.B. (1973). Hyperplasia of hepatic bile ducts in mice following long-term administration of butylated hydroxytoluene. *Food Cosmet. Toxicol.*, 11: 847.

of butylated hydroxytoluene alone or with diethylnitrosamine in mice. *Food Cosmet. Toxicol.*, 12: 367-371.

CLAPP, N.K., SATTERFIELD, L.C. & KLIMA, W.C. (1975). Modification of diethylnitrosamine lung tumorigenesis by concomitant treatment with butylated hydroxytoluene. Unpublished report from Oak Ridge National Lab., Oak Ridge, Tenn., USA, submitted to the World Health Organization by Eastman Chemical Product, Inc., Kingsport, Tenn., USA.

CLAYSON, D.B., IVERSON, F., NERA, E.A. & LOK, E. (1993). The importance of cellular proliferation induced by BHA and BHT. *Toxicol. Industr. Health*, 9(1-2): 231-242.

CLEGG, D.J. (1965). Absence of teratogenic effect of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in rats and mice. *Food Cosmet. Toxicol.*, 3: 387-403.

COHEN, L.A., POLANSKY, M., FURUYA, K., REDDY, M., BERKE, B. & WEISBURGER, J.H. (1984). Inhibition of chemically induced mammary carcinogenesis in rats by short-term exposure to BHT: Interrelationships among BHT concentration, carcinogen dose, and diet. *J. Natl. Cancer Inst.*, 72: 165-174.

COHEN, L.A., CHOI, K., NUMOTO, S., REDDY, M., BERKE, B. & WEISBURGER, J.H. (1986). Inhibition of chemically induced mammary carcinogenesis in rats by long-term exposure to butylated hydroxytoluene (BHT): interrelations among BHT concentration, carcinogen dose, and diet. *J. Natl. Cancer Inst.*, 76(4): 721-730.

COLLINGS, A.J. & SHARRATT, M. (1970). The BHT content of human adipose tissue. *Food Cosmet. Toxicol.*, 8: 409-412.

CONACHER, H.B., IVERSON, F., LAU, P.Y., & PAGE, B.D. (1986). Levels of BHA and BHT in human and animal adipose tissue: interspecies extrapolation. *Food Chem. Toxicol.*, 24: 1159-1162.

CONNING, D.M., KIRCH, D.A. & STYLES, J.A. (1969). The toxicity of BHT and several oxidation products *in vivo* and *in vitro*. Unpublished report (IHR/260) from the Industrial Hygiene Research Labs., Alderley Park, Cheshire, U.K., submitted to the World Health Organization by the Imperial Chemical Industries, Ltd.

COTTRELL, S., ANDREWS, C.M., CLAYTON, D. & POWELL, C.J. (1994). The dose-dependent effect of BHT (butylated hydroxytoluene) on vitamin K-dependent blood coagulation in rats. *Food Chem. Toxicol.*, 32(7): 589-594.

CRAMPTON, R.F., GRAY, T.J., GRASSO, P. & PARKE, D.V. (1977). Long-term studies on chemically induced liver enlargement in the rat. I. Sustained induction of microsomal enzymes with absence of liver damage on feeding phenobarbitone or butylated hydroxytoluene. *Toxicology*, 7: 289-306.

CREAVEN, P.J., DAVIES, W.H. & WILLIAMS, R.T. (1966). The effect of butylated hydroxytoluene, butylated hydroxyanisole and octyl gallate upon liver weight and biphenyl 4-hydroxylate activity in the rat. *J. Pharm Pharmacol.*, 18: 485.

CUMMING, R.B. & WALTON, M.F. (1973). Modification of the acute toxicity of mutagenic and carcinogenic chemicals in the mouse by prfeeding with antioxidants. *Food Cosmet. Toxicol.*, 11: 547-553.

DACRE, J.C. (1961). *Biochem. J.*, 78: 758.

DANIEL, J.W. & GAGE, J.C. (1965). The absorption and excretion of butylated hydroxytoluene (BHT) in the rat. *Food Cosmet. Toxicol.*, 3: 405-415.

DANIEL, J.W., GAGE, J.C., JONES, D.I. & STEVENS, M.A. (1967). Excretion of butylated hydroxyoluene (BHT) and butylated hydroxyanisole (BHA) by man. *Food Cosmet. Toxicol.*, 5: 475-479.

DANIEL, J.W., GAGE, I.C. & JONES, D.I. (1968). The metabolism of 3,5-di- *tert*-butyl-4-hydroxytoluene in the rat and in man. *Biochem. J.*, 106: 783-790.

DAUGHERTY, J.P., DAVIS, S. & YIELDING, K.L. (1978). Inhibition by butylated hydroxytoluene of excision repair synthesis and semiconservative DNA synthesis. *Biochem. Biophys. Res. Commun.*, 80(4): 963-969.

DAY, A.J., JOHNSON, A.R., O'HALLORAN, M.W & SCHWARTZ, C.J. (1959). *Aust. J. Exp. Biol. Med. Sci.*, 37: 295.

DEERBERG, F., RAPP, K.G., PITTERMANN, W. & REHM, S. (1980). Zum tumorspektrum der Han: Wis-Ratte. [Tumour spectrum of the Han:WIST rat] *Z. Versuchstierk*, 22: 267-280.

DEICHMANN, W.B., CLEMMER, J.J., RAKOCZY, R. & BIANCHINE, J. (1955). *A.M.A. Arch. Industr. Hlth.*, 11: 93.

DENZ, F.A. & LLAURADO, I.G. (1957). *Brit. J. Exp. Path.*, 38: 515.

DERTINGER, S.D., TOROUS, D.K. & TOMETSKO, A.M. (1993). *In vitro* system for detecting non-genotoxic carcinogens. *Environ. Molecular Mutagen.*, 21: 332-338.

DISSANAYAKE, M. & POWELL, S.M. (1989). Allergic contact dermatitis from BHT in leg ulcer patients. *Contact Dermatitis*, 21: 195.

FABER, W. (1990). Hemorrhagic effects of butylated hydroxytoluene (BHT). Unpublished report. Submitted to WHO by the BHT Special Program Panel, Chemical Manufacturers Association, Washington, D.C., USA.

FEUER, G., GAUNT, I.F., GOLBERG, L. & FAIRWEATHER, F.A. (1965). Liver response tests. VI Application to comparative study of food antioxidants and hepatotoxic agents. *Food Cosmet. Toxicol.*, 3: 457-469.

FLYVHOLM, M.-A. & MENNÉ, T. (1990). Sensitizing risk of butylated hydroxytoluene based on exposure and effect data. *Contact Dermatitis*, 23: 341-345.

FRAWLEY, J.P., KAY, J.H. & CALANDRA, J.C. (1965a). The residue of butylated hydroxytoluene (BHT) and metabolites in tissue and eggs of chickens fed diets containing radioactive BHT. *Food Cosmet. Toxicol.*, 3: 471-474.

FRAWLEY, J.P., KOHN. F.E., KAY, J.H. & CALANDRA, J.C. (1965b). Progress report n multigeneration reproduction studies in rats fed butylated hydroxytoluene (BHT). *Food Cosmet. Toxicol.*, 3: 377-386.

FRIEDMAN, F.K., MILLER, H., PARK, S.S., GRAHAM, S.A., GELBOIN, H.V. & CARUBELLI, R. (1989). Induction of rat liver microsomal and nuclear cytochrome P-450 by dietary 2-acetylaminofluorene and butylated hydroxytoluene. *Biochem. Pharmacol.*, 38(18): 3075-3081.

FUKAYAMA, M.Y. & HSIEH, D.P. (1985). Effect of butylated hydroxytoluene pretreatment on the excretion, tissue distribution and DNA binding of [^{14}C]aflatoxin B_1 in the rat. *Food Chem. Toxicol.*, 23: 567-573.

FUKUSHIMA, S., OGISO, T., KURATA, Y., HIROSE, M. & ITO, N. (1987a). Dose-dependent effects of butylated hydroxyanisole, butylated

in N-butyl-N-(4-hydroxybutyl)nitrosamine-initiated, unilaterally ureter-ligated rats. *Cancer Lett.*, 34: 83-90.

FUKUSHIMA, S., SAKATA, T., TAGAWA, Y., SHIBATA, M-A., HIROSE, M. & ITO, N. (1987b). Different modifying response of butylated hydroxyanisole, butylated hydroxytoluene, and other antioxidants in N,N-dibutylnitrosamine esophagus and forestomach carcinogenesis of rats. *Cancer Res.*, 4., 47:2113-2116.

FURUKAWA, K., MAEURA, Y., FURUKAWA, N.T., & WILLIAMS, G.M. (1984). Induction by butylated hydroxytoluene of rat liver y-glutamyl transpeptidase activity in comparison to expression in carcinogen-induced altered lesions. *Chem Biol. Interact.*, 48: 43-58.

GABRYELAK, T., PUMO, D.E. & CHIU, J.F. (1981). Changes in tumor-specific nuclear antigen activity in carcinogen-treated colon by tumor promotor and carcinogen inhibitors, *Cancer Res.*, 41: 3392-3394.

GAGE, J.C. (1964). The tissue retention of 3,5-Di-t-butyl-4-hydroxy-toluene (butylated hydroxytoluene, BHT). Unpublished report (No. IHR/157) from the Industrial Hygiene Research Labs., Alderley Park, Cheshire, U.K., submitted to the World Health Organization by the Imperial Chemical Industries, Ltd., U.K.

GAUNT, I.F., FEUER, G., FAIRWEATHER, F.A. & GILBERT, D. (1965a). Liver response tests. IV. Application to short-term feeding studies with butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). *Food Cosmet. Toxicol.*, 3: 433-443.

GAUNT, I.F., GILBERT, D. & MARTIN, D. (1965b). Liver response tests. V. Effect of dietary restriction on a short-term feeding study with butylated hydroxytoluene (BHT). *Food Cosmet. Toxicol.*, 3: 445-456.

GEYER, H., SCHEUNERT, I. & KORTE, F. (1986). Bioconcentration potential of organic environmental chemicals in humans. *Regul. Toxicol. Pharmacol.*, 6(4): 313-347.

GILBERT, D. & GOLBERG, L. (1965). *Food Cosmet. Toxicol.*, 3: 417.

GILBERT, D. & GOLBERG, L. (1967). BHT oxidase. A liver-microsomal enzyme induced by the treatment of rats with butylated hydroxytoluene. *Food Cosmet. Toxicol.*, 5: 481-490.

GOATER, T.O., KENYON, A.J. & & HURST, E.W. (1964). The subacute toxicity of Topanol(R) BHT. Unpublished report from the Industrial Hygiene Research Labs., Alderley Park, Cheshire, U.K., submitted to the World Health Organization by the Imperial Chemical Industries Ltd., U.K.

GOODMAN, D.L., McDONNELL, J.T., NELSON, H.S., VAUGHAN, T.R. & WEBER, R.W. (1990). Chronic urticaria exacerbated by the antioxidant food preservatives, butylated hydroxyanisoie (BHA) and butylated hydroxytoluene (BHT). *J. Allergy Clin. Immunol.*, 86: 570-575.

GRAY, T.J., PARKE, D.V., GRASSO, P.. CRAMPTON, RF (1972). Biochemical and pathological differences in hepatic response to chronic feeding of safrole and butylated hydroxytoluene to rats. *Biochem. J.*, 130(2): 91P.

HAESEN, S., DERIJCKE, T., DELEENER, A., CASTELAIN, P., ALEXANDRE, H., PRÉAT, V. & KIRSCH-VOLDERS, M. (1988). The influence of phenobarbital and butylated hydroxytoluene on the ploidy rate in rat hepato-carcinogenesis. *Carcinogenesis*, 9(10): 1755-1761.

HAGEMAN, G.J., VERHAGEN, H. & KLEINJANS, J.C. (1988). Butylated hydroxyanisole, butylated hydroxytoluene and *tert*-butylhydroquinone are not mutagenic in the *Salmonella/microsome* assay using new tester strains. *Mutat. Res.*, 208:207-211.

HAGIWARA, A., HIROSE, M., MIYATA, Y., FUKUSHIMA, S. & ITO, N. (1989). Modulation of N-butyl-N-(4-hydroxybutyl)nitrosamine-induced rat urinary bladder carcinogenesis by post-treatment with combinations of three phenolic antioxidants. *J. Toxicol. Pathol.*, 2: 33-39.

HAMMOCK, B.D. & OTA, K. (1983). Differential induction of cytosolic epoxide hydrolase, microsomal epoxide hydrolase and glutathione S-transferase activities. *Tox. Appl. Pharmacol.*, 71: 254-265.

HANNUKSELA, M. & LAHTI, A. (1986). Peroral challenge tests with food additives in urticaria and atopic dermatitis, *Int. J. Dermatol.*, 25(3): 178-180.

HARMAN, D. (1968). Free radical theory of aging: effect of free radical reaction inhibitors on the mortality rate of male LAF mice. *J. Gerontol.*, 23: 476-482.

HERMANN, R.S, KORANSKY, W., LEBERL, C. & NOACK, G. (1971). Hyperplasia and hypertrophy of rat liver induced by hexachloro-cyclohexane and butylhydroxytoluene. Retention of the hyperplasia during involution of the enlarged organ. *Virchows Arch. B. Zellpath.*, 9: 125-134.

HIRAGA, K. (1978). Life-span oral toxicity study of 3,5-di- *tert*-hydroxytoluene (BHT) in rats. *Ann. Rep. Tokyo Metropolitan Research Lab. Public Health*, 32: 83.

HIRAI, K., YAMAUCHI, M., WITSCHI, H, & COTE, M.G. (1983). Disintegration of lung peroxisomes during differentiation of type II cells to type I cells in butylated hydroxytoluene-administered mice. *Exper. Mol. Pathol.*, 39: 129-138.

HIROSE, M., SHIBATA, M., HAGIWARA, A., IMAIDA, K & ITO, N. (1981). Chronic toxicity of butylated hydroxytoluene in Wistar rats. *Food Cosmet. Toxicol.*, 19: 147-151.

HIROSE, M., MASUDA, A., KURATA, Y., IKAWA, E., MERA, Y. & ITO, N. (1986). Histologic and autoradiographic studies on the forestomach of hamsters treated with 2- tert-butylated hydroxyanisole, 3- tert-butylated hydroxyanisole, crude butylated hydroxyanisole, or butylated hydroxytoluene. *J. Natl. Cancer Inst.*, 76(1): 143-147.

HIROSE, M., MASUDA, A., IMAIDA, K., KAGAWA, M., TSUDA, H. & ITO, N. (1987). Induction of forestomach lesions in rats by oral administrations of naturally occurring antioxidants for 4 weeks. *Jpn. J. Cancer. Res.*, 78(4): 317-321.

HIROSE, M., YADA, H., HAKOI, K. TAKAHASHI, S. & ITO, N. (1993). Modification of carcinogenesis by alpha-tocopherol, t-butylhydro-quinone, propyl gallate and butylated hydroxytoluene in a rat multi-organ carcinogenesis model. *Carcinogenesis*, 14(11): 2359-2364.

HOLDER, G.M., RYAN, A.J., WATSON, T.R. & WIEBE, L.I. (1970a). The biliary metabolism of butylated hydroxytoluene and its derivatives in the rat. *J. Pharm. Pharmacol.*, 22: 832-838.

HOLDER, G.M., RYAN, A.J., WATSON, T.R. & WIEBE, L.I. (1970b). The metabolism of butylated hydroxytoluene in man. *J. Pharm. Pharmacol.*, 22: 375-376.

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (1986). Some naturally occuring and synthetic food components, furocoumarins and ultraviolet radiation. Lyon, (IARC Monographs on the Evaluation of Carcinogenic

IATROPOULOS, M.J., WILLIAMS, G.M., CONAWAY. C.C. (1994). Inhibition of the action of the food-borne carcinogen aflatoxin B_1 by butylated hydroxyanisole and butylated hydroxytoluene in male rats. Unpublished report to ILSI Research Foundation, 77 pp.

IMAIDA, K., FUKUSHIMA, S., SHIRAI, T., OHTANI, M., NAKANISHI, K. & ITO, N. (1983). Promoting activities of BHA and BHT on 2-stage urinary bladder carcinogenesis and inhibition of gamma-glutamyl transpeptidase-positive foci development in the liver of rats. *Carcinogenesis*, 4: 895-899.

IMAIDA, K., FUKUSHIMA, S., SHIRAI, T., MASUI, T., OGISO, T. & ITO, N. (1984). Promoting activities of BHA, BHT and sodium-L-ascorbate on forestomach and urinary bladder carcinogenesis initiated with methylnitrosourea in F-344 male rats. *Gann*, 75: 769-775.

IMAIDA, K., FUKUSHIMA, S., INOUE, K., MASUI, T., HIROSE, M. & ITO, N. (1988). Modifying effects of concomitant treatment with butylated hydroxyanisole or butylated hydroxytoluene on N,N-dibutylnitrosamine-induced liver, forestomach and urinary bladder carcinogenesis in F344 male rats. *Cancer Lett.*, 43: 167-172.

INAI, K., KOBUKE, T., NAMBU, S., TAKEMOTO, T., KOU, E., NISHINA, H., FUJIHARA, M., YONEHARA, S., SUEHIRO, S. & TSUYA, T. (1988). Hepatocellular tumorigenicity of butylated hydroxytoluene administered orally to $B6C3F_1$ mice. *Jpn. J. Cancer Res.*, 79(1): 49-58.

JOHNSON, A.R. & HEWGILL, F.R. (1961). *Aust. J. Exp. Biol. Med. Sci.*, 39: 353.

JOHNSON, A.R. & HOLDSWORTH, E.S (1968). *J. Nutr. & Diet.*, 5: 147.

JOHNSON, A.R, (1965). A re-examination of the possible teratogenic effects of butylated hydroxytoluene (BHT) and its effect on the reproductive capacity of the mouse. *Food Cosmet. Toxicol.*, 3: 371-375.

JOSEPHY, P.D., CARTER, M.H., & GOLDBERG, M.T. (1985). Inhibition of benzidine mutagenesis by nucleophiles: a study using the Ames test with hamster hepatic S9 activation. *Mut. Res.*, 143: 5-10.

KASHEI, K., YANG, E.K., CHOWDHURY, J.R., CHOWDHURY, N.R., DANNENBERG, A.J. (1994). Regulation of uridine diphosphate glucuronosyltransferase expression by phenolic antioxidants. *Cancer Res.*, 54: 5856-5859.

KARPLYUK, I.A. (1959). *Vop. Pitan.*, 18: 24.

KAWANO, S., NAKAO, T & HIRAGA, K. (1981). Strain differences in butylated hydroxytoluene-induced deaths in male mice. *Tox. Appl. Pharmacol.*, 61: 475-479.

KEHRER, J.P. & DIGIOVANNI, J. (1990). Comparison of lung injury induced in 4 strains of mice by butylated hydroxytoluene. *Toxicol. Lett.*, 52: 55-61.

KENNEDY, G., FANCHET, O.E. & CALANDRA, J.C. (1966). Three-generation reproduction study in albino rats. Butylated Hydroxytoluene. Final Report. Unpublished report from Bio-Test Labs., Inc., Northbrook, Ill., USA, submitted to the World Health Organization by Hercules, Inc., Wilmington, Delaware, USA.

KING, M.M., MCCAY, P.B. & KOSANKE, S.D. (1981). Comparison of the effects of butylated hydroxytoluene on N-nitrosomethylurea and 7,12-dimethylbenz[a]-anthracene-induced mammary tumours, *Cancer Letters*, 14: 219-226.

KITCHIN, K.T. & BROWN, J.L. (1987). Biochemical effects of two promoters of hepatocarcinogenesis in rats *Food Chem. Toxic.*, 25(8): 603-607.

KLEIN, A. & BRUSER, B. (1992). The effect of butylated hydroxytoluene with and without cortisol, on stimulated lymphocytes. *Life Sciences*, 50: 883-889.

KRASAVAGE, W.J. (1984). The lack of effect of tertiary butylhydroquinone on prothrombin time in male rats. *Drug Chem Toxicol.*, 7: 329-334.

LADOMERY, L.G., RYAN, A.J. & WRIGHT, S.E. (1963). *J. Pharm. Pharmacol.*, 15: 771

LADOMERY, L.G., RYAN, A.J. & WRIGHT, S.E. (1967a). The excretion of [^{14}C] butylated hydroxytoluene in the rat. *J. Pharm. Pharmacol.*, 19: 383-387.

LADOMERY, L.G., RYAN, A.J. & WRIGHT, S.E. (1967b). The biliary metabolite of butylated hydroxytoluene in the rat. *J. Pharm. Pharmacol.*, 19: 388-394.

LAKE, B.G., LONGLAND, R.C., GANGOLLI, S.D. & LLOYD, A.G. (1976). The influence of some foreign compounds on hepatic xenobiotic metabolism and the urinary excretion of D-glucuronic acid metabolites in the rat. *Toxicol. Appl. Pharmacol.*, 35:113-122.

LARSEN, J.C. & TARDING, F. (1978). Stimulation of DNA synthesis in mouse and rat lung following administration of butylated hydroxytoluene. *Archives of Toxicol.* Suppl., 1: 147-150.

LINDENSCHMIDT, R.C., TRYKA. A.F, GOAD, M.E., & WITSCHI, H.P. (1986). The effects of dietary BHT on liver and colon tumor development in mice. *Toxicology*, 38:151-160.

LINDENSCHMIDT, R.C., TRYKA, A.F. & WITSCHI, H. (1987). Modification of gastrointestinal tumor development in rats by dietary butylated hydroxytoluene. *Fundam. Appl. Toxicol.*, 8(4): 474-481.

MAEURA, Y. & WILLIAMS, G.M. (1984). Enhancing effect of butylated hydroxytoluene on the development of liver altered foci and neoplasms induced by N-2-fluorenylacetamide in rats. *Food Chem. Toxicol.*, 22: 191-198.

MAEURA, Y., WEISBURGER, J.H. & WILLIAMS, G.M. (1984). Dose-dependent reduction of N-2-fluorenylacetamide-induced liver cancer and enhancement of bladder cancer in rats by butylated hydroxytoluene. *Cancer Res.*, 44: 1604-1610.

MALKINSON, A.M. (1979). Prevention of butylated hydroxytoluene-induced lung damage in mice by cedar terpene administration. Pre-print of paper accepted for publication in *Toxicol. Appl. Pharmacol.*, 49: 551-560.

MALKINSON, A.M. (1991). Genetic studies on lung tumor susceptibility and histogenesis in mice. *Environ. Health Perspect.*, 93: 149-159.

MALKINSON, A.M. & THAETE, L.G. (1986). Effects of strain and age on prophylaxis and co-carcinogenesis of urethane-induced mouse lung adenomas by butylated hydroxytoluene. *Cancer. Res.*, 46: 1694-1697.

MALKINSON, A.M., THAETE, L.G., BLUMENTHAL, E.J. & THOMPSON, J.A. (1989). Evidence for a role of *tert*-butyl hydroxylation in the induction of pneumotoxicity in mice by butylated hydroxytoluene. *Toxicol. Appl. Pharmacol.*, 101: 196-204.

Comparative metabolism of 3,5-di- *tert*-butyl-4-hydroxytoluene (BHT) in mice and rats. *Food Chem. Toxicol.*, 22: 345-354.

McCARTHY, D.J., LINDAMOOD, C., GUNDBERG, C.M. & HILL, D.L. (1989). Retinoid-induced hemorrhaging and bone toxicity in rats fed diets deficient in vitamin K. *Toxicol. Appl. Pharmacol.*, 97: 300-310.

McCORMICK, D.L., MAJOR, N., & MOON, R.C. (1984). Inhibition of 7,12-dimethylbenz(a)anthracene-induced rat mammary carcinogenesis by concomitant or postcarcinogen antioxidant exposure. *Cancer Res.*, 44: 2858-2863.

McCORMICK, D.L., MAY, C.M., THOMAS, C.F. & DETRISAC, C.J. (1986). Anticarcinogenic and hepatotoxic interactions between retinyl acetate and butylated hydroxytoluene in rats. *Cancer Res.*, 46: 5264-5269.

MEYER, O. & HANSEN, E. (1980). Behavioural and developmental effects of butylated hydroxytoluene dosed to rats *in utero* and in the lactation period. *Toxicology*, 16: 247-258.

MEYER, O.A., KRISTIANSEN, E. & WURTZEN, G. (1989). Effects of dietary protein and butylated hydroxytoluene on the kidneys of rats. *Lab. Anim.*, 23: 175-179.

MIZUTANI, T., ISHIDA, I., YAMANOTOK, K. & TAJIMA, K. (1982). Pulmonary toxicity of butylated hydroxytoluene and related alkylphenols: Structural requirements for toxic potency in mice. *Toxicol. Appl. Pharmacol.*, 62: 273-281.

MIZUTANI, T., YAMAMOTO, K. & TAJIMA, K. (1983). Isotope effects on the metabolism and pulmonary toxicity of butylated hydroxytoluene in mice by deuteration of the 4-methyl group. *Toxicol. Appl. Pharmacol.*, 69: 283-290.

MIZUTANI, T., NOMURA, H., NAKANISHI, K. & FUJITA, S. (1987). Hepatotoxicity of butylated hydroxytoluene and its analogs in mice depleted of hepatic glutathione. *Toxicol. Appl. Pharmacol.*, 87: 166-176.

NAGAI, F., USHIYAMA, K. & KANO, I. (1993). DNA cleavage by metabolites of butylated hydroxytoluene. *Arch. Toxicol.*, 67: 552-557.

NAKAGAWA, Y. (1987). Effects of buthionine sulfoximine and cysteine on the hepatotoxicity of butylated hydroxytoluene in rats. *Toxicol. Lett.*, 37: 251-256.

NAKAGAWA, Y. & TAYAMA, K. (1988). Nephrotoxicity of butylated hydroxytoluene in phenobarbital-pretreated male rats. *Arch. Toxicol.*, 61: 359-365.

NCI (1979) National Cancer Institute. Bioassay of butylated hydroxytoluene (BHT) for possible carcinogenicity. *DHEW Report* No. NIH 79-1706. Technical Report Series No. 150.

NIEVEL, J.G. (1969). Effect of coumarin, BHT and phenobarbitone on protein synthesis in the rat liver. *Food Cosmet. Toxicol.*, 7: 621-634.

OKINE, L.K., LOWE, M.C., MIMNAUGH, E.G., GOOCHEE, J.M. & GRAM, T.E. (1986). Protection by methylprednisolone against butylated hydroxytoluene-induced pulmonary damage and impairment of microsomal monooxygenase activities in the mouse: lack of effect on fibrosis. *Exp. Lung Res.*, 10: 1-22.

OLSEN, P., GRY, J., KNUDSEN, L., MEYER, O, & POULSEN, E. (1984). Animal feeding study with nitrite-treated meat. In: N-nitroso compounds; Occurrence, biological effects, and relevance to human cancer. O'Neill, I.K., von Borstel, R.C., Miller, C.T., Long, J., & Bartsch, B. (eds.), *IARC Scient. Publ.* No.57: pp. 667-675. International Agency for Research on Cancer, Lyon.

OLSEN, P., MEYER, O., BILLE, N. & WURTZEN, G. (1986). Carcinogenicity study on butylated hydroxytoluene (BHT) in Wistar rats exposed *in utero*. *Food Chem. Toxicol.*, 24: 112.

OMAYE, S.T., REDDY, K.A. & CROSS, C.E. (1977). Effect of butylated hydroxytoluene and other antioxidants on mouse lung metabolism. *J. Toxicol. Environ. Health*, 3: 829-836.

PARTRIDGE, C.A., DAO, D.D. & AWASTHI, Y.C. (1982). Induction of glutathione-linked detoxification system by dietary antioxidants, *Fed. Proc.*, 41: Abstract 2152.

PERAINO, C., FRY, R.J., & STAFFELDT, E. (1973). Enhancement of spontaneous hepatic tumorigenesis in C_3H mice by dietary phenolbarbital. *J. Natl. Cancer Inst.*, 51: 1349-1350.

PERAINO, C., FRY, R.J., STAFFELDT, E. & CHRISTOPHER, J.P. (1977). Enhancing effects of phenobarbitone and butylated hydroxytoluene on 2-acetylaminofluorene-induced hepatic tumorigenesis in the rat. *Food and Cosmetics Toxicol.*, 15: 93-96.

PETO, R., PIKE, M.C., DAY, N.E., GRAY, R.G., LEE, P.N., PARISH, S., PETO, J., RICHARDS, S., & WAHRENDORF, J. (1980). Guidelines for simple, sensitive significance tests for carcinogenic effects in long-term animal experiments. In: Long-term and Short-term Screening Assays for Carcinogens: A Critical Appraisal. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Suppl. 2, pp. 311-426. International Agency for Research on Cancer, Lyon.

POWELL, C.J. & CONNOLLY, A.K. (1991). The site specificity and sensitivity of the rat liver to butylated hydroxytoluene-induced damage. *Toxicol. Appl. Pharmacol.*, 108: 67-77.

POWELL, C.J., CONNELLY, J.C., JONES, S.M., GRASSO, P., BRIDGES, J.W. (1986). Hepatic responses to the administration of high doses of BHT to the rat: their relevance to hepatocarcinogenicity. *Food. Chem. Toxicol.*, 24: 1131-1143

PRASAD, O. & KAMRA O.P. (1974). Radiosensitization of *Drosophila* sperm by commonly used food additives-butylated hydroxyanisole and butylated hydroxytoluene, *Int. J. Radiat. Biol.*, 25: 67-72.

PRÉAT, V., GERLACHE, J. DE, LANS, M., TAPER, H. & ROBERFROID, M. (1986). Comparative analysis of the effect of phenobarbital, dichlorodiphenyltrichloroethane, butylated hydroxytoluene and nafenopin on rat hepatocarcinogenesis. *Carcinogenesis*, 7(6): 1025-1028.

PRICE, S.C. (1994). The role of hepatocellular injury in the chronic toxicity of BHT: Two generation Wistar albino rat study. Robens Institute, U. of Surrey, Guildford, Surrey, U.K. Study No: 1/91/Tx. Final Report No: *R193/TOX/0020*. Vol. 1-8. Submitted to WHO by Robens Institute.

REDDY, B.S., HANSEN, D., MATHEWS, L., & SHARMA, C. (1983a). Effect of micronutrients, antioxidants and related compounds on the mutagenicity of 3,2'-dimethyl-4-aminobiphenyl, a colon and breast carcinogen *Food Chem. Toxicol.*, 21: 129-132.

REDDY, B.S., SHARMA, C., & MATHEWS, L. (1983b). Effect of butylated hydroxytoluene and butylated hydroxyanisole on the mutagenicity of 3,2'-dimethyl-4-aminobiphenyl. *Nutr. Cancer*, 5: 153-158.

2-acetylaminofluorene to DNA by butylated hydroxytoluene and butylated hydroxyanisole in vitro. *Cancer Lett.*, 47: 211-216.

RIKANS, L.E., GIBSON, D.D., McCAY, P.B., KING, M.M. (1981). Effects of butylated hydroxytoluene and acetylaminofluorene on NADPH-cytochrome P-450 reductase activity in rat liver microsome, *Food Cosmet. Toxicol.*, 19: 89-92.

ROBENS INSTITUTE (1989). Dose ranging experiment on the role of hepatocellular injury in the chronic toxicity of BHT. Final report 7/88/TX, Robens Institute of Health and Safety, University of Surrey, Guildford, Surrey, United Kingdom. Unpublished report. Submitted to WHO by European BHT Manufacturers Association (EBMA). CEFIC, Bruxelles, Belgium.

ROEBUCK, B.D., MACMILLAN, D.L., BUSH, D.M. & KENSLER, T.W. (1984). Modulation of azaserine-induced pancreatic foci by phenolic antioxidants in rats. *J. Natl. Cancer Inst.*, 72: 1405-1409.

RUZZENE, M., DONELLA-DEANA, A, ALEXANDRE, A., FRANCESCONI, M.A. & DEANA, R. (1991). The antioxidant butylated hydroxytoluene stimulates platelet protein kinase C and inhibits subsequent protein phosphorylation induced by thrombin. *Biochim. Biophys. Acta*, 1094: 121-129.

SAHEB, W. & WITSCHI, H. (1975). Lung growth in mice after a single dose of butylated hydroxytoluene. *Toxicol. & Appl. Pharm.*, 33: 309-319.

SATO, H., TAKAHASHI, M., FURUKAWA, F., MIYAKAWA, Y., HASEGAWA, R., TOYODA, K., HAYASHI, Y. (1987). Initiating potential of 2-(2-furyl)-3-(5-nitro-2- furyl)acrylamide (AF-2), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and 3,3',4',5,7-pentahydroxyflavone (quercetin) in two-stage mouse skin carcinogenesis. *Cancer Lett.*, 38(1-2): 49-56.

SHELEF, L.A. & CHIN, B. (1980) Effect of phenolic antioxidants on the mutagenicity of aflatoxins B_1. *Appl. Environ. Microbiol.*, 40: 1039-1043.

SHELLENBERGER, T.E., PARRISH, D.B. & SANFORD, P.E. (1957). *Poultry Sci.*, 36: 1313.

SHEU, C.W., CAIN, K.T., RUSHBROOK, C.J., JORGENSON, T.A. & GENEROSO, W.M. (1986). Tests for mutagenic effects of ammoniated glycyrrhizin, butylated hydroxytoluene, and gum Arabic in rodent germ cells. *Environ. Mutagen.*, 8(3): 357-367.

SHIBATA, M., HAGIWARA, A., MIYATA, Y., IMAIDA, K., ARAI, M. & ITO, N. (1979). Experimental study on carcinogenicity of butylated hydroxytoluene (BHT) in rats. Translation of the Proceedings of the 38th Annual Meeting of the Japanese Cancer Assoc., Tokyo, September 1979.

SHIBATA, M.A., YAMADA, M., TANAKA, H., KAGAWA, M. & FUKUSHIMA, S. (1989). Changes in urine composition, bladder epithelial morphology, and DNA synthesis in male F344 rats in response to ingestion of bladder tumour promoters. *Toxicol. Appl. Pharmacol.*, 99: 37-49.

SHIBATA, M.-A., ASAKAWA, E.. HAGIWARA, A., KURATA, Y. & FUKUSHIMA, S. (1991). DNA synthesis and scanning electron microscopic lesions in renal pelvic epithelium of rats treated with bladder cancer promoters. *Toxicol. Lett.*, 55: 263-272.

SHIRAI, T., HAGIWARA, A., KURATA, Y., SHIBATA, M., FUKUSHIMA, S., ITO, N. (1982). Lack of carcinogenicity of butylated hydroxytoluene on long-term administration to B6C3FI mice. *Food. Chem. Tox.*, 20: 861-865.

SHIRAI, T., FUKUSHIMA, S., OHSHIMA, M., MASUDA, A., & ITO, N. (1984). Effects of BHA, BHT and NaCl on gastric carcinogenesis initiated with N-methyl-N'-nitro-N-nitrosoguanidine in F-344 rats. *J. Natl. Cancer Inst.*, 72: 1189-1198.

SHIRAI, T., IKAWA, E., HIROSE. M., THAMAVIT, W., & ITO, N. (1985). Modification by five antioxidants of 1,2-dimethylhydrazine-initiated colon carcinogenesis in F-344 rats. *Carcinogenesis*, 6: 637-639.

SHIRAI, T., FUKUSHIMA, S., KAWABE, M., SHIBATA, M.-A., IWASAKI, S., TADA, M. & ITO, N. (1991). Selective induction of rat urinary bladder tumors by simultaneous administration of 3,2'-dimethyl-4-aminobiphenyl (DMAB) and butylated hydroxyanisole or butylated hydroxytoluene is associated with increased DMAB-DNA adduct formation. *Carcinogenesis*, 12(7): 1335-1339.

SINGLETARY, K.W. & NELSHOPPEN, J.M. (1991). Selective *in vivo* inhibition of rat mammary 7,12-dimethylbenz[alpha]anthracene - DNA adduct formation by dietary butylated hydroxytoluene. *Carcinogenesis*, 12(10): 1967-1969.

SINGLETARY, K.W., NELSHOPPEN, L.M., SCARDEFIELD, S. & WALLIG, M. (1992). Inhibition by butylated hydroxytoluene and its oxidative metabolites of DMBA-induced mammary tumorigenesis and of mammary DMBA-DNA adduct formation *in vivo* in the female rat. *Food Chem. Toxicol.*, 30(6): 455-465.

SOLLEVELD, H.A., HASEMAN, J.K., & MCCONNELL, E.E. (1984). Natural history of body weight gain, survival and neoplasia in the F344 rat. *J. Natl. Cancer Inst.*, 72: 929-940.

SONDERGAARD, D. & OLSEN, P (1982). The effect of butylated hydroxytoluene (BHT) on the rat thyroid. *Toxicology Letters*, 10: 239-244.

SPORN, A. & SCHOBESCH, O. (1961). *Igiena (Bucharest)*, 9: 113.

S.R.I. Stanford Research Institute (1972). Report 14 submitted to U.S. Food and Drug Administration.

STOKES, J.D. & SCUDDER, C.L. (1974). The effect of butylated hydroxyanisole and butylated hydroxytoluene on behavioral development of mice. *Devel. Psychobiol.*, 7: 343-350.

SUZUKI, H., NAKAO, T. & HIRAGA, K. (1983). Vitamin K content of liver and faeces from vitamin K-deficient and butylated hydroxytoluene (BHT)-treated male rats. *Toxicol. Appl. Pharmacol.*, 67: 152-155.

TAFFE, B.G. & KENSLER, T.W. (1988). Tumor promotion by a hydroperoxide metabolite of butylated hydroxytoluene, 2,6-di- tert-butyl-4-hydroperoxy-4-methyl-2,5-cyclohexadienone, in mouse skin. *Res. Commun. Chem. Pathol. Pharmacol.*, 61(3): 291-303.

TAFFE, B.G., ZWEIER, J.L., PANNELL, L.K. & KENSLER, T.W. (1989). Generation of reactive intermediates from the tumor promoter butylated hydroxytoluene hydroperoxide in isolated murine keratinocytes or by hematin. *Carcinogenesis*, 10(7): 1261-1268.

TAKAHASHI, O. (1986). Feeding of butylated hydroxytoluene to rats caused a rapid decrease in blood coagulation factors II (prothrombin), VII, IX and X. *Arch. Toxicol.*, 58(3): 177-181.

TAKAHASHI, O. (1987). Decrease in blood coagulation factors II (prothrombin), VII, IX and X in the rat after a single oral dose of

TAKAHASHI, O.(1990). Gastric retention and delayed absorption of a large dose of butylated hydroxytoluene in the rat. *Xenobiotica*, 20(12): 1319-1329.

TAKAHASHI, O. (1991). Some properties of rat platelet aggregation and effects of butylated hydroxytoluene, warfarin and aspirin. *Food Chem. Toxicol.*, 29(3): 173-183.

TAKAHASHI, O. (1992). Haemorrhages due to defective blood coagulation do not occur in mice and guinea-pigs fed butylated hydroxytoluene, but nephrotoxicity is found in mice. *Food Chem. Toxicol.*, 30(2): 89-97.

TAKAHASHI, O. & HIRAGA, K. (1978a). Dose-response study of hemorrhagic death by dietary butylated hydroxytoluene (BHT) m male rats. *Tox. Appl. Pharm.*, 43: 399-406.

TAKAHASHI, O. & HIRAGA, K. (1978b). Effects of low levels of butylated hydroxytoluene on the prothrombin index of male rats. *Food Cosmet. Toxicol.*, 24: 16, 475-477.

TAKAHASHI, O. & HIRAGA, K. (1979). Preventive effects of phylloquinone on hemorrhagic death induced by butylated hydroxytoluene in male rats. *J. Nutr.*, 109: 453-457.

TAKAHASHI, O. & HIRAGA, K (1981a). Inhibition of phylloquinone expoxide-dependent carboxylation of microsomal proteins from rat liver by 2,6-di- *tert*-butyl-4-methylene-2,5-cyclohexadienone. *Food Cosmet. Toxicol.*, 19: 701-706.

TAKAHASHI, O. & HIRAGA, K. (1981b). Haemorrhagic toxicosis in rats given butylated hydroxytoluene, *Acta Pharmacol. Toxicol.*, 49: 14-20.

TAKAHASHI, O. & HIRAGA, K. (1984). Effects of dietary butylated hydroxytoluene on functional and biochemical properties of platelets and plasma preceeding the occurrence of haemorrhage in rats. *Food Chem. Toxicol.*, 22: 97-103.

TAKAHASHI, O., HAYASHIDA, S., & HIRAGA, K. (1980). Species differences in the haemorrhagic response to butylated hydroxytoluene. *Food Cosmet. Toxicol.*, 18: 229-235.

TAKAHASHI M., FURUKAWA F., TOYODA, K., SATO, H., HASEGAWA, R. & HAYASHI, Y. (1986). Effects of four antioxidants on N-methyl-N'-nitro-N-nitrosoguanidine initiated gastric tumour development in rats. *Cancer Lett.*, 30(2): 161-168.

TANAKA, T., OISHI, S. & TAKAHASHI, O. (1993). Three generation toxicity study of butylated hydroxytoluene administered to mice. *Toxicol. Lett.*, 66: 295-304.

TATSUTA, M., MIKUNI, T., & TANIGUCHI, H. (1983). Protective effect of butylated hydroxytoluene against induction of gastric cancer by N-methyl-N'-nitro-N-nitrosoguanidine in Wistar rats. *Int. J. Cancer*, 32, 253-254.

THOMPSON, D.C. & TRUSH, M.A. (1988a). Enhancement of butylated hydroxytoluene-induced mouse lung damage by butylated hydroxyanisole. *Toxicol. Appl. Pharmacol.*, 96(1): 115-121.

THOMPSON, D.C. & TRUSH, M.A, (1988b). Studies on the mechanism of enhancement of butylated hydroxytoluene-induced mouse lung toxicity by butylated hydroxyanisole. *Toxicol. Appl. Pharmacol.*, 96(1): 122-131.

THOMPSON, D.C., CHA, Y.N. & TRUSH, M.A (1986). The peroxidative activation of butylated hydroxytoluene to BHT-quinone methide and stilbenequinone. *Adv. Exp. Med. Biol.*, 197: 301-309.

THOMPSON, J.A., MALKINSON, A.M., WAND, M.D., MASTOVICH, S.L., MEAD, E.W., SCHULLEK, K.M. & LAUDENSCHLAGER, W.G. (1987). Oxidative metabolism of butylated hydroxytoluene by hepatic and pulmonary microsomes from rats and mice. *Drug Metab. Dispos.*, 5: 833-840.

THOMPSON, J.A., SCHULLEK, K.M., FERNANDEZ, C.A. & MALKINSON, A.M. (1989). A metabolite of butylated hydroxytoluene with potent tumor-promoting activity in mouse lung. *Carcinogenesis*, 10(4): 773-775.

THORNTON, M., MOORE, M.A. & ITO, N. (1989). Modifying influence of dehydroepiandrosterone or butylated hydroxytoluene treatment on initiation and development stages of azaserine-induced acinar pancreatic preneoplastic lesions in the rat. *Carcinogenesis*, 10(2): 407-410.

TOKUMO, K., IATROPOULOS, M.J. & WILLIAMS, G.M. (1991). Butylated hydroxytoluene lacks the activity of phenobarbital in enhancing diethylnitrosamine-induced mouse liver carcinogenesis. *Cancer Lett.*, 59: 193-199.

TYE, R., ENGEL, J.D. & RAPIEN, I. (1965). Summary of toxicological data. Disposition of butylated hydroxytoluene (BHT) in the rat. *Food Cosmet Toxicol.*, 3: 547-551.

TYNDALL, R.L., COLYER, S. & CLAPP, N. (1975). Early alterations in plasma esterases with associated pathology following oral administration of diethylnitrosamine and butylated hydroxytoluene singly or in combination, *Int. J. Cancer*, 16: 184-191.

ULLAND, B.M., WEISBURGER, J.H., YAMAMOTO, R.S., WEISBURGER, E.K. (1973). Antioxidants and carcinogenesis: butylated hydroxytoluene, but not diphenyl-p-phenyl-enediamine, inhibits cancer induction by N-2-fluorenylacetamide and by N-hydroxy-N-2-fluorenyl acctamide in rats *Food Cosmet. Toxicol.*, 11: 199-207.

VAN STRATUM, P.G. & VOS, H.J. (1965). The transfer of dietary butylated hydroxytoluene (BHT) into the body and egg fat of laying hens. *Food Cosmet. Toxicol.*, 3: 475-477.

VERHAGEN, H., BECKER, H.H.G., COMUTH, P.A.W.V., MAAS, L.M., HOOR, F.TEN, HENDERSON, P.T. & KLEINJANS, J.C.S. (1989). Disposition of single oral doses of butylated hydroxytoluenc in man and in rat. *Fd. Chem. Toxicol.*, 27(12): 765-772.

VERSCHOYLE, R.D., WOLF, C.R. & DINSDALE, D. (1993). Cytochrome P450 2B isoenzymes are responsible for the pulmonary bioactivation and toxicity of butylated hydroxytoluene, O,O,S-trimethylphosphorothioate and methylcyclopentadienyl manganese tricarbonyl. *J. Pharmacol. Exp. Ther.*, 266(2): 958-963.

VORHEES, C.V., BUTCHER, R.E., BRUNNER, R.L., SOBOTKA, T.J. (1981). Developmental neurobehavioral toxicity of butylated hydroxytoluene in rats. *Food Cosmet. Toxicol.*, 19: 153-162.

WASEEM, M. & KAW, J.L. (1994). Pulmonary effects of butylated hydroxytoluene in mice. *Food Addit. Contain.*, 11(1): 33-38.

WESS, J.A. & ARCHER, D.L. (1982). Evidence from *in vitro* murine immunologic assays that some phenolic food additives may function as antipromotors by lowering intracellular cyclic GMP levels. *Proc. Soc. Exp. Biol. Med.*, 170: 427-430.

WILLIAMS, G.M., MAEURA, Y., & WEISBURGER, J.H. (1983). Simultaneous inhibition of liver carcinogenicity and enhancement of bladder carcinogenicity of N-2-fluorenylacetamide by butylated hydroxytoluene.

WILLIAMS, G.M., SHIMADA, T., McQUEEN, C., TONG, C., & VED BRAT, S. (1984). Lack of genotoxicity of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). *The Toxicologist*, 4: 104.

WILLIAMS, G.M., WANG, C.X. & IATROPOULOS, M.J. (1990a). Toxicity studies of butylated hydroxyanisole and butylated hydroxytoluene. II. Chronic feeding studies. *Food Chem. Toxicol.*, 28(12): 799-806.

WILLIAMS, G.M., MCQUEEN, C.A. & TONG, C (1990b). Toxicity studies of butylated hydroxyanisole and butylated hydroxytoluene. I. Genetic and cellular effects. *Food Chem. Toxicol.*, 28(12): 793-798.

WILLIAMS, G.M., TANAKA, T., MARUYAMA, H., MAEURA, Y., WEISBURGER, J.H. & ZANG, E. (1991). Modulation by butylated hydroxytoluene of liver and bladder carcinogenesis induced by chronic low-level exposure to 2-acetylaminofluorene. *Cancer Res.*, 51: 6224-6230.

WITSCHI, H. P. (1981). Enhancement of tumor formation in mouse lung by dietary butylated hydroxytoluene. *Toxicology*, 21: 95-104.

WITSCHI, H.P. (1986). Separation of early diffuse alveolar cell proliferation from enhanced tumour development in mouse lung. *Cancer Res.*, 46(6): 2675-2679.

WITSCHI, H. & COTE, M. G. (19761. Biochemical pathology of lung damage produced by chemicals. *Fed. Proc.*, 35(1): 89-94.

WITSCHI H.P. & KEHRER, J.P. (19821. Adenoma development in mouse lung following treatment with possible promoting agents. *J. Am. Coll. Toxicology*, 1: 171.

WITSCHI, H.P. & LOCK, S. (19791. Enhancement of adenoma formation in mouse lung by butylated hydroxytoluene. *Tox. Appl Pharm.*, 50: 391-400.

WITSCHI H.P. & MORSE, C.C. (19851. Cell kinetics in mouse lung following administration of carcinogens and butylated hydroxytoluene. *Tox. Appl. Pharm.*, 78: 464-472.

WITSCHI H.P., HAKKINEN, P.J. & KEHRER, J.P. (19811. Modification of lung tumor development in A/J mice, *Toxicology*, 21: 37-45.

YAMAMOTO, K., TAJIMA, K, OKINO, N. & MIZUTANI, T. (19881. Enhanced lung toxicity of butylated hydroxytoluene in mice by coadministration of butylated hydroxyanisole. *Res. Commun. Chem Pathol. Pharmacol.*, 59(2): 219-231.

YAMAMOTO, K., TAJIMA, K., TAKEMURA, M. & MIZUTANI, T. (1991). Further metabolism of 3,5-di- tert-butyl-4-hydroxybenzoic acid, a major metabolite of butylated hydroxytoluene, in rats. *Chem. Pharm. Bull.*, 39(2): 512-514.

YOSHIDA, Y. (1990). Study on mutagenicity and antimutagenicity of BHT and its derivatives in a bacterial assay. *Mutation Res.*, 242: 209-217.

See Also:
Toxicological Abbreviations
Butylated hydroxytoluene (ICSC)
Butylated hydroxytoluene (FAO Nutrition Meetings Report Series 38a)
Butylated hydroxytoluene (FAO Nutrition Meetings Report Series 40abc)
Butylated hydroxytoluene (WHO Food Additives Series 5)
Butylated hydroxytoluene (WHO Food Additives Series 10)
Butylated hydroxytoluene (WHO Food Additives Series 21)
Butylated hydroxytoluene (JECFA Evaluation)

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

WORLD HEALTH ORGANIZATION

SAFETY EVALUATION OF CERTAIN FOOD ADDITIVES

WHO FOOD ADDITIVES SERIES: 42

Prepared by the Fifty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)

World Health Organization, Geneva, 1999
IPCS - International Programme on Chemical Safety

EVALUATION OF NATIONAL INTAKE ASSESSMENTS OF BUTYLATED HYDROXYTOLUENE (BHT)

First draft prepared by
Dr Philippe Verger
Observatory of Food Consumption, Paris

1. INTRODUCTION

The Committee assessed the intake of butylated hydroxytoluene (BHT), for which maximum limits have been proposed in a wide range of solid foods in the draft General Standard for Food Additives (GSFA) being developed by the Codex Committee on Food Additives and Contaminants (CCFAC). An ADI of 0-0.3 mg/kg bw has been allocated to BHT (Annex 1, reference 116).

BHT is generally used as an antioxidant in products containing fats or oils. It can be use in conjunction with butylated hydroxyanisole, *tert*-butylated hydroquinone, and propyl gallate, providing a synergistic combination of antioxidants.

Information was provided by 10 countries: Australia, Brazil, China, Finland, France, Japan, New Zealand, Spain, the United Kingdom, and the United States. A combined assessment was provided by Australia and New Zealand (Aus-NZ). The submitted intake assessments were based on 'poundage', household economic surveys, sales data, model diets, or individual dietary records. Table 1 summarizes the submissions.

Table 1. Summary of submissions on butylated hydroxytoluene

Country	Budget method	Poundage data	FBS/HES/ sales data	Model diets	Individual dietary records
Australia-New Zealand	x			x	x
Brazil	x		x		
China	x	x		x	
Finland		x			
France			x		x
Japan				x	
Spain	x	x	x		
United Kingdom		x		x	x
United States		x		x	

FBS, food balance sheet; HES, household economic survey; sales, retail stores

2. SCREENING OF BUTYLATED HYDROXYTOLUENE BY THE BUDGET METHOD

National submissions of intakes derived by the budget method indicate whether BHT is used in solid foods. The Codex GSFA proposes that BHT be permitted in a wide range of solid foods. Table 2 summarizes data for each country on the permitted patterns of BHT use, the proportion of the solid food supply likely to contain BHT, the maximum levels of BHT permitted, and a comparison of the levels with the theoretical maximum level calculated by the budget method for that country.

The theoretical maximum level of use for BHT in solid foods was less than the national maximum permitted level of use in the four countries that submitted data and was also less than the GSFA level of 1000 mg/kg. Detailed assessments of the intake of BHT from its use in solid foods are therefore required.

3. ASSESSMENTS OF INTAKE OF BUTYLATED HYDROXYTOLUENE

3.1 Assessments based on data on poundage (disappearance)

Estimates of the amount of BHT available *per capita* based on poundage data are given in Table 3 for five countries and compared with the JECFA ADI of 0-0.3 mg/kg bw for BHT. The estimated intakes are all lower than the ADI but vary from 0.003 to 0.11 mg/kg bw per day (1-40% of the ADI) in recent studies. Results obtained by the poundage method show a large decrease in use of this additive in the United States between 1987 and 1995.

3.2 Assessments based on data from household economic surveys or sales

Four countries submitted data based on household surveys or sales, in which national maximum levels of use were assumed national in each country. The data are summarized in Table 4. The estimated intakes are all lower than the ADI, ranging from 0.052 mg/kg bw per day in Brazil to 0.1 mg/kg bw per day in Spain (17 and 33% of the ADI, respectively). Although high consumers generally cannot be identified from household economic surveys or sales data, the submission from France included estimates of high consumption derived by dividing household consumption by the number of members and then estimating high intake for individual consumers.

3.3 Assessments based on data from model diets

Five countries submitted data based on model diets, details of which are summarized in Table 5. The results cannot be compared directly because different assumptions were made. The model diets used in Aus-NZ and the United Kingdom were constructed to estimate the BHT intake of a high consumer, assuming a maximum permitted level of additive. The United States model diet was constructed to predict the BHT intake of a long-term consumer by using food consumption data derived from food frequency data for 1982-88 from the Market Research Corporation of America and average portion sizes from a three-day national food consumption survey conducted in 1987-88 by the US Department of Agriculture. Maximum additive levels were assumed. The Japanese model diet (based on a total diet survey) included analysed concentrations of food additives with national food consumption data to derive an estimate of the actual BHT intake of the average consumer.

The estimates of BHT intake based on the models of Aus-NZ and the United Kingdom do not exceed the ADI when additive levels at the national standards are assumed (0.02 and 0.09 mg/kg bw per day and 10 and 30% of the ADI, respectively), but largely exceed the ADI if GSFA levels of use are assumed (5.3 and 6.0 mg/kg bw per day, respectively, corresponding to 1800 and 2000% of the ADI). The last result is consistent with estimates of BHT intake based on the Chinese and United States models with the GSFA standard, which are 0.7 mg/kg bw per day (230% of the ADI) and 0.99 mg/kg bw per day (330% of the ADI) for average consumers and 4.4 mg/kg bw per day (1600% of the ADI) and 2.0 mg/kg bw per day (660% of the ADI) for high consumers, respectively. When national standards for BHT are used in the United States model, the ADI is also exceeded. The Japanese estimate of BHT intake is much lower than those from other model diets because the actual concentrations of BHT detected are used, which are much lower than the maximum permitted levels.

3.3 Assessments based on individual dietary records

Estimates of the intake of BHT based on individual dietary records were submitted by three countries. In each case, means and percentiles were derived from individual intake estimates adjusted for individual body weight (except in the United Kingdom). The assumptions made in deriving these estimates and the estimates themselves are summarized in Table 6. The estimates show that the intakes are lower than the ADI for both mean and high consumers in all countries. The estimates based on GSFA levels in Aus-NZ are higher than those based on national levels of use and exceed the ADI for both mean (0.7 mg/kg bw per day and 240% of the ADI) and high consumers (1.8 mg/kg bw per day and 580% of the ADI).

Table 7 summarizes the results of intake assessments based on the levels of use of the GSFA. The results for Aus-NZ, China, and the United Kingdom are consistent in showing a potential to exceed the ADI.

3.5 Food groups that are major sources of butylated hydroxytoluene

Assessment of intake based on GSFA levels of BHT, in conjunction with the data on food consumption supplied by countries, points to certain foods as major contributors to the overall intake of BHT. The ADI would be exceeded when 90 g/day of any food containing more than 200 mg/kg BHT, 45 g/day of any food containing more than 400 mg/kg BHT, or 18 g/day of any food containing more than 1000 mg/kg BHT was

ingested. Some of the GSFA levels are the result of a high permission

for one type of food within a food grouping which drives the permitted use level for the whole group to an unlikely high level. This is the case for edible fats and oils (75-200 mg/kg), confectionery (200-750 mg/kg), and frozen fish, fish fillets, and fish products (100-1000 mg/kg).

4. EVALUATION OF ESTIMATES OF INTAKE OF BUTYLATED HYDROXYTOLUENE

Screening of BHT by the budget method indicated that use of BHT as an additive requires further assessment. Inclusion of national proportions of the food supply that may contain BHT in the budget method of screening did not change this decision.

Estimates of the intake of BHT were submitted by 10 countries. All of the estimates based on model diets or individual consumption data combined with GSFA levels of use show that the ADI is consistently exceeded. The mean intake is estimated to be between 0.70 and 0.99 mg/kg bw (230 and 240% of the ADI for China and the United States), and the intake of high consumers is estimated to be 2.0-6.0 mg/kg bw (690-2000% of the ADI).

Intake estimates based on national levels of use are relatively consistent, ranging from 0 to 0.11 mg/kg bw per day (0-30% of the ADI) on the basis of poundage, 0.052-0.1 mg/kg bw per day (20-40% of the ADI) on the basis of household surveys or sales data, 0.02-0.09 mg/kg bw per day (10-30% of the ADI) on the basis of model diets and national levels of use (except for the United States), and 0.02-0.1 mg/kg bw per day (0.1-30% of the ADI) on the basis of individual consumption data.

Two exceptions must be noted. The first is estimates for mean and high consumption based on a model diet and authorized levels of use in the United States (0.39 and 0.78 mg/kg bw per day and 130 and 260% of the ADI, respectively). This result can be explained by the high levels of BHT authorized in that country. The second is the low estimate (0.00089 mg/kg bw per day or 0.003% of the ADI) in the study provided by Japan, which is based on the concentrations of BHT found in a total diet survey.

5. CONCLUSIONS AND RECOMMENDATIONS

The Committee recognized that the ADI for BHT is unlikely to be exceeded on the basis of the estimated intakes in the 10 countries for which data were available but that it might be exceeded when the proposed maximum limits in the GSFA are assumed.

The Committee recognized that BHT is likely to be used in conjunction with other antioxidants, such as *tert*-butylated hydroquinone and butylated hydroxyanisole, which act synergistically with BHT. Consequently, the amount of BHT used in practice will be lower and it will be used in fewer foods than assumed in the

estimates. All of the estimates except that from Japan are based on the assumption that BHT is the only antioxidant in foods where use is permitted and that all such foods contain it at maximum permitted levels. The actual intakes of BHT will depend on the relative proportions of antioxidants used in foods and on the proportion of foods in any one category that contains the additive.

Recommendations to the Codex Committee on Food Additives and Contaminants

The Committee identified certain food groups that could potentially contribute to a high intake of BHT. The Codex Committee may wish to review the appropriate levels of BHT in the following food groups: category 2.1, 'edible oils and fats'; category 9.2, 'fish and fish products'; and category 5.3, 'chewing gum'.

6. BIBLIOGRAPHY

Australia New Zealand Food Authority (1997) *Aus-NZ Food Standards Code*, Canberra.

Baines, J. Data sheets for Australia for TBHQ, BHA, BHT, sulfites, and benzoates. Personal communication. Australia-New Zealand Food Authority, Canberra, to J. Paakkanen, FAO, 5 January 1998.

Chen Junshi. Food additive intake from China. Personal communication. Chinese Academy of Preventive Medicine, Beijing, to J. Paakkanen, FAO, 16 March 1998.

Codex Committee on Food Additives and Contaminants (1997) *Report of the Twenty-ninth Session of the Codex Committee on Food Additives and Contaminants*, Rome, Food and Agriculture Organization of the United Nations (document ALINORM 97/12A, Appendix V).

Codex Committee on Food Additives and Contaminants (1998) Consideration of the Codex General Standard for Food Additives: Proposed draft revised annex A at step 3. Request for comments and information. Rome, Food and Agriculture Organization of the United Nations (document CX/FAC 98/9).

European Commission (1995) European Union Directive 95/2/CE, Brussels.

Fisher, C.E. Dietary exposure in the United Kingdom to the five additives referred to JECFA by CCFAC. Personal communication. Joint Food Safety and Standards Group, London, to J. Paakkanen, FAO, 29 April 1998.

Ishii, K. Use levels and intake estimates of certain food additives in Japan. Personal communication. Japan Food Additives Association, Tokyo, to J. Paakkanen, FAO, 10 May 1998.

Table 2. Estimation of theoretical maximum level for butylated hydroxytiluene (BHT) by the budget method

Country	% food supply containing BHT	National maximum level (mg/kg)	GSFA maximum level[a] (mg/kg)	Theoretical maximum level (mg/kg)
Australia-New Zealand	50	70	1000	24
Brazil	25	200	1000	48
China	20	200	1000	60
Spain	5	400	1000	240

GSFA, General Standard for Food Additives

[a] Maximum use level proposed is 1000 mg/kg for 9.2.1, 'frozen fish, fish fillet and fish products, including molluscs, crustacea and echinoderms'.

Table 3. Estimates of intake of butylated hydroxytoluene (BHT) based on poundage data

Country	Date	Consumers (millions)	Estimated intake of BHT (mg/kg bw per day)	% ADI[a]
China	1992	1200	0.016 70% of total population	5
Finland	1980	4.9	0	0
	1994	5.1	0	0
Spain	?	0.07	23 85% of total population	
United Kingdom	1984-86	56	0.003	1
United States[b]	1987	240	0.056 (mean)	19
			0.11 (90th percentile)	37
	1995	260	0.023 (mean)	7
			0.046 (90th percentile)	14

[a] JECFA ADI, 0-0.3 mg/kg bw

[b] Assuming that 100% of consumers ingest BHT and that the intake for 90th percentile consumers is twice the mean

Table 4. Estimates of intake of butylated hydroxytoluene (BHT) based on household economic surveys and sales data

Country	Date	Survey	Assumptions	Estimated intake of BHT (mg/kg bw per day)	% ADI[a]
Brazil[b]	1992-96	AC Nielsan Brazil; sales data	Maximum national use levels for all foods	0.052	17
	1984-94	Datamark	Maximum national use levels for all foods	0.08	26
France		Sales data	Maximum European Union levels of use Adjustment for catering outside the home	0.089	30
Spain	1993	Household survey	All foods in permitted groups contain BHT	0.1	33

[a] JECFA ADI, 0-0.5 mg/kg bw

[b] Assumed maximal level in final coconut and chewing gum products even though the use level in coconut is based on 60% fat content and that in chewing gum is based on 20% gum content, resulting in overestimates of BHT intake

Table 5. Model diets used to estimate intake of butylated hydroxytoluene (BHT)

Country	Date	Survey	Assumptions	Model	BHT intake (mg/kg bw per day)	% ADI[a]
Aus-NZ	1983	National, 24-h recall; adults, 25-64 years;	Two models: Aus-NZ/GSFA -- maximum levels (Aus-NZ or GSFA)	High consumer[b]/ Aus-NZ permissions[b]	0.02	10

		sample, 6254	-- 95th percentile high consumption level -- modified GSFA classification system	High consumer / Aus-NZ permissions	5.3	1800
China	1992	National household survey, 24-h recall; 30 provinces; sample, 91 818	One model -- maximum GSFA levels	Average consumer/ GSFA permissions	0.7	230
				High consumer/ GSFA permissions	4.9	1600
Japan	1994	National nutrition intake survey	One model: Japan -- Analysed food additive concentrations (zero values when not detected)	Average consumer levels/measured BHT	0.00085	0.003
United Kingdom	1986-87	National; 7-day weighed record; adults, 16-64 years	Three models: UK adult/child, GSFA -- maximum additive levels (EU)	High consumer[b]/ UK permissions/adult	0.09	30
			-- unit quantity diet (Codex model with GSFA levels)	High consumer[b]/ UK permissions/child	0.17	60
	1992	National; 7-day weighed record; children, 1.5-4.5 years	-- 97.5th percentile high consumption level (UK adult/child models) -- GSFA classification system	High consumer[b]/ GSFA permissions	6.0	2000
United States	1982-88	14-day menu obtained from MRCA food frequency data (1982-87) combined with portion sizes from USDA/NFCS (1987-88); ≥ 2 years	Two models: US and GSFA -- maximum additive levels (US or GSFA) -- 90th percentile high consumption level twice mean consumption -- all respondents are consumers -- GSFA classification system	Long-term consumer/USA permissions/mean	0.39	130
				Long-term consumer/USA permissions/90th percentile	0.78	260 0.99330
				Long-term consumer/GSFA permissions/mean		
				Long-term consumer/GSFA permissions/90th percentile	2.0	660

Table 5 (continued)

Aus-NZ, Australia-New Zealand; GSFA, General Standard for Food Additives; EU, European Union; MRCA, Market Research Corporation of America; USDA/NFCS, US Department of Agriculture/National Food Consumption Survey

[a] JECFA ADI, 0-0.3 mg/kg bw

[b] Assumed to consume one food with potentially highest BHT intake from two major food groups at the 97.5th percentile (United Kingdom) or 95th percentile (Aus-NZ) and from one food with potentially highest BHT intake from each of the other major food groups at a mean level for all respondents

Table 6. Estimates of intake of butylated hydroxytoluene (BHT) based on individual records

Country	Date	Survey	Model	BHT intake (mg/kg bw per day)	% ADI[a]
Aus-NZ	1983	National survey; 24-h recall; adults, 25-64 years; sample, 6254	Mean intake (population = consumers) Aus-NZ	0.02	7
			Mean (population = consumers) GSFA	0.7	240
			95th percentile (consumers) Aus-NZ	0	0
			95th percentile (consumers) GSFA	1.8	580
France	1993-94	National survey; 7-day record; 5-75 years; sample, 1116	Mean intake (population) EU	0.033	11
			90th percentile EU	0.071	24
			95th percentile EU	0.089	30
			Corrected mean intake (population) EU	0.001	00.3
United Kingdom	1986-87	National survey; 7-day weighed record; adults, 16-64 years; sample, 3000	Mean intake (population = consumers) EU	0.04	13
			97.5th percentile (consumers) EU	0.1	33

Aus-NZ, Australia-New Zealand; GSFA, General Standard for Food Additives; EU, European Union

[a] JECFA ADI, 0-0.3 mg/kg bw

Table 7. Summary of estimates of intake of butylated hydroxytoluene (BHT) based on additive levels permitted within the General Standard for Food Additives

Country	Model	Intake of BHT		% ADI[a]
		mg/person per day	mg/kg bw per day	
Australia-New Zealand	Individual records, mean intake	51	0.85	240
	Individual records, 95th percentile consumers	125	2.1	690
China	Model diet, mean intake	41	0.7	230
	Model diet, high consumers	290	4.9	1600
United States	MRCA, mean intake	59	0.99	330
	MRCA, pseudo-90th percentile consumers	120	2.0	660

MRCA, Market Research Corporation of America

[a] JECFA ADI, 0-0.3 mg/kg bw

Ishiwata, H., Nishijima, M., Fukasawa, Y., Ito, Y. & Yamada, T. (1997) Evaluation of the contents of BHA, BHT, propylene glycol, and sodium saccharin in foods and estimation of daily intake based on the results of official inspection in Japan in fiscal year 1994. *J. Food Hyg. Soc. Jpn*, 39, 89-100.

Ministry of Public Health (Spain) (1998) Calculation of certain food additives in Spain. Submitted to FAO.

National Food Administration (Finland) (1998) Estimated intake of certain food additives in Finland. Submitted to FAO.

Rulis, A.M. United States intake data on BHA, BHT, TBHQ, sulfites, and benzoates. Personal communication. Food and Drug Administration, Washington DC, to J. Paakkanen, FAO, 24 February 1998.

Toledo, M.C.F. Brazilian intake estimates of BHA, BHT, and TBHQ. Personal communication. University of Campinas, to J. Paakkanen, FAO, 26 February 1998.

United States Department of Agriculture (1996) Nationwide food consumption survey: 1989-91. Technical Information Service, Springfield, VA, United States.

Verger, P. Estimation of the theoretical maximum intake of certain food additives in France. CNERNA, Paris. Submitted to J. Paakkanen, FAO, 16 March 1998.

See Also:
Toxicological Abbreviations
Butylated hydroxytoluene (BHT) (WHO Food Additives Series 15)
Butylated hydroxytoluene (BHT) (WHO Food Additives Series 18)
Butylated hydroxytoluene (BHT) (WHO Food Additives Series 28)

ARTICLE RECORD

NAL CALL NO	382 So12
Author	Wessling, C.
ArticleTitle	Antioxidant ability of BHT- and alpha-tocopherol- impregnated LDPE film in packaging of oatmeal.
Source Info	Journal of the science of food and agriculture.Jan 15, 2001. v. 81 (2) (ABBREV TITLE = J. sci. food agric.)
Pages	p. 194-201.
Note	Includes references
CAB Subject	polyethylene film.
CAB Subject	alpha-tocopherol.
CAB Subject	butylated hydroxytoluene.
CAB Subject	packaging materials.
CAB Subject	oatmeal.
CAB Subject	infant foods.
CAB Subject	lipids.
CAB Subject	oxidation.
CAB Subject	aldehydes.
OtherSubject	low density polyethylene film
OtherSubject	hexanal
Other Author	Nielsen, T.
Other Author	Giacin, J.R.
Abstract	Cereals in general, and particularly oatmeals, are considered rather sensitive to oxidation owing to their relatively high fat content. The addition of antioxidants can sometimes prolong the shelf-life of products. The aim of the present study was to investigate how the rate of lipid oxidation of a packaged oatmeal product was affected by the nature and level of antioxidants incorporated in an LDPE film structure. The stability of the product, which was determined by hexanal analysis using GC-MS and by electronic nose analysis, showed very small variations over the chosen storage period. No oxidation, as determined by hexanal levels in the oatmeal, was initiated during storage, but small variations in volatile profile were seen among the samples analysed by the electronic nose. The product stored in the BHT-impregnated LDPE film had undergone the least change during 10 weeks of storage at 20 degrees C. alpha-Tocopherol-impregnated LDPE film did not appear to prolong the shelf-life of the oatmeal at all.