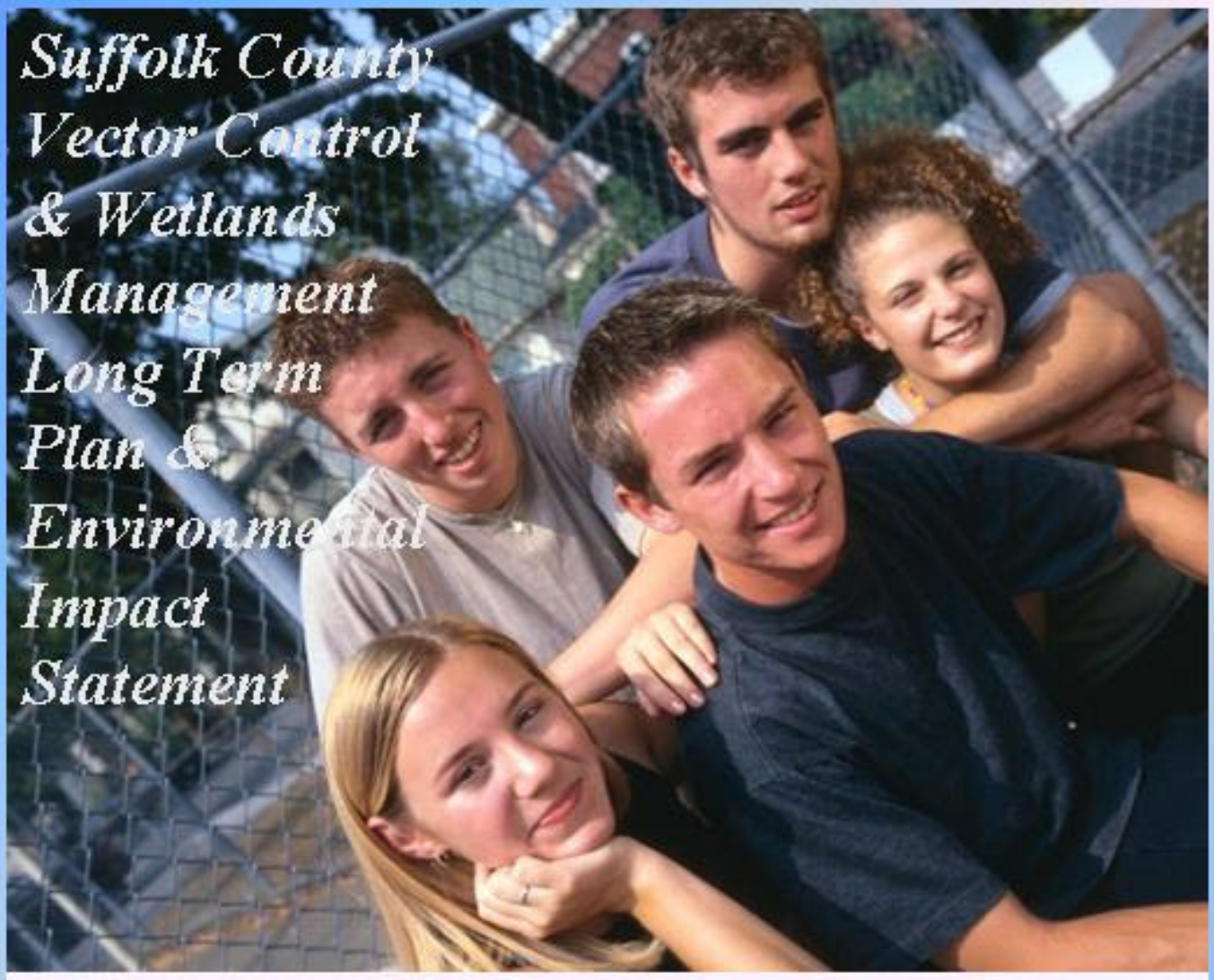

Task 3 Literature Review
Book 6 Part 1: Human Health and Domestic Animal Toxicity

Prepared for:

Suffolk County Department of Public Works
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LIST OF ACRONYMS AND ABBREVIATIONS

AChE	acetylcholinesterase
ADI	Acceptable Daily Intake
AGE	Aged Garlic Extract
ATSDR	Agency for Toxic Substances and Disease Registry
Bc	*Bacillus cereus*
Bs	*Bacillus sphaericus*
Bt	*Bacillus thuringiensis*
Btg	*Bt galleriae*
Bti	*Bacillus thuringiensis israelensis*
Btk	*Bt kurstaki*
bw	body weight
CARC	Cancer Assessment Review Committee
CDC	Centers for Disease Control
cfu	colony forming units
DDT	1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane
DEET	N,N-Diethyl-m-Toluamide
DNA	deoxyribonucleic acid
EIS	Environmental Impact Statement
EXTOXNET	Extension Toxicology Network
FEIS	Final Environmental Impact Statement
GEIS	Generic Environmental Impact Statement
GI	Gastrointestinal
GJIC	gap junction intercellular communication
GRAS	generally recognized as safe
HSDB	Hazardous Substances Data Bank
IARC	International Agency for Research on Cancer
IgM	immunoglobulin
IMS	Intermediate Syndrome
IRIS	Integrated Risk Information System
LD_{50}	Dose which is lethal to 50% of a sample population

LOAEL	Lowest observed adverse effect level
Medfly	Mediterranean fruit fly
mg/kg	milligrams per kilogram
mg/m^3	milligrams per cubic meter
MOE	Margin of Exposure
MRL	Minimal Risk Level
NCI	National Cancer Institute
NHL	Non-Hodgkin's Lymphoma
NIH	National Institutes of Health
NLM	National Library of Medicine
NOAEL	No observed adverse effect level
NOEL	No observed effect level
NPIC	National Pesticide Information Center
NTP	National Toxicology Program
PANNA	Pesticide Action Network North America
PBO	Piperonyl Butoxide
ppb	parts per billion
ppm	parts per million
RfD	Reference Dose
TESS	Toxic Exposure Surveillance System
USACHPPM	U.S. Army Center Health Promotion & Preventative Medicine
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
VPIS	Veterinary Poisons Information Service
WHO	World Health Organization

Executive Summary

This report presents the results of a review of the toxicological literature for the primary list of vector control agents being considered in the *Suffolk County Vector Control and Wetlands Management Long-Term Plan* (the *Plan*). The emphasis of this report is on potential toxicity to humans and domestic animals, primarily dogs.

Though the human health toxicity review presents information on effects in humans when available, this information is often limited. Information from laboratory animal studies is often relied on to predict possible human health effects and, therefore, is a large component of this human health literature review. Much of the toxicity data provided in this literature review was obtained from studies in which exposures are at much higher concentrations, a longer duration of exposure or by different routes of exposure (i.e., subcutaneous injection) than would be expected from vector control activities. Therefore, some of the information provided is not directly relevant to vector control activities. However, this information is included to ensure completeness and its relevance will be determined in the risk assessment.

The vector control agents addressed in this literature review are:

- Larvicides
  - *Bacillus thuringiensis israelensis (Bti)*
  - *Bacillus sphaericus (Bs)*
  - Methoprene
- Pyrethroid Adulticides
  - *Permethrin*
  - *Resmethrin*
  - *Sumithrin*
- Organophosphate adulticides
  - *Malathion*
    - Degradation/metabolic products of Malathion

  - Isomalathion
  - Malaoxon

- Synergist
  - *Piperonyl Butoxide (PBO)*
- Garlic Oil

This project-specific list of agents was developed by the project team as an early-work product. Many sources of information were reviewed including original journal articles, the New York City Final Environmental Impact Statement (FEIS) and the Westchester County Generic Environmental Impact Statement (GEIS), and toxicological review documents, such as those prepared by the World Health Organization (WHO), the Agency for Toxic Substances and Disease Registry (ATSDR) and the United States Environmental Agency (USEPA). Websites from various organizations and governmental agencies were also accessed.

A summary of the human health and domestic animal toxicity information obtained for each vector control agent on the primary list is provided below. The majority of the information compiled in the Domestic Animal Toxicity Literature Review is for dogs, since they are frequently used for scientific research. Some information was acquired for cats and, despite including horses in the literature search, no information was found for the effects of vector control pesticides on horses.

It is important to recognize that the toxic effects described in this report represent the range of effects that can occur and are not necessarily those that are expected to be associated with vector control activities. Whether toxic effects occur, and to what extent, depend upon many factors including the dose (amount that someone is exposed to), duration and route of exposure and individual susceptibility. A human health risk assessment, which will be conducted as part of the development of the Long-Term Plan, will evaluate the potential for adverse effects from vector control activities.

The following are Human Health Toxicity Summaries of the effects of the primary vector control agents contained in the Literature Review.

Larvicides

- ***Bacillus thuringiensis israelensis* (*Bti*)** is a naturally occurring soil bacterium that produces toxins that are toxic to mosquito larvae. These toxins disrupt digestion in the gut of mosquito larvae, leading to death. The toxins produced by *Bti* are specific to insects and therefore do not cause the same type of toxicity in mammals. However, during the manufacturing process, some other toxins (exotoxins) can be produced that may cause mammalian toxicity. Monitoring for these toxins occurs during the manufacturing process to ensure they are not present in the final product. *Bti* does not appear to be infectious in humans or other animals. *Bti* has been used in containers for drinking water in some Asian countries for the control of mosquitoes. Even though this practice results in a fairly high exposure, no adverse effects have been reported. Some studies with similar *bacillus* species have indicated a potential for allergic reactions in individuals who are exposed repetitively over a long period of time. Injections of *Bti* into the abdominal area of laboratory animals have caused toxicity under certain conditions.
- ***Bacillus sphaericus* (*Bs*)** is also a naturally occurring bacterium that produces toxins that are effective against mosquito larvae. As in the case with *Bti,* the toxins in *Bs* disrupt digestion in the gut of mosquito larvae, leading to death. Though there is less toxicity data available on *Bs* than *Bti,* evidence strongly suggests that *Bs* is not infectious in mammals. *Bs* has exhibited a very low mammalian toxicity. Very high doses are required in order to produce adverse effects.
- **Methoprene** is a biochemical larvicide that acts as a juvenile insect growth regulator. Methoprene prevents mosquito larvae from maturing into adult insects. Methoprene has been shown to produce liver and kidney toxicity in laboratory animals under certain exposure conditions. However, methoprene does not appear to be carcinogenic or to cause endocrine or reproductive effects.

Pyrethroid Adulticides

Pyrethroids are synthetic pesticides that are similar to pyrethrins, which are found naturally in pyrethrum flowers, such as chrysanthemums. Pyrethroids, as well as the naturally occurring pyrethrins, exert their pesticidal effects by disrupting nervous system in insects, which ultimately

leads to death. In general, pyrethrins and pyrethroids are more toxic to insects than they are to mammals because they are more quickly metabolized and broken down by mammalian systems. Typical symptoms of acute high dose exposures to pyrethroids can include excitability, confusion, exaggerated startle response, twitching and tremors. The efficacy of pyrethroid formulations can be improved by the addition of PBO, a chemical synergist. Of the ten primary list control agents, **permethrin, resmethrin,** and **sumithrin** are synthetic pyrethroids.

- **Permethrin** is a broad spectrum synthetic pyrethroid used against a variety of pests, including adult mosquitoes. It is a component in many shampoos used to control head lice. Aside from the neurological effects briefly described above, permethrin has shown some effects on the immune system and may mimic some hormones (e.g., estrogen). The liver has also been shown to be a target organ. The USEPA has classified permethrin as a possible human carcinogen, based on limited evidence from animal studies. A recent area of research has been the investigation of the potential role of permethrin and other pesticide exposures in the symptoms experienced by Gulf War veterans. Neurological and reproductive effects have been observed in animal studies with co-exposure to permethrin, DEET (N,N-diethyl-m-toluamide) and pyridostigmine, simulating exposures received by Gulf War veterans.
- **Resmethrin** is also a broad spectrum pyrethroid used against a variety of pests, including adult mosquitoes. The toxicity of resmethrin is similar to permethrin. Upon long-term exposure in laboratory animals, liver and kidney toxicity have been noted. Most of the long-term animal studies have not indicated that resmethrin is carcinogenic.
- **Sumithrin** is also known as phenothrin. It is also a broad spectrum pesticide that is used against a variety of pests, including adult mosquitoes and is used in some head lice shampoos. Compared to permethrin and resmethrin, there is much less toxicity information on sumithrin. Higher doses of sumithrin are necessary to produce neurotoxin effects as compared to permethrin and resmethrin. There is some information from cell cultures that sumithrin may have the potential to mimic estrogen, however, animal studies have not supported this data.

Organophosphate Adulticides

Organophosphate pesticides exert toxicity through the inhibition of acetyl cholinesterase (AChE) in insects as well as mammals. For this reason, organophosphates are non-selective in their target species. In insects, the inhibition of AChE interferes with the nerve-muscle communication, which ultimately causes paralysis of the insect. Malathion is the only primary list mosquito control agent belonging to the organophosphate class. Two other chemicals that are included in this primary list, malaoxon and isomalathion, are degradation products of malathion. Information on their toxicity is included in the malathion literature review.

- **Malathion** is used to control a variety of insects, including adult mosquitoes. Malathion, like other organophosphates, is a cholinesterase inhibitor and, therefore, its primary toxic effect is on the nervous system. Isomalathion and malaoxon are also cholinesterase inhibitors and appear to be more potent than malathion itself. Inhibition of cholinesterase leads to various forms of toxicity affecting muscles, the central nervous system and endocrine glands. Exposure to the skin or eyes may produce some irritant effects. Some studies have shown that under certain conditions, malathion may cause allergic reactions and affect the endocrine system. The USEPA considers malathion to have suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential. The International Agency for Research on Cancer (IARC) and the ATSDR consider the evidence to be insufficient to determine carcinogenic potential.

Synergists

A synergist is a chemical that is added to a pesticide product to enhance its potency. Synthetic pyrethroids are frequently used in combination with the synergist PBO. PBO is added to pyrethroid formulations in order to slow the metabolism of pyrethroids, thereby enhancing the effectiveness of the product. PBO is the single primary list mosquito control agent belonging to the synergist class.

- **Piperonyl butoxide (PBO)** is utilized as a chemical synergist in pyrethroid formulations. The same property (metabolic inhibition) that enables PBO to be used as a synergist may also lead to toxic effects. Repeated exposure to PBO has led to enlarged livers in laboratory animals. Since PBO inhibits enzymes that are involved in

the metabolism of other chemicals, it can be expected that co-exposure will affect that chemicals' toxicity. Some reproductive effects have been noted in animal studies at relatively high doses. IARC considers there to be insufficient evidence to classify PBO as to its carcinogenic potential. The USEPA considers PBO to be a possible carcinogen. The USEPA recommends the use of a "margin of exposure (MOE)" approach in evaluating potential human risk; since there is evidence PBO may exert this effect via a threshold mechanism as opposed to a direct effect on DNA (deoxyribonucleic acid).

Garlic Oil

- **Garlic oil** is a natural pesticide that can be formulated as a powder, distilled liquid, or emulsifiable oil. Garlic oil is obtained from the cloves of the plant *Allium sativum.* Garlic has historically been used as a medicinal herb and much of the toxicological literature on garlic relates to its beneficial effects. Excess intake of garlic can have adverse side effects, such as garlic odor on breath or skin, occasional allergic reactions, stomach disorders and diarrhea, decrease in serum protein and calcium levels, bronchial asthma and contact dermatitis. However, much of the literature on garlic relates to its health benefits, such as cancer prevention, inhibition of chemical toxicity and reduction in cholesterol levels.

The following are Domestic Animal Toxicity Summaries of the effects of the primary vector control agents contained in the Literature Review.

Larvicides

- ***Bacillus thuringiensis israelensis (Bti)*** is a microbial pesticide that produces chemicals toxic to insects. There is little available information regarding the effects of *Bti* on domestic animals. However, the information that is available indicates that *Bti* is practically nontoxic.
- ***Bacillus sphaericus* (*Bs*)** is a bacterium that produces toxins that are effective against mosquito larvae. This literature search did not find any information regarding its effects on domestic animals.

- **Methoprene**, a juvenile hormone mimetic, interferes with the processes of metamorphosis and development. Though laboratory studies have revealed toxicity at high doses, studies have not revealed clinical signs of toxicosis from methoprene in dogs under exposures that might be encountered under realistic conditions. In addition, methoprene has been found to be non-irritating to the skin and eyes of dogs.

Pyrethroid Adulticides

- **Permethrin** can cause feline toxicosis when cats are inadvertently treated with topical flea-control products intended for use on dogs or when the cats were exposed to companion animals that had been treated with the permethrin products. Permethrin toxicosis in dogs is far less common than permethrin toxicity in cats. Short-term studies have not shown any clinical signs of toxicity. During sub-chronic studies dogs have exhibited signs of permethrin poisoning at the highest administered doses.
- **Resmethrin** has not been shown to have any adverse effects of exposure when administered in short-term studies. The symptoms of resmethrin exposure via any route may include: incoordination, twitching, loss of bladder control, and seizures. At sufficient doses over a long-term study resmethrin can cause central nervous system dysfunction, increases in liver and kidney weights and liver lesions.
- **Sumithrin** administered to dogs at high doses has resulted in the following: decreases in erythrocyte count, haemoglobin concentration, haematocrit and total blood protein, and increases in mean liver weight. However, these effects have not been significant.

Organophosphate Adulticides

- **Malathion** is an organophosphate insecticide that kills by disrupting the nervous system of adult insects via cholinesterase inhibition. Malathion, whether administered orally or dermally, has relatively low toxicity for dogs. Cholinesterase inhibition is a clinical indicator that dogs have been exposed. No mortality has been observed when dogs have been exposed to malathion.
- **Isomalathion** appears to be up to six times more toxic to mammals than malathion.

- **Malaoxon** - This literature search did not find any information regarding its effects on domestic animals.

Synergists

- **Piperonyl Butoxide (PBO)** is a chemical that does not have any pesticidal effects, but enhances the pesticidal properties of other chemicals. Some studies have shown that oral exposure to PBO can cause reduced weight gains, liver, kidney and adrenal weight gains, changes in blood chemistry, and even death (at the highest doses) in dogs, while other studies have shown that there are no relevant clinical signs for PBO exposure.

Garlic Oil

- **Garlic Oil** - There was no information collected regarding the effects of garlic oil on domestic animals.

1. Introduction

This report consists of a review of the literature relating to pesticides used in vector control activities and human health, as well as impacts to domestic animals. The pesticides that are included in this literature review are those that were selected as the primary list of mosquito control agents, as discussed in the following section. Information for each pesticide is presented individually, with a summary of studies that have been conducted and a table of various criteria that have been developed. The Human Health Toxicity Review is presented first, followed by the Domestic Animal Toxicity Review, which represents information specific to domestic animals (e.g., dogs, cats and horses).

Though the human health toxicity review presents information on effects in humans when available, this information is often limited. Information from laboratory animal studies is often relied upon to predict possible human health effects and, therefore, is a large component of this literature review. In the domestic animal toxicity review the majority of the information collected is for dogs, due to the fact that they are frequently used for scientific research. Some information was found on cats, however, despite including horses in the search, there was no information collected for the effects of vector control pesticides on horses. In addition, all of the information collected pertained to laboratory experiments or was anecdotal information of the effects of active ingredients when used for flea control. None of the information for dogs and cats was in the context of vector control activities.

It is important to realize that much of the toxicity data provided in this literature review was obtained from studies in which exposures at much higher concentrations, for a longer duration of exposure, or by different routes of exposure (i.e., subcutaneous injection) than would be expected from vector control activities. Therefore, some of the information provided is not directly relevant to exposures that may be received from vector control activities. However, this information has been included for completeness and its relevance will be determined in the risk assessment. When discussing human health risks, the body of evidence has been evaluated. For example, though one study may suggest a possible carcinogenic effect, the study is evaluated in context with other available data. The toxicity information presented here is not intended to indicate that these toxic effects are expected from vector control activities. Instead this toxicity

review is intended to provide a foundation from which the risk assessment will be conducted. The risk assessment will evaluate potential human health impacts from vector control activities. Although pesticide products also contain inert ingredients, materials that are added to the product, but do not have any pesticidal activity, very little information is available on the identity and toxicity of these materials. Therefore, this toxicological literature review focuses on the primary active ingredients.

2. Overview of Primary List Mosquito Control Agents

The project team performed an extensive survey of mosquito control agencies outside Suffolk County to develop an inclusive list of agents and chemicals potentially used in Suffolk County to control mosquito populations (CA/CE, 2004). This survey placed particular emphasis on information pertaining to control agents utilized by regional mosquito control programs. Based on this information, the project team identified a primary list of 9 mosquito control agents and two pesticide degradation products for detailed data and literature review. The 11 control agents are presented below in Table 1. Since the two degradation products are related to malathion, their toxicity is included in the malathion toxicity literature review.

Table 1 – Primary List of Mosquito Control Agents Identified for Detailed Review

AGENT	CLASS	TRADE NAME
Bti (*Bacillus thuringiensis israelensis*)	Larvicide	Vectobac, Teknar
Bs (*Bacillus sphaericus*)	Larvicide	Vectolex
Methoprene	Larvicide	Altosid
Garlic Oil	Repellant	Garlic Barrier
Malathion	Adulticide	Fyfanon, Atrapa
Resmethrin	Adulticide	Scourge
Sumithrin	Adulticide	Anvil
Permethrin	Adulticide	Permanone
Malaoxon	Degradate	
Isomalathion	Degradate	
Piperonyl butoxide (PBO)	Synergist	

References for Section 2

CA/CE, 2004. *Mosquito Control Agents.* Cashin Engineering and Cameron Engineering and Associates. August.

3. Data and Information Sources

General reviews published by the WHO, ATSDR and USEPA, as well as the New York City FEIS and the Westchester County GEIS, were used to begin the literature search. Approximately 200 original articles were reviewed from the recent literature, identified primarily from online databases, such as PubMed. In the original scope of work the literature reviews presented in the New York City FEIS and the Westchester County GEIS were to be relied on with an additional review of subsequent literature. However, in actually conducting this literature review, it was often necessary to go back to the original articles and, in some cases, additional past articles that were not identified in the previous Environmental Impact Statements (EIS).
Information, compiled from reports and scientific papers, was obtained from a variety of sources including:

- Agency for Toxic Substances and Disease Registry (ATSDR) - http://www.atsdr.cdc.gov/
- Centers for Disease Control and Prevention (CDC) - www.cdc.gov/mmwr/
- Extension Toxicology Network (EXTOXNET) - http://extoxnet.orst.edu/
- International Agency for Research on Cancer (IARC) - http://www.iarc.fr/
- National Cancer Institute (NCI) - http://www.cancer.gov/
- National Institutes of Health (NIH)
- National Library of Medicine (NLM)
    - Hazardous Substance Data Bank (HSDB) - http://toxnet.nlm.nih.gov/
    - Toxnet - http://toxnet.nlm.nih.gov/
    - PubMed - http://www.ncbi.nlm.nih.gov/pubmed/
- National Pesticide Information Center (NPIC) - http://npic.orst.edu/
- National Toxicology Program (NTP) –http://ntp-server.niehs.nih.gov/
- Pesticide Action Network North America (PANNA) - http://www.panna.org/
- United States Environmental Protection Agency (USEPA) Web Sites -

  - Office of Pesticides Programs: http://www.epa.gov/pesticides/
  - Integrated Risk Information System (IRIS)
  - Regional Offices
- United States Army Center Health Promotion & Preventative Medicine (USACHPPM) - http://chppm-www.apgea.army.mil/
- United States Geological Survey (USGS) – http://www.usgs.gov/
- Web of Knowledge
- Web of Science
- World Health Organization Web Site (WHO) - http://www.who.int/en/

4. Human Health Toxicity Literature Reviews

Contained in this section are the literature reviews conducted for each vector control agent in the primary list. The agents have been grouped as follow:

- Larvicides (*Bt*, *Bs*, methoprene)
- Pyrethroid Adulticides (permethrin, resmethrin, sumithrin)
- Organophosphate Adulticides (malathion)
- Garlic Oil
- Synergists (PBO)

4.1 Larvicides

Larvicides are insecticide formulations that target insects in the larval or pupal stage of development. The three larvicides included in this review include two biological pesticides (*Bti* and *Bs*) and the insect juvenile hormone mimicker, methoprene.

4.1.1 *Bacillus thuringiensis israelensis (Bti)*

Background

Bacillus thuringiensis (Bt) is a naturally occurring bacterium found in soil (EXTOXNET, 1996). *Bt* was first isolated in Japan in 1902 from diseased silkworm larvae (Jenkins, 1992). *Bt's* use as an insecticide in the U.S. began in 1938. It is used to control pests on food and forage crops, forests and for mosquito control (Jenkins, 1992).

Bt is considered a microbial larvicide that exerts its pesticidal effect through toxins (delta-endotoxins) that are produced during the formation of spores (NPTN, 2000). When insects ingest the spores, the toxin is dissolved and then reacts with the membrane of the digestive system causing cell death (McClintock et al., 1995). The spores may then germinate (vegetative form) and multiply in the insect, leading to death (EXTOXNET, 1996; Jenkins, 1992). Because the spores must be ingested to cause toxicity to insects, *Bt* is only effective during the feeding, or larval, stage of development (NPTN, 2000). Various strains or sub-species of *Bt* exist with some specificity in their toxicity to different insects. *Bti* is a strain that has been used for vector control purposes since 1983 (Westchester County, 2002).

Absorption/Distribution/Excretion

After oral, inhalation or intravenous exposure of rats, *Bt* is gradually eliminated from the body (WHO, 1999). Many studies have shown that after oral doses, *Bt* remains in the GI tract (GI) and is not systemically distributed (McClintock et al., 1995). After injecting *Bt* into the abdominal area (intraperitoneal) of mice, however, *Bt* could be detected in blood for eight days, though no infection resulted (de Barjac et al., 1980 [as cited in WHO, 1999]). Clearance may take longer in preparations that contain more vegetative *Bt* cells than spores. In one study in which mice were injected with *Bti*, *Bti* was detected in blood 80 days following exposure (Siegel and Shadduck, 1990). It was noted that this preparation contained approximately 95 percent vegetative cells, which take a longer time to clear from the body than spores. In another study, after mice were injected intravenously with *Bti*, it was found in the lungs, kidneys, lymph, blood and brain (Harde, 1991 [as cited in McClintock et al., 1995]). The majority of *Bti* was cleared by day 57.

Intranasal and intratracheal administration of various *Bt* subspecies to mice have shown *Bt* to be distributed in the lungs blood, lymph and kidneys (McClintock et al., 1995). Elimination occurs slowly over several days.

Data evaluated by the USEPA on the protein responsible for the pesticidal activity of *Bt* (Cry3Bb1) demonstrates that the protein is expected to be rapidly degraded (within minutes) by mammalian gastric fluid (USEPA, 2003).

Toxicity

According to the USEPA Reregistration Eligibility Document for *Bt*, studies of this bacterium have not shown it to be pathogenic to mammalian species (USEPA, 1998). Toxicity studies submitted to the USEPA in support of the reregistration did not show any adverse effects on body weight (bw) gain or tissue or organ damage upon necropsy of treated animals (McClintock et al., 1995). However, at very high doses (greater than 10^8 colony-forming units) lethality has been observed in laboratory animals (McClintock et al., 1995). The mechanism of this lethality has not been determined.

Any potential health impacts do not appear to be of an infectious nature. There is potential toxicity from toxins, which are also produced by the bacterium. Monitoring for these toxins is conducted during the manufacture of *Bt* products to minimize their presence in the finished

product (USEPA, 1998). One such toxin is thuringiensin (Beta-exotoxin), which manufacturers must demonstrate is absent in the finished product before it is registered by the USEPA. Another heat-sensitive toxin has been found to exert toxicity on non-target species, including mammals. This toxin exerts its effect by inhibiting RNA polymerase. This material appears to be formed during the manufacturing process (USEPA, 1998).

The WHO has concluded that because of the specific nature of the mode of action of *Bt* products, they are unlikely to pose a health risk to humans or other non-target animals, as long as they are free of exotoxins or other non-*Bt* microorganisms. This conclusion is supported by the lack of reports of adverse health effects in workers who manufacture *Bt* products (WHO, 1999). According to Jenkins (1992), *Bt* products are often the insecticides of choice for aerial spraying because they are natural, host-specific and are not persistent in the environment. The protein involved in the pesticidal activity of *Bt* (Cry3Bb1) has been tested and shown not to produce toxicity in mammalian species (USEPA, 2003).

Neurological Effects

There are no reports of neurologic effects after exposure to *Bt* in either animals or humans (Jenkins, 1992).

Skin and Eye Irritation

Human Evidence

According to the WHO, although there is considerable opportunity for occupational exposure via the skin or inhalation, the only adverse effects that have been observed are eye and skin irritation (WHO, 1999). It appears this irritation may be due to other ingredients in the formulation or due to the dryness of the product (USEPA, 1998).

Workers exposed during spray events of *Bt kurstaki* (*Btk*) for gypsy moth control reported transient chapped lips, dry skin, eye irritation and nasal drip, especially during the beginning of a spray. An examination of hospital records during the time period of the spraying did not produce any evidence of illness in the community from the treatment event (Noble et al., 1992 [as cited in WHO, 1999]). Similarly, public health surveillance during *Bti* spraying in the USA, Canada and New Zealand, have rarely identified cases of potential harmful effects (WHO, 1999).

There was a report of a research worker who developed an inflammation of the lymph nodes and a local reaction at the site of an accidental needle stick while handling *Bti*. *Bt* as well as another bacterium were isolated from the infected area (Warren et al., 1984 [as cited in WHO, 1999]).

Animal Evidence

Bt has been tested on the skin of mice and rabbits and shown to have no toxic effects, other than occasional mild irritation (McClintock et al., 1995). de Barjac et al. (1980 [as cited in WHO, 1999]) inoculated mice and guinea pigs subcutaneously with *Bti* and found no evidence of infection or mortality. In a similar study, mice developed abscesses at the injection site when a commercial *Bti* product was used (Siegel et al., 1987 [as cited in WHO, 1999]). This was attributed to contamination with heat-stable foreign material. No evidence of infection or mortality was observed.

Injection of *Bti* into the abdominal area (intraperitoneal) of mice that had their thymus gland removed resulted in significant mortality. However, when the study was repeated with another formulation of *Bti*, no mortality was observed, leading the authors to conclude that the mortality observed in the first study was not caused by *Bti* itself, but something in the formulation used (Siegel et al., 1987 [as cited in McClintock et al., 1995]). Additionally, McClintock et al. (1995) reported that, of 5 different isolates of *Bti*, only one caused significant mortality when injected intraperitoneally at high doses. This supports the conclusion that a contaminant in the formulation is responsible for mortality via this mode of administration.

Applying commercial *Bti* to the eyes of rabbits did not result in irritation or infection (Siegel and Shadduck, 1990; Siegel et al., 1987 [as cited in WHO, 1999]). However, a laboratory culture of *Bti* did produce severe conjunctive congestion and discharge. This was attributed to the nature of the preparation used, which was a dry, clumpy paste (Siegel at al., 1987 [as cited in WHO, 1999]). McClintock et al., (1995) reported that ocular exposure to various subspecies of *Bt* in rabbits resulted in mild redness that resolved within 7 days. *Bti* could be detected in the eyes of rabbits up to one week after exposure (Siegel and Shadduck, 1990).

Gastrointestinal Effects

Human Evidence

Though the endotoxins that are responsible for the pesticidal properties of *Bt* exert their effect through the gut of insects, this is not expected to occur for mammals because the receptors present on the membrane of the insect gut are not present in mammals (USEPA, 1998). An enterotoxin that can cause diarrhea has been reported to be formed from spores of commercial *Bt* (Damgaard, 1995 [as cited in WHO, 1999]).

According to the WHO, *Bti* is used in containers for drinking water in some Asian countries for the control of mosquitoes. The authors note that even though this practice results in a fairly high exposure, no adverse effects have been reported (Menon and De Mestral, 1985 [as cited in WHO, 1999]).

Eight human volunteers ingested 1 gram of *Btk* formulation daily for 5 days. Some of the volunteers also inhaled 100 mg of *Btk* powder for the same time period. Examination immediately after and 4 to 5 weeks following the exposure revealed no evidence of adverse clinical or health effects (Fisher and Rosner, 1959 [as cited in WHO, 1999]).

Ingestion of foods containing another strain, *Bt galleriae (Btg)* did cause nausea, vomiting, diarrhea and colic-like pains, as well as fever in some of the volunteers (Pivovarov et al., 1977 [as cited in WHO, 1999]). A later review of this study concluded that the observed toxicity may have been due to contamination with an exotoxin (Ray, 1990 [as cited in WHO, 1999]).

A related bacterium, *Bacillus cereus (Bc)*, produces a GI illness which causes diarrhea and vomiting. The bacteria form toxins that produce these effects during vegetative growth. Since this bacteria and *Bt* are closely related there has been concern that *Bt* strains may be able to produce similar toxins during vegetative growth. The WHO notes that the public health significance of the potential for the production of these *Bc*-like toxins is not known (WHO, 1999).

Animal Evidence

Bti was given in the diet to rats at a dose of 4g/kg per day for 3 months without any resulting toxicity (McClintock et al., 1995). A longer term (2 years) study was conducted with *Btk* in

which it was administered to rats in the diet at 8400 mg/kg daily. Decreased body weight gain was observed in females (McClintock et al., 1995).

Respiratory Effects

Human Evidence

In a study of farm workers who were exposed to *Bt*, there was no evidence of respiratory symptoms related to occupational exposure (Bernstein et al., 1999).

Animal Evidence

Rats exposed to *Bti* spores (2×10^6 spores) for 30 minutes did not show any evidence of lesions on the lungs (Siegel et al., 1987 [as cited in WHO, 1999]).

Immunologic Response

According to the USEPA, there are no known toxins or metabolites of *Bt* that are immunotoxic (USEPA, 1998).

Human Evidence

Bt has been isolated in various human specimens, but this does not necessarily indicate *Bt* is responsible for infection (WHO, 1999). Even though *Bt* products have been extensively used for many years, there have only been two incidents reported of potential allergic reactions to *Bt*-based products. The first appeared to be due to a pre-existing condition (Kawasaki Syndrome). The second incident involved an individual with severe food allergies (McClintock et al., 1995).

During 1985 and 1986 aerial spraying for gypsy moth control was conducted in Lane County Oregon. The product used was *Btk*. Approximately 80,000 people lived in the treated area during the first season of spraying and approximately 40,000 the second year. A public health surveillance program was undertaken to determine whether *Btk* could be causing any illness in the area. In three of the 55 cases in which *Bt* was isolated, it could not be determined whether or not *Bt* caused the illness. In the other cases *Bt* was ruled out as a causative agent (Green et al., 1990). A similar study in British Columbia, Canada, isolated *Btk* from air, water and human nasal swab samples during an aerial spray program for gypsy moths. This study found *Btk* in air, water and nasal swab samples, both before and after the spray event. The authors suggest that the presence of *Btk* in the water and air samples prior to the spray event may be due to either

other commercial products in use that contain *Bt* or its presence naturally. The frequency of *Btk* positive nasal swabs increased significantly after the spraying took place. This incidence also increased outside the spray area, which may be indicative of the *Bt* spores remaining in the air and being carried outside the spray area. Human health surveillance efforts did not detect short-term health effects in the adult population, in emergency room visits, or in aggravation of asthma in children (Valadares de Amorim et al., 2001).

In a study that investigated farm workers who were occupationally exposed to *Btk*, various indicators of immune response were evaluated. There was a significant positive response in skin prick tests 1 and 4 months after exposure to *Btk* spray. Specific IgG and IgE antibodies were present, and in a greater frequency, in the high exposure group compared to the lower exposure groups. Antibodies specific to vegetative *Bt* organisms were also isolated in a couple of individuals. The authors of this study (Bernstein et al., 1999) conclude that repetitive exposure to *Bt* sprays may lead to allergic skin sensitization and the induction antibodies.

Regarding potential allergic responses to the protein responsible for the pesticidal activity of *Bt* (Cry3Bb1), the USEPA has concluded that because this protein is expected to be broken down quickly in the GI tract, an allergic response following oral ingestion would not be expected. In addition, the USEPA has concluded that the acute oral toxicity data that has been submitted on this protein does not indicate other immune system toxicity (USEPA, 2003).

Animal Evidence

Several studies were reported by the WHO (1999) that investigated the potential for *Bti* to cause infection in immunocompromised animals. Though the clearance rates differed, immune-suppressed animals did not experience an increased rate of infection (WHO, 1999; Siegel and Shadduck, 1990).

Endocrine Disruption

None of the toxins or metabolites of *Bt* are known to act as endocrine disruptors (USEPA, 1998).

Developmental and Reproductive Effects

There was no information available on developmental or reproductive effects of Bti in humans or animals.

Cancer

Mutagenicity studies with *Bt* have produced negative results (Jenkins, 1992). In a 2-year bioassay in which rats were given dietary doses of 8,400 mg/kg of *Bt*, no tumors were reported (Abbott Laboratories, 1982 [as cited in WHO, 1999]; EXTOXNET, 1996).

Studies conducted in cells from the root of *allium* plants (onion family) were exposed to two *Bt* toxins. One toxin (beta exotoxin), which is formed only by some *Bt* subspecies, was found to arrest cell division and to cause clastogenesis in one *allium* species. The other (delta endotoxin), which is formed by all *Bt* subspecies, just slightly depressed mitotic activity (Panda et al., 1979).

Table 2
Toxicity Study Summary Table- *Bacillus thuringiensis*

Compound	Purity	Test Species (Strain)	Test Species Age/Lifecyle	Route	Dose	Study Duration	Endpoint	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Comments	Reference
Cry3Bb1 var		mice		oral	36, 396, 3780 mg/kg bw	14 days	clinical signs, body weight gain, necropsy findings	3780		proteins that are toxic are usually so at low levels in acute manner. This demonstrates lack of toxicity	USEPA, 2003
Cry3Bb1 var		mice		oral	39, 2980 mg/kg bw	14 days	clinical signs, body weight gain, necropsy findings	2980		proteins that are toxic are usually so at low levels in acute manner. This demonstrates lack of toxicity	USEPA, 2003
Cry3Bb1 var		mice		oral	400, 1100, 3200 mg/kg bw	14 days	clinical signs, body weight gain, necropsy findings	3200		proteins that are toxic are usually so at low levels in acute manner. This demonstrates lack of toxicity	USEPA, 2003
Bacillus thurin-giensis israelensis		CD-1 mice	26-33 days old	ip injection	$2.72x10^7$ cfu Bti-82161	80 days	enlarged spleen		$2.72x10^7$ cfu		Siegel and Shadduck, 1990
					$2x10^7$ cfu ABG-6193	27 days	histological eval	$2x10^7$ cfu		no effects noted	
		New Zealand Rabbit	3 kg	ocular instillation	$5.43x10^6$ cfu	8 weeks	infection	$5.43x10^6$ cfu		*Bti* recovered up to 1 week post-exposure	
Bacillus thuringiensis		rats		dietary	8400 mg/kg bw per day	13 weeks		8400 mg/kgbw per day		no effects noted	Ray, 1991; as cited in EXTOXNET, 1996
Bacillus thuringiensis kurstaki		rats		dietary	8400 mg/kg bw per day	2 years		8400 mg/kgbw per day		decreased body wgt in females during weeks 10-104, no other effects noted	Abbott Laboratories, 1982; as cited in EXTOXNET, 1996 and WHO, 1999
Bacillus thuringiensis Beta-exotoxin		rats		oral	170 mg/kg bw	acute	LD50		170 mg/kg bw		Majors and Burrato, 1984; as cited in McClintock et al., 1995
Bacillus thurin-giensis israelensis		rabbit		oral	2×10^9 spores	acute	LD50		$>2 \times 10^9$ spores	no infectivity noted	McClintock et al., 1995
		rat		oral	2.67 g/kg bw	acute	LD50		>2.67 g/kg bw		
		rat		oral	$2.3x\ 10^{10}$ spores	acute	LD50		$2.3x\ 10^{10}$ spores	no infectivity or pathogenicity noted	
Bacillus thurin-giensis israelensis		rat		dermal	2000 mg/kg bw	acute	LD50		>2000 mg/kg bw	no infectivity or pathogenicity noted	McClintock et al., 1995
		rabbit		dermal	6.28 g/kg	acute	LD50		>6.28 g/kg	no infectivity or toxicity noted	
		rat		intratracheal	$8 x10^7$ spores/ animal	acute	LD50				
Bacillus thurin-giensis israelensis		rat		feeding/oral	4 g/kgbw per day	3-month		4 g/kgbw		no toxicity noted	McClintock et al., 1995
Bacillus thurin-giensis israelensis		Swiss mice		inhalation	$2x10^8$ spores for 15 min	15 days		$2x10^8$ spores		no Bti recovered in lungs	de Barjac et al., 1980; as cited in WHO, 1999
Bacillus thurin-giensis israelensis		Sprague-Dawley rats		inhalation	$2x10^6$ spores for 30 min	27 days	lung lesions	$2x10^6$ spores		no Bti detected 7 days post-exposure	Siegel et al., 1987; as cited in WHO, 1999
Bacillus thurin-giensis israelensis		Swiss mice		dermal	$5.1x10^7$ cfu		inflammation	$5.1x10^7$ cfu			de Barjac et al., 1980; as cited in WHO, 1999
Bacillus thurin-giensis israelensis		New Zealand rabbits		dermal scarification	$3.3x10^{13}$ cfu		inflammation or infection	$3.3x10^{13}$ cfu			de Barjac et al., 1980; as cited in WHO, 1999
Bacillus thurin-giensis israelensis		Swiss mice		subcutaneous injection	$8.5x10^7$ cfu		infection or mortality	$8.5x10^7$ cfu			de Barjac et al., 1980; as cited in WHO, 1999
		guinea pigs			$1.7x10^8$ cfu		infection or mortality	$1.7x10^8$ cfu			
Bacillus thurin-giensis israelensis		BALB/c mice	female	subcutaneous injection	$1x10^9$ cfu		infection or mortality	$1x10^9$ cfu		abcess occurred at injection site but not attributed to Bti	Siegel et al., 1987; as cited in WHO, 1999
Bacillus thurin-giensis israelensis		New Zealand rabbits		eye	$3.7x10^7$ cfu		irritation or conjunctivitis	$3.7x10^7$ cfu			de Barjac et al., 1980; as cited in WHO, 1999
Bacillus thurin-giensis israelensis	commercial dry powder formulation	New Zealand rabbits		eye	50 mg/day	9 days	irritation or conjunctivitis	50 mg/kgbw			Siegel et al., 1987; as cited in WHO, 1999
	laboratory grown						irritation or conjunctivitis		50 mg/kgbw	severe conjunctival congestion and discharge, due to formulation	

Reference List

Bacillus thuringiensis

Abbott Laboratories. 1982. Toxicology Profile: Dipel, *Bacillus thuringiensis* Insecticide Chemical and Agricultural Products Division, North Chicago, IL, 1982. 10-149. As cited in EXTOXNET, 1996 and WHO, 1999.

Bernstein, IL, J Bernstein, M Miller, S Tierzieva, D Bernstein, Z Lummus, M Selgrade, D Doerfler, and V Seligy. 1999. *Immune Responses in Farm Workers After Exposure to Bacillus Thuringiensis Pesticides.* Environmental Health Perspectives, Volume 107, Number 7, July 1999.

Damgaard, PH. 1995. *Diarrhoeal enterotoxin production by strains of Bacillus thuringiensis isolated from commercial Bacillus thuringiensis–based insecticides.* FEMS Immunol. Med. Microbiol., 12: 245-250. As cited in WHO, 1999.

de Barjac, H, I Larget, L Benichou, V Cosmao, G Viviani, H Ripouteau, and S Papion. 1980. *Test d'innocuite sur mammiferes avec du serotype H 14 de Bacillus thuringiensis.* Geneva, World Health Organization (WHO/VBC/80.761). As cited in WHO, 1999.

EXTOXNET. 1996. *Extension Toxicology Network Pesticide Information Profile for Bacillus thuringiensis.* Cooperative Extension Offices of Cornell University, Oregon State University, University of Idaho, University of California at Davis, Institute for Environmental Toxicology, Michigan State University. Revised June 1996.

Fisher, R and L Rosner. 1959. *Toxicology of the microbial insecticide, Thuricide.* J. Agric. Food Chem., 7: 686-688. As cited in WHO, 1999.

Green, M, M Heumann, R Sokolo, LR Foster, R Bryant, and M Skeels. 1990. *Public Health Implications of the Microbial Pesticide Bacillus thuringiensis: An Epidemiological Study, 1985-86.* American Journal of Public Health Vol. 80, No. 7, July 1990.

Harde, T. 1991. *Bacillus thuringiensis var. israelensis acute intravenous toxicity/pathogenicity study in rats given Bti Batch BIF 61. Project I.D. No. 90070. Unpublished study prepared by Novo Nordisk A/S. EPA MRID No. 419801-04C. 1991.* As cited in McClintock et al., 1995.

Jenkins, J. 1992. *Environmental Toxicology and Chemistry Memo Subject: B.t.* Department of Agricultural Chemistry, Oregon State University. Printed 1992.

Majors, KP and B Burrato. 1984. *Acute oral toxicity evaluation of ABG-6146 (beta-exotoxin formulation) in mice and rats.* Study No. T-83-437. Unpublished study for Abbott Laboratories, 1985. As cited in McClintock et al, 1995.

McClintock, JT, CR Schaffer, and RD Sjoblad. 1995. *A Comparative Review of the Mammalian Toxicity of Bacillus thuringiensis-Based Pesticides.* Pestic. Sci. 1995, 45, 95-105.

Menon, AS and J DeMestral. 1985. *Survival of Bacillus thuringiensis var. kurstaki in waters.* Water Air Soil Pollut., 25: 265-274. As cited in WHO, 1999.

National Pesticide Telecommunications Network (NPTN). 2000. *Technical Fact Sheet Bacillus thuringiensis* . Reviewed 10/24/2000.

Noble, MA, PD Riben, and CJ Cook. 1992. *Microbiological and epidemiological surveillance programme to monitor the health effects of Foray 48B BTK spray.* Vancouver, Canada, Ministry of Forests of the Province of British Columbia, *pp. 1-63.* As cited in WHO, 1999.

Panda, B, R Sahu, C Sharma. 1979. *Cytogenetic Hazards From Agricultural Chemicals, 2.Selective Clastogenesis and Spindle Inhibition in Some Plant Mitotic Systems by the B-Exotoxin and the General Ineffectiveness of the B-Endotoxin Protein of Bacillus thuringiensis.* Mutation Research, 67 (1979) 161-166, 1979.

Pivovarov, YP, SA Ivashina, and VP Padalkin. 1977. *[Hygienic assessment of investigation of insecticide preparations.]* Gig I Sanit., 8:45-48 (in Russian). As cited in WHO, 1999.

Ray, DE. 1991. *Pesticides derived from plants and other organisms.* In: Hayes, W.J. & Laws ER ed. Handbook of Pesticide Toxicology. New York, London, Academic Press, pp 11-18. As cited in WHO, 1999 and as cited in EXTOXNET, 1996.

Siegel, JP, JA Shadduck, and J Szabo. 1987. *Safety of the entomopathogen Bacillus thuringiensis var. israelensis for mammals.* J. Econ Entomol., 80: 717-723. As cited in WHO, 1999 and McClintock et al., 1995.

Siegel, JP and JA Shadduck. 1990. *Clearance of Bacillus sphaericus and Bacillus thuringiensis ssp. israelensis from mammals.* J. Econ. Entomo., 83: 347-355.

United States Environmental Protection Agency (USEPA). 1986. *Pesticide Fact Sheet Number 93: Bacillus thuringiensis*. Office of Pesticides and Toxic Substances, Washington, D.C., 1986. 10-149.

United States Environmental Protection Agency (USEPA). 1998. *Reregistration Eligibility Decision, Bacillus thuringiensis.* Office of Prevention, Pesticides and Toxic Substances. EPA738-R-98-004, March 1998.

United States Environmental Protection Agency (USEPA). 2003. *Bacillus thuringiensis Cry3Bb1; Notice of Filing Pesticide Petition to Establish a Tolerance Exemption for a Certain Pesticide Chemical in or on Food.* Federal Register, October 22, 2003 (Volume 68, Number 204) [Notices] pg 60371-60375.

Valadares de Amorim, G, B Whittome, B Shore, and D Levin. 2001. *Identification of Bacillus thuringiensis subsp. kurstaki Strain HD1-Like Bacteria from Environmental and Human Samples after Aerial Spraying of Victoria, British Columbia, Canada, with Foray 48B,* 2001. Applied and Environmental Microbiology, p. 1035. Vol. 67, No. 3. Mar. 2001.

Warren, RE, D Rubenstein, DJ Ellar, JM Kramer,and RJ Gilbert. 1984. *Bacillus thuringiensis var. israelensis: Protoxin activation and safety. Lancet: March 24: 678-679.* As cited in WHO, 1999.

Westchester County. 2002. *Comprehensive Mosquito-Borne Disease Surveillance and Control Plan*, Generic Environmental Impact Statement. February, 2002.

World Health Organization (WHO). 1999. *Environmental Health Criteria 217, Microbial Pest Control Agent Bacillus thuringiensis.* International Programme on Chemical Safety. Geneva.

4.1.2 *Bacillus sphaericus (Bs)*

Background

Bs is similar to *Bt* in that it is a naturally occurring bacterium found in soils. *Bs* and *Bt* are biological pesticides that exert their toxic effects on insects via a protein or endotoxin. These proteins are relatively specific for different target insects and must be ingested during the larval stage of development to be effective.

Absorption/Distribution/Excretion

After administration to the respiratory system, peak concentrations of *Bs* can be detected in the lymph system and spleen 1 day after exposure, with no *Bs* detected 7 days after exposure. *Bs*, however, can still be detected in the lungs 49 days later (David, 1989 [as cited in McClintock et al., 1995]).

After injection of *Bs* into the abdominal area (i.e., i.p. injection) of mice, *Bs* could be recovered in the spleen 67 days post-exposure (Siegel and Shadduck, 1990).

The presence of these bacteria after exposure is not indicative of an infection (WHO, 1999).

Toxicity

In a review of the toxicity studies for *Bt*, McClintock et al., (1995) concluded that the overall evidence on *Bt* subspecies and *Bs* clearly indicate that they are not infectious or pathogenic.

In a 1998 review conducted to establish an exemption from the requirement of a tolerance for *Bs* in foods, the USEPA concluded that residues of *Bs* on food would not be expected to result in harm, considering the low mammalian toxicity of *Bs* and its ubiquitous occurrence naturally. Several studies have shown that oral, intratracheal and intravenous administration of *Bs* does not produce mortality, pathogenicity or other toxicity at doses ranging from 1×10^7 to 1×10^8 colony forming units (cfu) (USEPA, 1998). For more information on these studies, please refer to the Toxicological Data Table for *Bs*.

The acute oral and dermal lethal doses (LD_{50}s) for *Bs* are greater than 5000 and 2000 mg/kg bw, respectively (USEPA, 1998). Additionally a four hour inhalation study, which exposed laboratory rats to 0.09 mg of *Bs*/L in air did not detect any clinical signs of toxicity during a 14 day post-exposure observation period (USEPA, 1998).

Neurological Effects

No reports of neurological effects were found in the available literature. However, there was a study that did report lesions in the brain and bleeding following intracerebral injection of *Bs* (Shadduck et al., 1980). There was no evidence of bacterial infection in these lesions.

Skin and Eye Irritation

Human Evidence

No information on skin and eye irritation in humans was found.

Animal Evidence

Applying commercially produced *Bs* to the eyes of rabbits did not result in irritation or infection. However, *Bs* could be detected in the eyes of rabbits up to eight weeks after exposure (Siegel and Shadduck, 1990). When *Bs* was injected into the eyes of rabbits, moderate to severe lesions did result (Shadduck et al., 1980). Though less severe, these lesions also resulted when the *Bs* solution was subjected to extreme heat before intraocular injection.

Injection of 6.7×10^9 cfu *Bs* under the skin of mice did not produce any effects, except in one animal with one strain of *Bs* (Shadduck et al., 1980). Injections of a strain of *Bs* into the abdominal area of mice lead to significant mortality in high dose groups, though previous studies using different strains had not produced such mortality (Siegel and Shadduck, 1990; Shadduck et al., 1980). The exact mechanism for this mortality is not understood, however, it appears to be related to the *Bs* spore. Examination of tissues from animals that died did not reveal lesions that could be responsible for the mortality. Injection of less than 1×10^8 cfu of *Bs* did not cause mortality (Siegel and Shadduck, 1990). Similar injection of the *Bs* toxin also did not result in mortality.

Gastrointestinal Effects

No information was available concerning effects of *Bs* on the GI system.

Respiratory Effects

No information was available concerning effects of *Bs* on the respiratory system.

Immunologic Response

No information was available concerning effects of *Bs* on the immune system.

Endocrine Disruption

No information was available concerning endocrine effects of *Bs*.

Developmental and Reproductive Effects

No information was available concerning developmental or reproductive effects or of *Bs*.

Cancer

No information was available on possible carcinogenic effects of *Bs*.

Table 3
Toxicity Study Summary Table- *Bacillus sphaericus*

Compound	Purity/ Strain	Test Species (Strain)	Test Species Age/Lifecyle	Route	Dose	Study Duration	Endpoint	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	LOAEL Statistical Significance	Comments	Reference
Bacillus sphaericus	SSII-1, 1404-9, 1593-4	CF-W mice		s.c. injection intracerebral injection	6.7×10^9 cfu 1×10^8 to 1×10^9 cfu	14 days	hemorrhage	6.7×10^9 cfu	3×10^8 cfu		1 animal developed abcess	Shadduck et al., 1980
Bacillus sphaericus	SSII-1, 1404-9, 1593-4	Sprague-Dawley rats		I.p. injection intracerebral injection	3.2 to 4.7×10^8 cfu 1×10^8 to 1×10^9 cfu	9 days	mild perivascular cuffs, meningitis	3.2 to 4.7×10^8 cfu	1×10^8		no detectable lesions	Shadduck et al., 1980
Bacillus sphaericus	SSII-1, 1404-9, 1593-4	New Zealand white rabbits		intracerebral injection intraocular injection	1×10^8 to 1×10^9 cfu 1×10^3		mod. endophthalmitis panophthalmitis	1×10^9 cfu	1×10^3			Shadduck et al., 1980
Bacillus sphaericus		CD-1 mice New Zealand Rabbit	26-33 days old 3 kg	ip injection ocular instillation	8×10^8 cfu 4×10^5 to 4×10^9 filtered & unfilt 4.48×10^8 cfu	24 hr 24 hr 24 hr 8 weeks	mortality mortality infection	 4×10^7 cfu 4×10^9 cfu 4.48×10^8 cfu	8×10^8 cfu 4×10^8 cfu	 unfiltered filtered	histology revealed no lesions *Bs* recovered up to 8 wks post exp	Siegel and Shadduck, 1990
Toxin extract of *Bacillus sphaericus 2362*		CD-1 mice	26-33 days old	ip injection	0.7mg/0.5ml	3 weeks	mortality	0.7mg/0.5ml				Siegel and Shadduck, 1990
Bacillus sphaericus		rats		oral	1×10^8 cfu	20 days	mortality, pathogenicity, or toxicity	1×10^8 cfu			rapid clearance	USEPA, 1998
Bacillus sphaericus		rats		intratracheal	1×10^8 cfu	49 days	mortality, pathogenicity, or toxicity	1×10^8 cfu				USEPA, 1998
Bacillus sphaericus		rats		intravenous	1×10^7 cfu	35 days	mortality, pathogenicity, or toxicity	1×10^7 cfu				USEPA, 1998
Bacillus sphaericus		rats		oral	5000 mg/kgbw		mortality	5000 mg/kgbw				USEPA, 1998
Bacillus sphaericus		rabbits		dermal	2000 mg/kgbw	24hr/14 day	mortality	2000 mg/kgbw				USEPA, 1998
Bacillus sphaericus		rats		inhalation	0.09 mg/L	4hr/14 day	mortality	0.09 mg/L				USEPA, 1998

cfu: Colony forming unit

Reference List

Bacillus sphaericus

David, RM. 1989. *Acute pulmonary toxicity/pathogenicity study of Bacillus sphaericus technical powder in rats.* MBA Study No. G-7161.225. Unpublished study for Abbott Laboratories. EPA MRID No. 411034-02, 1989. As cited in McClintock et al., 1995.

McClintock, JT, CR Schaffer, and RD Sjoblad. 1995. *A Comparative Review of the Mammalian Toxicity of Bacillus thuringiensis-Based Pesticides.* Pestic. Sci. 1995, 45, 95-105.

New York State Department of Environmental Conservation (NYSDEC). 1996. *Bacillus sphaericus (VectoLex) Registration of New Active Ingredient 8/96.* Letter to Mr. Marvin Alphin, Abbott Laboratories from Norman Nosenchuck. 8/2/96.

Shadduck, J, S Singer, and S Lause. 1980. *Lack of Mammalian Pathogenicity of Entomocidal Isolates of Bacillus sphaericus.* Environ. Entomol. 9:403 407. 1980.

Siegel, JP and JA Shadduck. 1990. *Clearance of Bacillus sphaericus and Bacillus thuringiensis ssp. israelensis from mammals.* J. Econ. Entomo., 83: 347-355.

United States Environmental Protection Agency (USEPA). 1998. *Bacillus sphaericus (VectoLex) Tolerance Requirement Exemption 8/98.* Federal Register September 10, 1998, pp 48594-48597.

World Health Organization (WHO). 1999. *Environmental Health Criteria 217, Microbial Pest Control Agent Bacillus thuringiensis.* International Programme on Chemical Safety. Geneva.

4.13 Methoprene

Background

Methoprene is a larvicide that is available in a granular form, emulsifiable concentrate and in briquettes. It was first registered in 1975 and is considered a biochemical pesticide (USEPA, 2002). Methoprene is an insect juvenile hormone analogue, which interferes with metamorphosis in insects, impairing development into adult insects. This toxic mechanism is selective to insects (WHO, 1984). In addition to mosquitoes, methoprene insecticides are used to control fleas, flies, moths and beetles. Methoprene has two molecular structures, R and S form. S-Methoprene is the form that is responsible for insecticidal activity (WHO, 2001 and USEPA, 2002).

Absorption/Distribution/Excretion

Studies on the absorption and distribution of methoprene have mostly utilized the oral route of exposure. Concentrations in blood appear to peak 2 hours after exposure in rats and 5.5 hours in guinea pigs (Chamberlain et al., 1975 [as cited in WHO, 2001]; Ekdawi and Yu, 1996 [as cited in WHO, 2001]). In organs that receive a lot of circulating blood, peak concentrations are reached 6-12 hours after oral exposure. In other tissues that receive less blood flow, such as muscle and fat, peak concentrations may not be reached until 12 hours after an oral exposure. Studies using oral administration of radio labeled methoprene indicate that some organs in the body, such as the adrenal cortex, appear to incorporate biotransformed methoprene into their tissue (Chasseaud et al., 1974 [as cited in WHO, 2001]; Hawkins et al., 1977 [as cited in WHO, 2001]). Fat tissue appears to store unchanged methoprene (Ekdawi and Yu, 1996 [as cited in WHO, 2001]).

Methoprene is excreted via the urine, feces and expired air. Mice receiving 1.2 mg/kg bw by gavage excreted 64 percent in the urine and 12 percent in the feces after 24 hours. Ninety-six hours after administration, 68 percent and 14 percent was excreted in the urine and feces, respectively (Cohen and Trudell, 1972 [as cited in WHO, 2001]). In another study in which expired air was also measured following an oral dose of 25 mg/kg bw in rats, 26 percent was excreted in expired air, 13 percent in urine and 5 percent in feces. After 120 hours, 39 percent was excreted in expired air, 20 percent in urine and 18 percent in feces, with 17 percent remaining in the carcass (Chasseaud et al., 1974 [as cited in WHO, 2001]; Hawkins et al., 1977

[as cited in WHO, 2001]). In cows receiving 0.61 mg/kg oral dose, approximately 8 percent of the dose was excreted in milk (Chamberlain et al., 1975 [as cited in WHO, 2001]). Hens excreted up to 19 percent of an orally administered dose ranging from 59 to 64 mg/kg bw (Davison, 1976 [as cited in WHO, 2001]).

Studies in rats, guinea pigs, cows and poultry indicate that methoprene undergoes extensive biotransformation once absorbed. Most of what is excreted in the urine is metabolites, whereas a large percentage (approximately 77 percent) of what is excreted in the feces is unchanged, and probably reflects unabsorbed parent compound (WHO, 2001). The portion of methoprene that is expired in air is mostly in the form of carbon dioxide (Chasseaud et al., 1974 [as cited in WHO, 2001]; Hawkins et al., 1977 [as cited in WHO, 2001]).

Toxicity

Methoprene is generally considered to be of low toxicity, with little acute toxicity (USEPA, 2002). In evaluating the reregistration eligibility of methoprene, the USEPA did not identify an endpoint for acute toxicity. Acute LD_{50}s have been reported to be greater than 2 g/kg bw in the laboratory animals species tested (USEPA, 2002).

Symptoms of acute exposure to methoprene include aggressive behavior, depression, tremors, lacrimation, salivation, dilated pupils, diarrhea, distended abdomen and increased respiratory frequency (Jorgenson and Sasmore, 1972a [as cited in WHO, 2001]). Very high doses (e.g., 10 g/kg bw) may lead to shallow respiration, difficulty walking, vomiting, convulsions and death (Hill, 1972a [as cited in WHO, 200]). Dogs receiving up to 5 g/kg bw were observed for 21 days with no adverse effects being noted (Hill, 1972b [as cited in WHO, 2001]).

Neurological Effects

No information was obtained concerning neurological effects of methoprene.

Skin and Eye Irritation

Human Evidence

No information was available on skin or eye irritation in humans.

Animal Evidence

Studies in rabbits in which methoprene is applied directly to the eyes has resulted in either slight or no irritation (Hill, 1973 [as cited in WHO, 2001]; Brown, 1984 [as cited in WHO, 2001]). The USEPA 1991 Reregistration Eligibility Document concluded that methoprene was not an eye irritant (USEPA, 1991). Moderate to severe eye irritation was reportedly observed following applications of 2 percent ground briquette formulation or 4 percent ground pellet formulation (Hiles and Collins, 1984, Schindler and Baldwin, 1991a [as cited in WHO, 2001]). In all these studies, the observed irritation was transient, with full recovery after one to three days.

A few studies have been conducted to evaluate the potential for methoprene to cause dermal irritation. Most of these studies indicate little, if any, dermal irritation when methoprene is applied to the skin (WHO, 2001; USEPA, 1991). Studies have been inconclusive on whether methoprene causes skin sensitization. Some have indicated that methoprene is not a skin sensitizer (Nakayoshi, 1975 [as cited in WHO, 2001]; Blaszcak, 1994a [as cited in WHO, 2001]; Blaszcak, 1994b [as cited in WHO, 2001]), though others have indicated that it does have the potential to cause sensitization (Schindler and Baldwin, 1991b [as cited in WHO, 2001]). A USEPA evaluation concluded that methoprene is not a skin sensitizer (USEPA, 1991).

Gastrointestinal Effects

No information was found regarding the GI effects of methoprene.

Respiratory Effects

No information was obtained on possible respiratory effects of methoprene.

Immunologic Response

No information was found regarding immunologic effects of methoprene.

Endocrine Disruption

Human Evidence

There was no information available on endocrine effects in humans.

Animal Evidence

The studies that have been conducted to evaluate the potential for methoprene to have endocrine disrupting effects have been negative (WHO, 2001).

Developmental and Reproductive Effects

Human Evidence

There was no information available on developmental or reproductive effects in humans.

Animal Evidence

In a three generation reproductive study, rats were exposed to methoprene in the diet at concentrations of 500 or 2500 parts per million (ppm) (25 and 75 mg/kg bw per day). At the highest concentration tested, mean pup weight and weight gain were reduced and an increase in the number or pups born dead was observed (Killeen and Rapp, 1974 [as cited in WHO, 2001]). However, the USEPA did not consider these effects to be significant and concluded that no adverse effects were observed at the highest dose tested (USEPA, 1991).

The USEPA does not consider methoprene to be a developmental toxicant based upon developmental toxicity studies that have been conducted in mice and rabbits t (USEPA, 1991). A study in mice exposed animals to oral doses ranging from 50 to 600 mg/kg bw per day on gestation days 7 to 14. An increase in the number of implantations and live fetuses was observed at 200 and 600 mg/kg, which the authors considered indicative of a modest advancement of development and not toxicologically. No fetotoxicity was observed, however, at weaning, an increase in lung, liver and kidney weight was observed at the highest dose tested (Nakasawa et al., 1975a [as cited in WHO, 2001]). A study in rabbits noted an increase in the incidence of fetal deaths and an increase in the proportion of female fetuses at a dose of 2000 mg/kg bw. Maternal weight gain was also decreased at this dose, indicative of maternal toxicity. The no observed adverse effect level (NOAEL) for this study was determined to be 190 mg/kg bw per day (Nakasawa et al., 1975b [as cited in WHO, 2001]).

Cancer

Human Evidence

There was no information available on carcinogenicity of methoprene in humans.

Animal Evidence

In vitro mutagenicity studies have been conducted, with and without activation, which do not indicate that methoprene is mutagenic, however, the range of doses tested was limited (WHO, 2001).

The few long-term animal studies that have been conducted did not produce evidence of carcinogenicity. These studies are discussed in further detail under liver and kidney toxicity. The WHO has concluded that methoprene is unlikely to pose a carcinogenic risk to humans (WHO, 2001). Similarly, the USEPA has concluded that methoprene is not oncogenic based on the long-term exposure studies that have been conducted (USEPA, 2001).

Other Toxicity

Liver and Kidney Toxicity

Studies in laboratory animals have shown that oral doses of methoprene can cause liver or kidney toxicity. Rats receiving methoprene in the diet at a dose of 170 mg/kg bw per day for 90 days had increased liver and kidney weights, as well as kidney tubule lesions. The kidney effects were considered by the authors to be reversible (Jorgenson and Sasmore, 1972b [as cited in WHO, 2001]). Increased liver weight was also observed in a dose dependent manner in dogs receiving 25 to 500 mg/kg bw per day for two weeks in the diet (Jorgenson and Sasmore, 1972a [as cited in WHO, 2001]). Swelling and vacuolization was also observed in hepatocytes at doses of 250 and 500 mg/kg bw per day. In another study, dogs received doses ranging from 6.2 to 120 mg/kg bw per day for 90 days. Liver weights were elevated and serum alkaline phosphatase was also increased (Jorgenson and Sasmore, 1972b [as cited in WHO, 2001]). The NOAEL for this study was 8.6 mg/kg bw per day.

Increased liver and kidney weights were also observed in rabbits that received dermal doses of methoprene. Technical grade methoprene was applied to the skin at doses of 100 to 2700 mg/kg bw per day for 30 days. Redness in the area of application was observed at the highest dose used and occasionally at lower doses. Reduced weight gain and increased white blood cell counts were observed in all treatment groups. Kidney weights were increased in a dose dependent manner and liver weight was increased at the highest dose tested (Nakasawa, 1975 [as cited in WHO, 2001]).

In one inhalation study, rats were exposed to aerosol concentrations of methoprene at 2 mg/l or 20 mg/l in air for four hours a day for five days a week for three weeks. A slight nasal discharge, as well as increased serum alkaline phosphatase activity was noted at the highest concentration used. Bilirubin concentrations in the blood were also increased in a dose dependent manner (Olson, 1972 [as cited in WHO, 2001]).

A few longer term exposure studies have been conducted. Mice were given methoprene in the diet for 78 weeks. Doses ranged from 38 to 380 mg/kg bw per day. An unidentified brown pigment was observed in the livers of mice receiving 150 and 380 mg/kg per day (Wazeter and Goldenthal, 1975a [as cited in WHO, 2001]). The USEPA used this study to derive their oral chronic reference dose (RfD) of 0.4 mg/kg bw per day (USEPA, 2002).

In another study, rats were given methoprene in the diet resulting in doses of 12 to 250 mg/kg bw per day for two years. The only treatment related effect noted was an increase in relative liver weight at the highest dose tested (250 mg/kg bw per day). The NOAEL in this study was determined to be 50 mg/kg bw per day (Wazeter and Goldenthal, 1975b [as cited in WHO, 2001]).

Table 4
Toxicity Study Summary Table- Methoprene

Compound	Purity	Test Species (Strain)	Test Species Age/Lifecyle	Route	Dose Duration	Study Duration	Endpoint	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	LOAEL Statsitical Significance	Comments	Reference
Methoprene, racemic	68.90%	Sprague Dawley rats	28 days	diet	0,250,500, 1000, 5000 ppm	90 days	incr relative liver and kidney (males only) weight, kidney lesions 1000 and 5000 ppm	17	34		kidney lesions considered reversible. 5000 ppm considered excessive. EPA considered inapp for endpt selection	Jorgenson and Sasmore, 1972b; as cited in WHO, 2001 and USEPA, 2002
Methoprene, racemic	68.90%	Beagle dogs		diet	1000, 5000, 10000, 20000 ppm	2 weeks	dose dependent increase in relative liver wgt	120	250		1 week recovery period may have masked some treatment effects	Jorgenson and Sasmore, 1972a; as cited in WHO, 2001
Methoprene, racemic	68.90%	Beagle dogs	19 weeks old	diet	250, 500, 5000 ppm	90 days	serum alkaline phosphatase activity increased, incr relative liver wgt	8.6	86		Used by WHO for ADI, EPA considered inappropriate for endpoint selection	Jorgenson and Sasmore, 1972b; as cited in WHO, 2001 and USEPA, 2002
Methoprene, racemic	86.90%	Charles River CD-1 mice		diet	250, 1000, 2500 ppm	78 weeks	inidentified brown pigment in liver	37.5	150		used by EPA to set RfD, WHO did not consider pigment to be adverse effect	Wazeter and Goldenthal, 1975a; as cited in WHO, 2001 and USEPA, 2002
Methoprene, racemic	96%	Japanese rabbits		dermal	100,300,900 2700 mg/kgbw per day over 10 cm circular area	30 days	redness, reduced wgt, incr white blood cell count, incr kidney and liver wgt		97		WHO considered study of limited value USEPA considered this dose to be NOAEL	Nakasawa, 1975; as cited in WHO, 2001 and USEPA, 1991
Methoprene, racemic	68.90%	rats		inhalation, aerosol	0,2,20 mg/L air 4 hr/day, 5 day/wk	3 weeks	incr bilirubin, incr serum alkaline phosphatase	1.4 mg/L 20 mg/L (3408 mg/kg bw day)	14 mg/L	p<0.001 bilirubin p<0.05 alkaline phosphatase	 USEPA evaluation	Olson, 1972; as cited in WHO, 2001 and USEPA, 2002
Methoprene, racemic	86.90%	Charles River CD rats		diet	250, 1000, 5000 ppm	2 years	liver wgt and lesions	44	217		USEPA considers highest dose tested to be NOAEL	Wazeter and Goldenthal, 1975b; as cited in WHO, 2001
Methoprene, racemic	86.90%	Long Evans rats	weanling	diet	500, 2500 ppm	3-generation	pup wgt, incr in pups born dead in F3	29 50	75 250	WHO EPA	EPA appears to use same study for inter. Exp, but calculated diff NOAEL and LOAEL	Killeen and Rapp, 1974; as cited in WHO, 2001 and USEPA, 2002
Methoprene, racemic	96%	ICR mice	mated mice	intubated	50,200,600 mg/kg bw day	gestation day 7-14	effects on fetus incr liver, kidney, lung wgt in male offspring	570 190	 570		NOAEL is highest dose tested observed modest advancement of development organ wgt not considered by EPA	Nakasawa et al., 1975a; as cited in WHO, 2001 and USEPA, 2002
Methoprene, racemic	96%	Japanese rabbits	pregnant dams	gavage	50, 200, 2000 mg/kg bw day	gestation day 7-18	maternal toxicity (wgt gain) fetotoxicity (fetal death)	190 1900	1900		WHO evaluation USEPA evaluation, fetal effects only at maternally toxic doses	Nakasawa et al., 1975b; as cited in WHO, 2001 and USEPA, 2002
Methoprene, racemic		mice	19-21 days	subcutaneous	0.015, 0.15 mg/kg bw per day	3 days	incr in relative uterine wgt	0.15			no effect noted	Rooks, undated; as cited in WHO, 2001
Methoprene, racemic		castrated male rats	21 days old	subcutaneous	0.37, 3.7 mg/kg per day	7 days	incr relative wgt of seminal vesicles ventral prostate or levator ani	3.7			no effect noted	Rooks, undated; as cited in WHO, 2001
Methoprene, racemic		adrenalectomized male rats	21-23 days old	subcutaneous	0.9, 9	6 days	thymus weight	9			no effect noted	Rooks, undated; as cited in WHO, 2001

Table 5

Noncancer Criteria (Oral/Dermal)- Methoprene

Chemical of Potential Concern	Exposure Duration	Criteria	Value	Units	Species	Endpoint	NOAEL/ LOAEL	Units	Combined Uncertainty/Modifying Factors	Study Source	Reference for Criteria
S-Methoprene	chronic, oral	ADI	5.00E-02	mg/kg/day	dogs	incr liver wgt	8.6E+00	mg/kg/day	0.5 X ADI of racemic mix	Jorgenson and Sasmore, 1972b	WHO, 2001
Methoprene, racemic	chronic, oral	ADI	9.00E-02	mg/kg/day	dogs	incr liver wgt	8.6E+00	mg/kg/day	100	Jorgenson and Sasmore, 1972b	WHO, 2001
Methoprene	chronic, oral	RfD	4.00E-01	mg/kg/day	mice	liver lesions, pigment	3.75E+01	mg/kg/day	100	Wazeter and Goldenthal, 1975a	US EPA, 2002

NA: Not Applicable or Not Available.

NOAEL: no observed adverse effect level

LOAEL: lowest observed adverse effect level

RfD: USEPA reference dose

ADI: World Health Organization Acceptable Daily Intake

Reference List

Methoprene

Blaszcak, DL. 1994a. *Closed Patch Repeated Insult Dermal Sensitization Study of Altosid Pellets in Guinea Pigs.* Pharmaco LSR Study No 93-0860. Pharmaco LSR Inc., East Millstone, New Jersey. Submitted to WHO by Novartis Animal Health Australasia Ltd, Pendle Hill, NSW, Australia. As cited in WHO, 2001.

Blaszcak, DL. 1994b. *Closed Patch Repeated Insult Dermal Sensitization Study of Altosid 150 Day Briquet in Guinea Pigs.* Pharmaco LSR Study No. 93-0860. Pharmaco LSR Inc., East Millstone, New Jersey, USA. Submitted to WHO by Novartis Animal Health Australasia Ltd, Pendle Hill, NSW, Australia. As cited in WHO, 2001.

Brown, JM. 1984. *Primary Eye Irritation of S-Methoprene in Rabbits.* Final Report No. 36. SRI Project LSC-7182. SRI International, California, USA. Submitted to WHO by Novartis Animal Health Australasia Ltd, Pendle Hill, NSW, Australia. As cited in WHO, 2001.

Chamberlain, WF, LM Hunt, DE Hopkins, AR Ngrfich, JA Miller, and BN Gilbert. 1975. *Absorption, Excretion, and Metabolism of Methoprene by A Guinea Pig, A Steer, and A Cow.* J.Agric. Food Chem., 23, 738-742. As cited in WHO, 2001.

Chasseaud, LF, DR Hawkins, ER Franklin, and KT Weston. 1974. *The Metabolic Fate of ($5^{14}C$)-Isopropyl 11-Methoxy-3,7,11-Trimethyl-dodeca-2,4-Dieonate (Altosid) in the Rat.* Huntingdon Research Centre, United Kingdom. Submitted to WHO by Novartis Animal Health Australasia Ltd. Pendle Hill, NSW, Australia. As cited in WHO, 2001.

Cohen, EN and T Trudell. 1972. *Metabolism of Altosid in Mice.* Letter from E.N. Cohen to J. Siddal, Stanford University Medical Center, USA. Submitted to WHO by Novartis Animal Health Australasia Ltd, Pendle Hill, NSW, Australia. As cited in WHO, 2001.

Davison, KL. 1976. *Carbon-14 Distribution and Elimination in Chickens Given Methoprene-^{14}C.* J. Agric Food Chem., 24, 641-643. As cited in WHO, 2001.

Ekdawi, ML and CC Yu. 1996. *Pharmacokinetics of Methoprene in Rats.* Project No. 489855, Sandoz Agro Inc., Des Plaines, Illinois, USA. Submitted to WHO by Novartis Animal Health Australasia Ltd, Pendle Hill, NSW, Australia. As cited in WHO, 2001.

Hawkins, DR, KT Weston, LF Chasseaud, and ER Franklin. 1977. *Fate of Methoprene (Isopropyl (2E,4E)-11 Methoxy-3,7,11-Trimethyl-2,4 Dodecadienoate) in Rats.* J.Agric. Food Chem., 25, 398-403. As cited in WHO, 2001.

Hiles, RA and WT Collins. 1984. *Final Report Eye Irritation of Altosid® SR-5 in Rabbits.* Study No., 3122.1. Springborn Institute for Bioresearch Inc., Spencerville, Ohio, USA. Submitted to WHO by Novartis Animal Health Australasia Ltd, Pendle Hill, NSW, Australia. As cited in WHO, 2001.

Hill, R. 1972a. *Effect of a Single Oral Dose of 10 g/kg of ZR 515 on Dogs.* Report No. 70-D-72-ZR 515-PO-TX. Syntex Research, California, USA. Submitted to WHO by Novartis Animal Health Australasia Ltd, Pendle Hill, NSW, Australia. As cited in WHO, 2001.

Hill, R. 1972b. *Acute Oral Toxicity of ZR for Dogs.* Report No. 99-D-72-ZR 515-PO-TX. Syntex Research, California, USA. Submitted to WHO by Novartis Animal Health Australasia Ltd, Pendle Hill, NSW, Australia. As cited in WHO, 2001.

Hill, R. 1973. *Primary Eye Irritation Study with Altosid® Using Rabbits.* Report No. 251-B-72-ZR 515-Ey-LL. Syntex Research, California, USA. Submitted to WHO by Novartis Animal Health Australasia Ltd, Pendle Hill, NSW, Australia. As cited in WHO, 2001.

Jorgenson, TA and DP Sasmore. 1972a. *Toxicity Studies of ZR-515 (Altosid™ Technical)-(1) Acute IP in Rats, (2) Repeated IP in Rats, (3) Two-Week, Range-Finding Dietary Studies in Rats and Dogs.* Report No. 1 SRI Project LSC-1833.1. Submitted to WHO by Novartis Animal Health Australasia Ltd, Pendle Hill, NSW, Australia. As cited in WHO, 2001.

Jorgenson, TA and DP Sasmore. 1972b. *Toxicity Studies of Altosid Technical (1) Ninety-Day Subacute in Rats, (2) Ninety Day Subacute in Dogs.* SRI Project No. LSC-1833.2, 11/72. Submitted to WHO by Novartis Animal Health Australasia Ltd, Pendle Hill, NSW, Australia. As cited in WHO, 2001 and USEPA, 2002.

Killeen, JC and WR Rapp. 1974. *A Three Generation Reproduction Study of Altosid™ in Rats.* Project No. 73R-892. BioDynamics Inc. Submitted to WHO by Novartis Animal Health Australasia Ltd, Pendle Hill, NSW, Australia. As cited in WHO, 2001 and USEPA, 2002.

Nakasawa, M. 1975. *Rabbit Subacute Dermal Toxicity Test of Altosid.* Project No. NRI-PL-74-2465. Nomura Research Laboratory, Japan. Submitted to WHO by Novartis Animal Health Australasia Ltd., Pendle Hill, NSW, Australia. As cited in WHO, 2001 and USEPA, 1991.

Nakasawa, M, A Nomura, T Furuhashi, J Mihori, and E Ikeya. 1975a. *Determination of Teratogenic Potential of Altosid Administered Orally to Mice.* Nomura Research Institute, Japan. Submitted to WHO by Novartis Animal Health Australasia Ltd, Pendle Hill, NSW, Australia. As cited in WHO, 2001 and USEPA, 2002.

Nakasawa, M, H Matsumiya, and I Ishikawa. 1975b. *Test of Altosid Toxicity. III. Determination of Teratogenic Potential of Altosid Administered Orally to Rabbits.* Report No. NRI-PL-74-2465. Nomura Research Institute, Japan. Submitted to WHO by Novartis Animal Health Australasia Ltd, Pendle Hill, NSW, Australia. As cited in WHO, 2001 and USEPA, 2002.

Nakayoshi, H. 1975. *Test of Altosid Toxicity. V. Skin Sensitization Test of Altosid in Guinea Pigs.* Report No. NRI-PL-74-2466. Nomura Research Institute, Japan Submitted to WHO by Novartis Animal Health Australasia Ltd, Pendle Hill, NSW, Australia. As cited in WHO, 2001.

Olson, WA. 1972. *Three-week Subacute Inhalation Exposure-Rats to Altosid (Technical Grade).* Project No. 777-103. Hazelton Laboratories, Virginia, USA. Submitted to WHO by Novartis Animal Health Australasia Ltd, Pendle Hill, NSW, Australia. As cited in WHO, 2001 and USEPA, 2002.

Rooks, WH, II. undated. *Report on the Mammalian Endocrine Testing Performed on ZR-515.* Syntax Research Center, Palo Alto, California, USA. Submitted to WHO by Novartis Animal Health Australasia Ltd, Pendle Hill, NSW, Australia. As cited in WHO, 2001.

Schindler, JE and RC Baldwin. 1991a. *Zoecon Sample No. R437N SAN 810I 20CS: Eye Irritation Study in Rabbits.* Study No. LSC 2673-M035-91. SRI International, Menlo Park, California, USA. Submitted to WHO by Novartis Animal Health Australasia Ltd, Pendle Hill, NSW, Australia. As cited in WHO, 2001.

Schindler, JE and RC Baldwin. 1991b. *Zoecon Sample No. R43N SAN 810I 20CS: Skin Sensitisation Study in Guinea Pigs.* Study No. LSC 2673-M036-91. SRI International, Menlo

Park, California, USA. Submitted to WHO by Novartis Animal Health Australasia Ltd, Pendle Hill, NSW, Australia. As cited in WHO, 2001.

United States Environmental Protection Agency (USEPA). 1991. *Reregistration Eligibility Document Isopropyl (2E, 4E)-11 Methoxy-3, 7, 11-Trimethyl-2, 4-Dodecadienoate (Referred to as Methoprene).* List A Case 0030. Office of Pesticide Programs. March, 1991.

United States Environmental Protection Agency (USEPA). 2001. *June 2001 Update of the March 1991 Methoprene R.E.D. Fact Sheet.* Pesticide Fact Sheet. Office of Pesticide Programs.

United States Environmental Protection Agency (USEPA). 2002. *IIFG Decision Document on Tolerance Reassessment for Methoprene.* Memorandum from Kathryn Boyle and Kerry Leifer to Robert Forrest. August 1, 2002. Office of Prevention, Pesticides and Toxic Substances.

Wazeter, FX and EI Goldenthal. 1975a. *Eighteen Month Oral Carcinogenic Study in Mice.* Report No. 322-003. International Research and Development Corporation, dated 14 March. Submitted to WHO, by Novartis Animal Health Australasia Ltd, Pendle Hill, NSW, Australia. As cited in WHO, 2001.

Wazeter, FX and EI Goldenthal. 1975b. *Chronic Oral Toxicity Studies With Altosid Technical.* Report No. 322-001. International Research and Development Corporation, dated 14 March. Submitted to WHO by Novartis Animal Health Australasia Ltd, Pendle Hill, NSW, Australia. As cited in WHO, 2001.

Westchester County. 2002. *Comprehensive Mosquito-Borne Disease Surveillance and Control Plan Generic Environmental Impact Statement.* Prepared by AKRF, Inc. Gradient Corporation and PBS&J.

World Health Organization (WHO). 1984. *Data Sheets on Pesticides No. 47 Methoprene.* Food and Agriculture Organization, IPCS INCHEM.

World Health Organization (WHO). 2001. *Pesticide Residues in Food 2001 Toxicological Evaluations Methoprene and S-Methoprene.* IPCS INCHEM.

4.2 Pyrethroid Adulticides

Pyrethroids are synthetic pesticides that are similar to pyrethrins, which are found naturally in pyrethrum flowers, such as chrysanthemums. The synthetic counterparts were prepared to build upon the insecticidal properties of the natural material and make them less biodegradable so that they are longer acting (ATSDR, 2003). There are many pyrethroid products currently on the market with broad insecticidal uses from ants and cockroaches to mosquitoes. They are among the most common pesticides currently in use worldwide (Yamada et al., 2003).

Pyrethroids are categorized as Type I and Type II. Type I are considered less toxic and include permethrin, resmethrin and sumithrin (Westchester County, 2002). Type II pyrethroids include derivatives that have a cyano chemical group (ATSDR, 2003).

Commercial pyrethroid products, with the exception of deltamethrin, often contain different isomers. Isomers are chemicals that have the same composition, but a different atomic geometric arrangement. This geometric arrangement can have an effect on toxicity and, therefore, different commercial products of the same pyrethroid may have differing toxicity, depending upon their isomeric composition (ATSDR, 2003).

4.2.1 Permethrin

Background

Permethrin is a Type I pyrethroid. Pesticide products containing permethrin require either a warning or caution label. Permethrin products are used for a variety of agricultural as well as residential applications. It is also used in the treatment of ectoparasites, such as lice and scabies (ATSDR, 2003).

Absorption/Distribution/Excretion

Permethrin is readily absorbed from oral ingestion and inhalation, but only absorbed through the skin to a limited extent (Westchester County, 2002; ATSDR, 2003). Based upon measurement of urinary metabolites, it has been estimated that 30 to 60 percent of an oral dose is absorbed (ATSDR, 2003). A much smaller amount is absorbed following dermal exposure. Animal, as well as human, evidence indicates that approximately 0.5 to 1 percent of the dermal dose is absorbed (ATSDR, 2003). However, excretion of urinary metabolites continued for 7 to 19 days

indicating that permethrin may be stored in the skin and slowly released into circulation (van der Rhee et al., 1989 [as cited in ATSDR, 2003]).

Once absorbed permethrin is distributed in the body, with higher concentrations found in nervous system tissue than blood (ATSDR, 2003). Peak concentrations appear in blood, nerve tissues and the liver 4 hours after an oral dosage (Anadon et al., 1991 [as cited in ATSDR, 2003]). Pyrethroids do not appear to cross the placenta (ATSDR, 2003).

Permethrin is metabolized in the liver as well as other tissues by microsomal mixed function oxidase enzymes and microsomal carboxyesterases (ATSDR, 2003). Metabolic breakdown products include carboxylic acids and alcohols (Westchester County, 2002). These metabolites are excreted in the urine, feces and exhaled air. Urinary metabolites begin to appear 30 minutes following inhalation exposure in humans (Leng et al., 1997 [as cited in ATSDR, 2003]). Elimination half-lives in humans are approximately 6.4 to 16.5 hours, with almost complete elimination within 5 days (Westchester County, 2002 and ATSDR, 2003). Some studies have shown that exposure to permethrin may enhance the activity of metabolic enzymes. Such enhanced metabolism reduces the effectiveness of the pesticide and is suspected as a factor in the development of resistance by target insects (Heder et al., 2001). It has been hypothesized that variation in human susceptibility to localized toxic effects, such as numbness on the skin, may be due to differences in the activity of carboxyesterases between individuals (Leng et al., 1999).

Toxicity

Neurological Effects

The pesticidal properties of permethrin result from its ability to interfere with the nervous system. Specifically, permethrin interferes with the change in electrical charge as a nerve impulse is transmitted, by slowing the closing of sodium channels on the membrane of nerve cells. This impairs the ability to terminate the transmission of the signal and may lead to repetitive nerve impulses. Certain isomers of permethrin appear to be more toxic. Trans isomers of pyrethroids are typically less toxic than their cis counterparts. Neurological effects are typically short term because pyrethroids are generally rapidly eliminated from the body (ATSDR, 2003).

Human Evidence

In an evaluation of 48 human cases of poisoning in Taiwan, neurological symptoms, including confusion, coma and seizures, were noted in 33 percent of the patients (Yang et al., 2002). All patients survived except one who developed complications. Neurological symptoms were temporary in all surviving cases.

Studies of patients using 1 percent permethrin products to treat lice or scabies have not shown nervous system effects (Bowerman et al., 1987 [as cited in Westchester County, 2002]; Haustein and Hilawa, 1989 [as cited in Westchester County, 2002]). One report suggested an association between "vegetative", unresponsive, behavior and permethrin exposure (Mitsche et al., 2000). This case involved an individual that had been an animal keeper for 13 years, who had used pyrethroid insecticides to treat animals.

When applied to the skin, irritant effects have been shown to progress to partial numbness. Nervous system effects did not occur below 0.01 mg/cm2 (Flannigan and Tucker, 1985 [as cited in Westchester County, 2002]).

Animal Evidence

Symptoms that have been reported in laboratory animal studies include aggressiveness, agitation, whole-body tremors, decreased motor activity and increased reaction to auditory stimuli (McDaniel and Moser, 1993 [as cited in Westchester County, 2002]). LD_{50}'s range from 584 mg/kg to 3801 mg/kg, depending upon the formulation and the vehicle in which permethrin is administered (ATSDR, 2003). Domestic cats appear to be more sensitive to pyrethroids than dogs, presumably because of differences in metabolism. There are reports of cat deaths when permethrin products designed for dogs were mistakenly used (Meyer, 1999 [as cited in ATSDR, 2003]).

Rats receiving a single oral dose of 200 mg/kg were observed to have decreased motor activity (Crofton and Reiter, 1988 [as cited in ATSDR, 2003]). In another study, an increase in group total activity and individual nonambulatory activity was noted in mice following a single oral dose of 50 mg/kg (Mitchell et al., 1988 [as cited in ATSDR, 2003]). Rats exposed to oral doses of 432 mg/kg-day for 14 days experienced muscle tremors, which were not observed at 216 mg/kg-day (Metker et al., 1977 [as cited in WHO, 1990]).

Similar effects have been noted in longer term oral exposure studies. Hyperexcitability was observed in rats exposed to 1000 mg/kg-day for 4 weeks. Such effects were not seen at 500 mg/kg-day (Clapp et al., 1977[as cited in WHO, 1990]). An increased sensitivity to sound was also observed in rats exposed to doses above 60 mg/kg-day (Sheets, 2000 [as cited in Westchester County, 2002]). Ninety day exposure to 500 mg/kg-day caused transient tremors, which subsided after the first week of exposure (Killeen and Rapp, 1976b [as cited in WHO, 1990]). Hyperexcitability and tremors were observed in a six month dietary study when animals were exposed to 3000 mg/kg-day. Such effects were not noted at doses of 1500 mg/kg-day (Kadota et al., 1975 [as cited in WHO, 1990]). Long-term studies have been conducted in which permethrin was administered in the diet of rats and mice. Doses for the rats ranged from 19 to 104 mg/kg-day and for mice ranged from 29 to 348 mg/kg-day. The only toxicity noted was in rats at the highest dose tested, in which body tremors, hyperexcitability and decreased body weight gain were noted during the first two weeks, but not afterwards (Ishmael and Litchfield, 1988 [as cited in ATSDR, 2003]).

Changes in brain chemistry have been noted in rats following dermal exposure to 0.13 mg/kg-day (Abou-Donia et al., 2001a) and after high doses via injection in mice (Bloomquist, 2001 [as cited in Westchester County, 2002]). In an *in vitro* study using isolated synaptosomal membranes from the brain of rats, it was observed that exposure to low concentrations (10 to 50 micromolar concentrations) of permethrin resulted in an increase in ATPase activity. This activity was dramatically reduced at high concentrations (100 micromolar). ATPase is a family of enzymes that enable cells to use energy. It was theorized by the authors of this study that the initial increase in activity is a response by the cell to the high sodium ion concentrations caused by the delay in the closing of the sodium channel (Kakko et al., 2003).

Recent studies have been conducted to investigate a potential role of permethrin exposure in Parkinson's disease and Gulf War Syndrome. Parkinson's disease is characterized by a loss of specific neurons that release the neurotransmitter, dopamine, as well as reduced dopamine levels (Gillette and Bloomquist, 2003). Gulf War syndrome is characterized by a variety of neurological symptoms that have been reported by veterans of the Persian Gulf War. Symptoms include muscle and joint pain, difficulty in walking, headache, chronic fatigue and decreased concentration (Abou-Donia et al., 2001b). Pesticides have been implicated because exposure is known to have occurred via DEET, pyridostigmine bromide and permethrin-impregnated

clothing (Gillette and Bloomquist, 2003). Many laboratory animal studies have found that low level exposure to permethrin can affect dopamine levels, as well as the re-uptake of dopamine by the dopamine transporter, once it is released. Mice given permethrin by injection at doses up to 1.5 mg/kg had an increase in the uptake of dopamine by the dopamine transporter (Karen et al., 2001). However, at 25 mg/kg, the uptake was significantly reduced. The authors suggest that this observation is due to toxicity to the neurons at the higher dose, which impairs overall function. Similar effects were observed in another study that used mice. In this study, levels of the neurotransmitter dopamine were affected at doses as low as 0.2 mg/kg (Gillette and Bloomquist, 2003). In addition, this study found that the effects were delayed (14-28 days) and persistent after treatment. Dermal exposure to permethrin (0.13 mg/kg bw) alone and in combination with DEET (40 mg/kg bw) and pyridostigmine (oral dose of 1.3 mg/kg bw) was also found to have neurobehavioral effects and effects on AChE activity in specific areas of the nervous system after 45 days of treatment (Abou-Donia et al., 2001b). Similar results were observed when permethrin (0.13 mg/kg bw), DEET (40 mg/kg bw) and malathion (44.4 mg/kg bw) were applied dermally, alone and in combination every day for 30 days to laboratory rats (Abdel-Rahman et al., 2004b). The permeability of the blood brain barrier was found to be decreased following DEET exposure and to a greater degree with combined exposure to DEET and permethrin, though permethrin alone did not affect permeability at the doses tested (0.013 to 1.3 mg/kg bw) (Abou-Donia et al., 2001a). Adding stress (five minutes of restraint) augmented impacts to the blood brain barrier and neuronal cell death (Abdel-Rahman et al., 2002; Abdel-Rahman et al., 2004a). These observations are relevant in that the blood brain barrier regulates the entry of chemicals into the central nervous system and helps to maintain normal brain function. Therefore, any alteration of this barrier may impair proper central nervous system function (Abou-Donia et al., 2001a).

Skin and Eye Irritation

Human Evidence

There have been some reports of skin irritation in a small percentage of people using permethrin products (Westchester County, 2002). In a large scale study which follow 18,950 people who had used a commercially available cream rinse (1 percent permethrin) for treatment of lice found that approximately 2 out of 1000 people reported skin and/or eye irritation. No other serious

effects were reported (Andrews et al., 1992 [as cited in Westchester County, 2002]). Temporary redness and mild itching may result in approximately 1 percent of those using permethrin shampoos or cream rinse (Bowerman et al., 1987 [as cited in Westchester County, 2002]). Mild itching and burning and stinging have been reported after application of a 5 percent permethrin product used to control scabies (Schultz et al., 1990 [as cited in Westchester County, 2002]). Symptoms were more common in cases with open sores. When permethrin products are left on the skin for longer periods of time more people may experience mild irritation. For example, 2 out of 229 soldiers using impregnated clothing for protection from malaria carrying mosquitoes in Columbia experienced mild skin irritation (Soto et al., 1995 [as cited in Westchester County, 2002]) and 2 out of 17 volunteers developed skin rashes when exposed dermally to 1 percent permethrin for nine days (WHO, 1990). Application of 0.13 mg/cm^2 to the skin resulted in mild skin sensations that did not persist beyond 24 hours (Flannigan and Tucker, 1985; Flannigan et al., 1985a; Flannigan et al., 1985b [as cited in Westchester County, 2002]).

Animal Evidence

Some studies in laboratory rabbits have shown irritant effects while others have not. Redness of the skin was reported after application of 0.13 mg /cm^2 (Flannigan et al., 1985a [as cited in Westchester County, 2002]). However, application of 25 percent and 100 percent permethrin in other studies did not show any irritant effects to rabbits (WHO, 1990). A study in guinea pigs using a 1 percent application also did not show irritant effects (WHO, 1990).

Gastrointestinal Effects

Human Evidence

A recent review of 48 cases of accidental/intentional poisonings from the ingestion of permethrin products in Taiwan found that the most common symptom was GI effects (73 percent of cases), including sore throat, mouth ulcerations, difficulty in swallowing, abdominal pain and vomiting (Yang et al., 2002). Because the pesticide products also contained xylene and a surfactant, the exact role each component had in the causation of these symptoms cannot be determined.

Animal Evidence

No studies were located that reported GI effects in animal studies.

Respiratory Effects

Human Evidence

A study involving 87 plant nursery workers exposed to permethrin found that 20 percent experienced throat irritation or increased mucus production (Kolmodin-Hedman et al., 1982 [as cited in Westchester County, 2002]).

Animal Evidence

In a laboratory study using beagle dogs exposed to 500 mg/m^3 for 6 hours a day, 5 days per week for 13 weeks, did not find effects on the lungs (WHO, 1990).

Immunologic Response

Human Evidence

Allergic reactions to the skin have been reported. Minor skin reddening was reported in 2 out of 17 volunteers who were exposed for 9 days to a 1 percent permethrin solution (WHO, 1990). Redness was also reported in 3 of 10 volunteers treated with 1 percent permethrin for head lice (WHO, 1990). There was also a report of an animal keeper who had used permethrin and S-bioallethrin over a period of 13 years. She had a positive dose dependent allergic response upon skin testing. Pyrethroid metabolites were measured in her hair and urine (Mitsche et al., 2000).

A case report was found in the literature regarding a family of six that experienced allergic reactions following aerial agricultural application of a permethrin product (Fuortes, 1999). In this case the pesticide POUNCE was applied at a rate of 8 lbs/acre to a corn field adjacent to their residence. This product is a granular formulation consisting of a crystalline core coated with permethrin and kaolin. Some of the product landed on the family's home and property. Immediately after the application, the parents reported coughing and mild bronchial irritation. This persisted and the family had to leave the home. Upon returning to clean, respiratory irritation continued. Five days following the application, three of the children developed hives (urticaria). Samples of soil and a swipe sample of an automobile parked outside found 0.024 ppm and approximately 500 ng/square inch of permethrin, respectively. These symptoms were short lived and the family was able to return to their home after the home was professionally cleaned.

Animal Evidence

Allergic reactions did not result when 0.1 percent permethrin solution was injected into guinea pigs (Metker et al., 1977, Metker, 1978 [as cited in WHO, 1990]). However, effects on antibodies in mice following permethrin exposure did occur (Stelzer and Gordon, 1984 [as cited in Westchester County, 2002]). The immune response of splenocytes from mice treated with 0.4 mg/kg-day for 10 days was significantly reduced (Blaylock et al., 1995). Immune function was not observed to be affected in another study in which permethrin was orally administered to male rats at doses ranging from 12.6 to 125.7 mg/kg-day for 28 days (Institoris et al., 1999).

Dermal doses between 22 to 220 mg/kg-day applied daily and intermittently for durations of 7 to 30 days suggested that dermal application of permethrin may cause systemic immune effects. Effects on antibody production and phagocytic ability of macrophages were observed. Intermittent exposure was less likely to produce immune system effects, presumably due to the rapid metabolism of permethrin (Punareewattana et al., 2001). A single dermal dose applied to the skin of mice was also found to produce immunologic effects. Doses equivalent to 220 to 1100 mg/kg were found to result in reduced thymus and spleen weights. The thymus appeared to be more sensitive than the spleen and the reduced organ weight appeared to result from cell death and decreased proliferation (Prater et al., 2002). Another study explored the possibility that sunlight exposure may enhance the immunotoxic effects of permethrin. This study exposed mice to permethrin (25 microliters of a 91.6 percent solution) and a chemical that is present in the skin after exposure to sunlight (urocanic acid, 6.6mg/kg bw). This study found that co-exposure to permethrin and urocanic acid did exacerbate permethrin toxicity to the thymic gland (Prater et al., 2003).

Endocrine Disruption

Human Evidence

There is some evidence from *in vitro* studies that permethrin may mimic some growth hormones and estrogens (Garey and Wolff, 1998 [as cited in Westchester County, 2002]). Using cultures of human breast cancer cells, researchers tested the ability of various pyrethroids to mimic estrogen and increase cell proliferation. Permethrin was found to increase cell proliferation, indicative of an estrogenic response (Go et al., 1999). Permethrin was also found to induce human estrogen receptors in an *in vitro* screening study (Kojima et al., 2004).

Permethrin has been found to interrupt the ability of growth hormones to interact with specific cells (Tyler et al., 2000 [as cited in Westchester County, 2002]).

Animal Evidence

Cultures using animal cells have not shown permethrin to cause endocrine disruption (Saito et al., 2000, Sumida et al., 2001 [as cited in Westchester County, 2002]).

Developmental and Reproductive Effects

Human Evidence

The use of permethrin products for the treatment of lice and scabies and in products that protect against malaria transmitting mosquitoes, has not been noted to produce toxicity to nursing mothers, premature infants and children (Dolan et al., 1993 [as cited in Westchester County, 2002], Haustein and Hilawa, 1989 [as cited in Westchester County, 2002]).

Animal Evidence

A three-generation reproductive study was conducted in rats. Reduced fertility was noted only at very high doses, greater than 2500 mg/kg-day (Wauchope et al., 1992 [as cited in Westchester County, 2002]). In another study, lactating cows were fed permethrin at doses between 0 and 50 mg/kg for a total of 28 days. No effects on growth or milk production were noted. Permethrin was excreted via breast milk but at levels less than 0.5 percent of the dose (WHO, 1990).

Toxicity to mouse embryos was investigated in a study which exposed the embryos to several pesticides at concentrations which attempted to reflect potential environmental concentrations. Permethrin was the only pesticide tested that did not significantly affect the development of the embryos nor lead to increased cell death (Greenlee et al., 2004).

Exposure to permethrin, DEET and pyridostigmine administered together was found to cause cellular death in the testes of treated rats (Abou-Donia et al., 2003). Permethrin and DEET were administered dermally, while pyridostigmine was given orally daily for 28 days. The doses administered were relevant to the exposures that may have occurred to soldiers serving in the Persian Gulf War. This effect on the testes was significantly enhanced with the introduction of stress.

Cancer

Human Evidence

There is no evidence that permethrin causes cancer based on human data (Westchester County, 2002). The USEPA has classified permethrin as a possible human carcinogen based upon limited data from animal studies (USEPA, 2002a). The IARC evaluated permethrin in 1991 and determined that permethrin was not classifiable as to its carcinogenicity in humans and that there was inadequate evidence for carcinogenicity in laboratory animals (IARC, 1991).

Since these evaluations on the weight of evidence for carcinogenicity of permethrin, the association between pesticide exposure and prostate cancer was evaluated in the Agricultural Health Study Cohort. This cohort consists of a group of pesticide applicators from Iowa and North Carolina. Ever having used permethrin for animal use was found to have a significantly elevated risk of prostate cancer (odds ratio of 1.38). This risk was even greater in individuals who had a family history of prostate cancer (odds ratio 2.31) (Alavanja et al., 2003).

Using human prostate cancer cell lines, researchers have found that trans-permethrin has the ability to activate an oncogene (erbB-2) that is typically over expressed in some prostate and breast cancers (Tessier and Matsumura, 2001). Similarly, permethrin was found to induce the expression of an oncogene (WNT10B Proto-oncogene) in human breast cancer cells (Kasat et al., 2002). Permethrin was also found to cause DNA damage in cultures using cells from human nasal biopsies (Tisch et al., 2002).

Animal Evidence

There are conflicting results in studies that investigate the ability of permethrin to cause genotoxicity. Mice receiving intraperitoneal administration of permethrin at doses up to 275 mg/kg did not increase the percent of micronuclei in bone marrow (Chruscielska and Kalhorn, 1999 [as cited in ATSDR, 2003]). However, the number of chromosome aberrations was increased when permethrin was administered orally at doses ranging from 12.6 to 125.7 mg/kg-day for 28 days (Institoris et al., 1999). Permethrin was found to be mutagenic in drosophila larvae (Kale et al., 1995 [as cited in ATSDR, 2003]).

Oxidative damage to DNA after dermal exposure to permethrin was investigated. DEET and permethrin alone, and in combination, were applied to the skin of laboratory rats at doses of 400

and 1.3 mg/kg, respectively. Urinary excretion of a biomarker of DNA oxidative damage was measured. DEET alone caused a significant increase in excretion of this biomarker, indicating damage to DNA had occurred (Abu-Qare and Abou-Donia, 2000). Permethrin alone did not cause a significant increase. A similar study was performed adding co-administration of pyridostigmine (oral dose). Permethrin again was not found to enhance urinary excretion of a biomarker of oxidative stress, although DEET with and without administration of pyridostigmine did (Abu-Qare et al., 2001).

No carcinogenic effects were seen at oral doses up to 2500 mg/kg-day in either rats or mice (USEPA, 2000 [as cited in Westchester County, 2002]; WHO, 1990). Dermal exposure in rats to 1.3 mg/kg of permethrin did not produce biological markers indicative of carcinogenic effects (Abu-Qare and Abou-Donia., 2000; Abu-Qare et al., 2001). A two year bioassay in rats receiving oral doses up to 104 mg/kg-day of permethrin (40/60 cis/trans) did not provide evidence of a carcinogenic response (Ishmael and Litchfield, 1988 [as cited in ATSDR, 2003]). Male mice receiving an oral dose of 295 mg/kg-day of the same isomer ratio had a significantly increased incidence of benign lung tumors (Ishmael and Litchfield, 1988; [as cited in ATSDR, 2003]). In another cancer bioassay, doses of permethrin (25/75 cis/trans) up to 250 mg/kg per day did not produce cancer in rats, though female mice at this dose level did have an increase in benign lung tumors (ATSDR, 2003).

A review by the WHO concluded that the literature does not support the consideration of pyrethroids as carcinogens (WHO, 2001 [as cited in ATSDR, 2003]).

Other Toxicity

Cytotoxic Effects

In vitro studies have shown various pyrethroids to have cytotoxic effects, resulting from inhibition of cell division (mitosis). In one study using cultures of Chinese hamster lung cells, permethrin (97 percent purity) arrested growth of cells in these cultures at a concentration of 25 ug/ml (Hadnagy et al., 1999). The apparent mechanism of mitotic inhibition was interference with the mitotic spindle apparatus. The results of this study also indicated that the observed cytotoxic effect may have been due to impurities in the formulations and not the pyrethroid tested specifically (Hadnagy et al., 1999).

Table 6
Toxicity Study Summary Table- Permethrin

Compound	Purity	Test Species (Strain)	Test Species Age/Lifecyle	Route	Dose Duration	Study Duration	Endpoint	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	LOAEL Statistical Significance	Comments	Reference
Permethrin	tech grade 40:60, 94.5-96.7%	Long Evans Rats		diet	0,1,5,25 mg/kg/d 24 mnth	24mnth	chronic toxicity/ carcinogenicity	5	25		clinical signs, dec body wgt, inc ovary wgt	WHO, 1999
Permethrin	98.20%	Wistar male rats	4 weeks	gavage	12.6,50.3,125.7 mg/kg-d	28 days	immunotoxicity chromosome aberrations	125.7	 12.6	 P<0.05	no immuno tox noted all doses had some effect.	Institoris, et al., 1999
Permethrin	91.60%	C57BL/6N female mice	21.5 g	dermal in corn oil	22 to 220 mg/kg-day Exp1:daily for 10d Exp2:ev. Other day for 7d Exp3:ev other day for 14d Exp4: daily for 30d	30 days plus 30 day obs	antibody production macrophage function	22	66 22	P<0.05 P<0.05	only seen in Exp1 effect at this level transient	Punareewattana et al, 2001
Permethrin	91.60%	C57BL/6N Fem mice	5 weeks	dermal in corn oil	220 to 1100 mg/kg	48 h	spleen and thymus weights T-cell proliferation	220	440 25uM	P<0.01 P<0.01		Prater et al., 2002
Permethrin	technical	C57BL/6 male mice	38 g	I.p. corn oil	0.1 to 200 mg/kg 3 X over 2 wks	28 days	dopamine levels and uptake	0.1	0.2	NR		Gillette and Bloomquist, 2003
Permethrin	technical	C57BL/6 mice	7-9 months	I.p. corn oil	0.2 to 200 mg/kg 3 X over 2 wks	15 days	dopamine levels and uptake behavioral effects	0.8	1.5	P<0.05		Karen et al., 2001
Permethrin	99%	Sprague-Dawley rats	200-240 g	dermal ethanol	1.3 mg/kg 1X 1.3 with 400 mg/kg DEET 1x	72 hours	urinary 8-hydroxy2deoxy guanosine	1.3 alone	1.3 with DEET		DEET still incr excretion even with permethrin. Greatest levels 24hr after dosing	Abu-Qare and Abou-Donia, 2000
Permethrin	99%	Sprague-Dawley rats	200-240 g	dermal ethanol	1.3 mg/kg 1X 1.3 with 400 mg/kg DEET 1.3 + 400 + 13 pyridostigmine	72 hours	urinary excretion of 3-nitro tyrosine	1.3 alone	1.3 with DEET all three	P<0.05	permethrin alone had no effect in combination with other two did not impact excretion	Abu-Qare et al., 2001
Permethrin	93.6%	Sprague-Dawley male rats	225-250 g	dermal ethanol	0.13 mg/kg/day 0.13 w/ 1.3 pyridostigmine & 40 DEET	45 days	neurobehavioral effects acetylcholine esterase activity		0.13	P<0.001	effects seen alone and in combin nation with the other chemicals	Abou-Donia et al., 2001b
Permethrin	93.6%	Sprague-Dawley male rats	200-250 g	dermal ethanol	0.013. 0.13, 1.3 mg/kg/day alone & w/ 4,40,400 mg/kg/d DEET	60 days	neurobehavioral effects Blood brain barrier blood testes barrier	0.013 1.3 1.3	0.13 0.13 w/40 DEET	P<0.001 P<0.05	behavioral not sensorimotor only with DEET blood testes barrier not affected	Abou-Donia et al., 2001a
Permethrin	93.6%	Sprague-Dawley male rats	225-250 g	dermal ethanol	0.13 mg/kg/day alone & w/ 40 DEET, 44.4 malathion & all 3	30 days	neurobehavioral effects acetylcholine esterase activity	0.013	0.13 in combination 0.13			Abdel-Rahman et al., 2004b
Permethrin	93.6%	Sprague-Dawley male rats	225-250 g	dermal ethanol	0.13 mg/kg/day w/ 40 mg/kg-d DEET & 1.3 pyridostigmine w/ & w/o stress	28 days	testicular apotosis		0.13 in combination with other chemicals	P<0.05 P<0.05	significantly enhanced with stress	Abou-Donia et al., 2003

Table 7

Noncancer Criteria (Oral/Dermal)- Permethrin

Chemical of Potential Concern	Exposure Duration	Criteria	Value	Units	Species	Endpoint	NOAEL	LOAEL	Units	Combined Uncertainty/Modifying Factors	Study Source	Reference for Criteria
permethrin	acute	RfD-oral	0.25	mg/kg/day	rat	dec. body wgt, neuro	2.50E+01	NA	mg/kg/day	100	McDaniel & Moser, 1993	Westchester County, 2002
						logic, behavioral						
permethrin	acute	RfD-dermal	1.5	mg/kg/day	rat	increased liver weight	1.50E+02	NA	mg/kg/day	100	USEPA, 2000	Westchester County, 2002
permethrin	acute	MRL	0.30	mg/kg/day	rat	neurolgic impariment	2.5E+01		mg/kg/day	100	McDaniel & Moser, 1993	ATSDR, 2003
permethrin	intermediate	MRL	0.20	mg/kg/day	rat	neurolgic impariment	1.55E+01		mg/kg/day	100	USEPA, 1994b	ATSDR, 2003
permethrin	subchronic	RfD-oral	0.155	mg/kg/day	rat	neurologic,behavioral	1.55E+01	NA	mg/kg/day	100	FMC Corp, 1993	Westchester County, 2002
permethrin	subchronic	RfD-dermal	1.5	mg/kg/day	rat	increased liver weight	1.50E+02	NA	mg/kg/day	100	USEPA, 2000	Westchester County, 2002
permethrin	chronic	RfD	0.05	mg/kg/day	rat	increased liver weight	5.00E+00	2.50E+01	mg/kg/day	100	FMC Corp, 1977	USEPA, 1992
permethrin	chronic	ADI	0-0.5	mg/kg/day	dog	dec body wgt	5.00E+00		mg/kg/day	100	Kalinowski et al, 1982	WHO, 1999
permethrin	chronic	RfD-oral	0.05	mg/kg/day	rat	increased liver weight	5.00E+00	NA	mg/kg/day	100	USEPA, 1992	Westchester County, 2002
permethrin	chronic	RfD-dermal	0.05	mg/kg/day	rat	increased liver weight	5.00E+00	NA	mg/kg/day	100	USEPA, 1992	Westchester County, 2002

NA: Not Applicable or Not Available.

NOAEL: no observed adverse effect level

LOAEL: lowest observed adverse effect level

RfD: USEPA reference dose

ADI: World Health Organization Acceptable Daily Intake

MRL: Agency for Toxic Substances and Disease Registry Minimum Risk Level

Table 8

Cancer Criteria (Oral/Dermal)- Permethrin

Chemical of Potential Concern	Oral Cancer Slope Factor	Oral to Dermal Adjustment Factor	Adjusted Dermal Cancer Slope Factor (1)	Units	Weight of Evidence/ Cancer Guideline Description	Source	Reference
Permethrin	0.0184	1	0.0184	$(mg/kg\text{-}day)^{-1}$	possible human carcinogen	USEPA, 2000	Westchester
					limited evidence in animals		County, 2002

Table 9
Noncancer Criteria (Inhalation)- Permethrin

Chemical of Potential Concern	Exposure Duration	Criteria	Value	Units	Endpoint	Species	NOAEL	LOAEL	Units	Combined Uncertainty/Modifying Factors	Study Source	Reference for Criteria
Permethrin	acute	RfC	0.0025	mg/L	rat	tremors, convulsions	2.50E-01	NA	mg/L	100	USEPA, 2000	Westchester
						incr. liver enzyme						County, 2002
Permethrin	subchronic	RfC	0.0025	mg/L	rat	tremors, convulsions	2.50E-01	NA	mg/L	100	USEPA, 2000	Westchester
						incr. liver enzyme						County, 2002
Permethrin	chronic	RfC	0.00025	mg/L	rat	tremors, convulsions	2.50E-01	NA	mg/L	1000	USEPA, 2000	Westchester
						incr. liver enzyme						County, 2002
Permethrin	chronic	RBC	1.80E+02	ug/m^3	NA	NA	NA	NA	NA	NA		USEPA Region III, 2004
Permethrin	chronic	PRG	1.80E+02	ug/m^3	NA	NA	NA	NA	NA	NA		USEPA Region IV, 2002b

NA: Not Applicable or Not Available.
RfC: USEPA Reference Concentration
PRG: USEPA Region 9 Preliminary Remediation Goal

Table 10
Cancer Criteria (Inhalation)- Permethrin

Chemical of Potential Concern	Inhalation Cancer Unit Risk	Units	Weight of Evidence/ Cancer Guideline Description	Source	Reference for Criteria
Permethrin	5.260E-06	$(ug/m^3)^{-1}$	possible human carcinogen limited evidence in animals	USEPA, 2000	Westchester County, 2002

Reference List

Permethrin

Abdel-Rahman, A, SM Abou-Donia, EM El-Masry, AK Shetty, and MB Abou-Donia. 2004a. *Stress and Combined Exposure to Low Doses of Pyridostigmine Bromide, DEET, and Permethrin Produce Neurochemical and Neuropathological Alterations in Cerebral Cortex, Hippocampus, and Cerebellum.* Journal of Toxicology and Environmental Health, Part A, 67:163-192, 2004.

Abdel-Rahman, A, AM Dechkovskaia, LB Goldstein, SH Bullman, W Khan, EM El-Masry, and MB Abou-Donia. 2004b. *Neurological Deficits Induced by Malathion, DEET, and Permethrin, Alone or in Combination in Adult Rats.* Journal of Toxicology and Environmental Health, Part A, 67:331-356, 2004.

Abdel-Rahman, A, AK Shetty, and MB Abou-Donia. 2002. *Disruption of the Blood-Brain Barrier and Neuronal Cell Death in Cingulate Cortex, Dentate Gyrus, Thalamus, and Hypothalamus in a Rat Model of Gulf-War Syndrome.* Neurobiology of Disease 10, 306-326, 2002.

Abou-Donia, MB, HB Suliman, AK Wasiuddin, and AA Abdel-Rahman. 2003. *Testicular Germ-Cell Apoptosis in Stressed Rats Following Combined Exposure to Pyridostigmine Bromide, N,N-Diethyl m-Toluamide (DEET), and Permethrin.* Journal of Toxicology and Environmental Health, Part A, 66:57-73, 2003.

Abou-Donia MB, LB Goldstein, AM Dechkovskaia, SL Bullman, KH Jones, EA Herrick, AA Abdel-Rahman, and WA Khan. 2001a. *Effects of Daily Dermal Application of DEET and Permethrin, Alone and in Combination, on Sensorimotor Performance, Blood-Brain Barrier, and Blood-Testis Barrier in Rats.* Journal of Toxicology and Environmental Health, Part A, 62:523-541, 2001.

Abou-Donia, MB, LB Goldstein, KH Jones, AA Abdel-Rahman, TV Damodaran, AM Dechkovskaia, SL Bullman, BE Amir, and WA Khan. 2001b. *Locomotor and Sensorimotor Performance Deficit in Rats Following Exposure to Pyridostigmine Bromide, DEET, and Permethrin, Alone and in Combination.* Toxicological Sciences 60, 305-314, 2001.

Abu-Qare, A and M Abou-Donia. 2000. *Increased 8-Hydroxy-2'-Deoxyguanosine, a Biomarker of Oxidative DNA Damage in Rat Urine Following a Single Dermal Dose of DEET (N, N-Diethyl-M-Toluamide), and Permethrin, Alone and in Combination*. Toxicological Letters20;117(3):151-60.

Abu-Qare, AW, HB Suliman, and MB Abou-Donia. 2001. *Induction of Urinary Excretion of 3-Nitrotyrosine, a Marker of Oxidative Stress, Following Administration of Pyridostigmine* B*romide, DEET (N,N-Diethyl-M-Toluamide) and Permethrin, Alone and in Combination in Rats.* Toxicological Letters. 121(2):127-34.

Agency for Toxic Substances and Disease Registry (ATSDR). 2003. *Toxicological Profile for Pyrethrins and Pyrethroids.* US Department of Health and Human Services, Agency for Toxic Substances and Disease Registry. September 2003.

Alavanja, MCR, C Samanic, M Dosemeci, J Lubin, R Tarone, CF Lynch, C Knott, K Thomas, JA Hoppin, J Barker, J Coble, DP Sandler, and A Blair. 2003. *Use of Agricultural Pesticides and Prostate Cancer Risk in the Agricultural Health Study Cohort.* American Journal of Epidemiology, Vol. 157, No. 9, 157:800-814, 2003.

Anadon, A, MR Martinez-Larranaga, MJ Diaz, et al. 1991. *Toxicokinetics of* P*ermethrin in the Rat*. Toxicol Appl Pharmacol 110:1-8. As cited in ATSDR, 2003.

Andrews, EB, MC Joseph, MJ Magenheim, HH Tilson, PA Doi, and MW Schultz. 1992. *Postmarketing Surveillance Study of Permethrin Crème Rinse*. American Journal of Public Health 82(6):857-861. As cited in Westchester County, 2002.

Blaylock BL, M Abdel-Nasser, SM McCarty, et al. 1995. *Suppression of Cellular* I*mmune Responses in Balb/c Mice Following Oral Exposure to Permethrin.* Bull Environ Contam Toxicol 54:768-774. As cited in ATSDR, 2003.

Bloomquist, J. 2001. *Insecticide Exposure in Parkinsonism*. *(Annual report, January 1-December 31, 2000*) National Technical Information Service (Springfield, VA) NTIS ADA388559. 70p. January 2001. As cited in, Westchester County, 2002.

Bloomquist, JR, RL Barlow, JS Gillette, W Li, and ML Kirby. 2002. *Selective Effects of Insecticides on Nigrostriatal Dopaminergic Nerve Pathways.* NeuroToxicology 23, 537-544, 2002.

Bowerman, JG, MP Gomez, RD Austin, and DE Wold. 1987. *Comparative Study of* P*ermethrin 1% Crème Rinse and Lindane Shampoo for the Treatment of Head Lice.* The Pediatric Infectious Disease Journal 6(3):252-255. As cited in, Westchester County, 2002.

Chen, H, J Xiao, G Hu, J Zhou, H Xiao, and X Wang. 2002. *Estrogenicity of Organophosphorus and Pyrethroid Pesticides.* Journal of Toxicology and Environmental Health, Part A, 65:1419-1435, 2002.

Chruscielska, K and D Kalhorn. 1999. *Genotoxicity of Pyrethroids: Part 2. Studies on Mutagenic Activity of Commercial Formulations in Mouse Micronucleus Test*. Pestycydy 4:47-50. As cited in ATSDR, 2003.

Clapp, MJL, PB Banham, IS Chart, J Glaister, C Gore, and A Moyes. 1977. *28 Day* F*eeding Study in Rats* (Report No. CTL/P/355) (Unpublished data submitted to WHO by ICI Central Toxicology Laboratory). As cited in WHO, 1990.

Crofton and Reiter. 1988. *The Effects of Type I and II Pyrethroids on Motor Activity and the Acoustic Startle Response in the Rat*. Fundam Appl Toxicol 10:624-634. As cited in ATSDR, 2003.

Dolan, G, FO Kuile, V Jacoutor, NJ White, C Luxemburger, L Malankirii, T Chongsuphajaisiddhi, and F Nosten. 1993. *Bed Nets for the Prevention of Malaria and* A*nemia in Pregnancy*. Transactions of the Royal Society of Tropical Medicine and Hygiene 87(6):620-626. As cited in Westchester County, 2002.

EXTOXNET. 1996. *Pesticide Information Profiles*, *Permethrin.* Extension Toxicology Network, Revised June 1996.

Flannigan, SA and SB Tucker. 1985. *Variation in Cutaneous Sensation Between* S*ynthetic Pyrethroid Insecticides*. Contact Dermatitis 13:140-147. As cited in Westchester County, 2002.

Flannigan, SA, SB Tucker, MK Marcus, CE Ross, EJ Fairchild, BA Grimes, and RB Harrist. 1985a. *Primary Irritant Contact Dermatitis from Synthetic Pyrethroid* I*nsecticide Exposure*. Archives of Toxicology 56:288-294. As cited in Westchester County, 2002.

Flannigan, SA, SB Tucker, MM Key, CE Ross, EJ Fairchild, BA Grimes, and RB Harrist. 1985b. *Synthetic Pyrethroid Insecticides: A Dermatological Evaluation*. British Journal of Industrial Medicine 42(6):363:372. As cited in Westchester County, 2002.

Fuortes, L, MD. 1999. *Urticaria Due to Airborne Permethrin Exposure.* Vet Human Toxicol 41 (2), April 1999.

Garey, J and MS Wolff. 1998. *Estrogenic and Antiprogestagenic Activities of Pyrethroid Insecticide.* Biochemical and Biophysical Research Communications. Vol. 251: 855-859. As cited in Westchester County, 2002.

Gillette, JS, and JR Bloomquist. 2003. *Differential Up-regulation of Striatal Dopamine Transporter and Alpha-synuclein by the Pyrethroid Insecticide Permethrin.* Toxicology and Applied Pharmacology 192, 287-293, 2003.

Go, V, J Garey, MS Wolff, and BG Pogo. 1999. *Estrogenic Potential of Certain Pyrethroid Compounds in the MCF-7 Human Breast Carcinoma Cell Line.* Environmental Health Perspectives. 1999 March;107(3):173-7.

Hadnagy, Wolfgang, N Seemayer, KH Kuhn, G Leng, and H Idel. 1999. *Induction of Mitotic Cell Division Disturbances and Mitotic Arrest by Pyrethroids in V79 Cell Cultures.* Toxicology Letters 107 (1999) 81-87.

Haustein, U and B Hilawa. 1989. *Treatment of Scabies with Permethrin Versus Lindane and Benzyl Benzoate.* Acta Dermato-Venereologiga 69(4):348-351. As cited in Westchester County, 2002.

Heder, AF, KI Hirsch-Ernst, D Bauer, GF Kahl, and H Desel. 2001. *Induction of Cytochrome P450 2B1 by Pyrethroids in Primary Rat Hepatocyte Cultures.* Biochem Pharmacol. 2001 July 1;62(1):71-9.

Institoris, L, U Undeger, O Siroki, M Nehez, and I Desi. 1999. *Comparison of Detection Sensitivity of Immuno- and Genotoxicological Effects of Subacute Cypermethrin and Permethrin Exposure in Rats,* Toxicology 137, 47-55, 1999.

International Agency for Research on Cancer (IARC). 1991. *IARC Monographs Permethrin*. Vol. 53(1991) (p. 329).

Ishmael, J and MH Litchfield. 1988. *Chronic Toxicity and Carcinogenic Evaluation of* P*ermethrin in Rats and Mice*. Fundam Appl Toxicol 11:308-322. As cited in ATSDR, 2003.

Kadota, T, J Miyamoto, and N Ito. 1975. *Six-Month Subacute Oral Toxicity of NRDC in Sprague-Dawley Rats.* Unpublished data submitted WHO by Sumitomo Chemical Co. As cited in WHO, 1990.

Kakko, I, T Toimela, H Tahti. 2003. *The Synaptosomal Membrane Bound ATPase as a Target For the Neurotoxic Effects of Pyrethroids, Permethrin and Cypermethrin.* Chemosphere 51 (2003) 475-480.

Kale, PG, BT Petty, S Walker, et al. 1995. *Mutagenicity Testing of Nine Herbicides and Pesticides Currently Used in Agriculture*. Environ Mol Mutagen 25:148-153. As cited in ATSDR, 2003.

Karen, DJ, W Li, PR Harp, JS Gillette, and JR Bloomquist. 2001. *Striatal Dopaminergic Pathways as a Target for the Insecticides Permethrin and Chlorpyrifos.* NeuroToxicology 22, 811-817, 2001.

Kasat, K, V Go, and BG Pogo. 2002. *Effects of Pyrethroid Insecticides and Estrogen on WNT10B Proto-oncogene Expression.* Environ Int. 2002 November; 28(5):429-432.

Killeen, JC and WR Rapp. 1976a. *A Three Month Oral Toxicity Study of FMC 33297 in Beagle Dogs*. Bio-Dynamics Inc. Project. Unpublished report submitted to WHO by FMC Corporation. As cited in Westchester County, 2002.

Killeen, JC and WR Rapp. 1976b. *AThree Month Oral Toxicity Study of FMC 33297 in Rats.* Bio-Dynamics Inc. Project. Unpublished report submitted to WHO by FMC Corporation. As cited in Westchester County, 2002.

Kojima, H, E Katsura, S Takeuchi, K Niiyama, and K Kobayashi. 2004. Screening for Estrogen and Androgen Receptor Activities in 200 Pesticides by In Vitro Reporter Gene Assays Using Chinese Hamster Ovary Cells. *Environmental Health Perspectives*, Volume 112, No. 5, pages 524-531. April.

Kolmodin-Hedman, B, A Swenson, and M Akerblom. 1982. *Occupational Exposure to Some Synthetic Pyrethroids (Permethrin and Fenvalerate).* Archives of Toxicology 50(1):27-33. As cited in Westchester County, 2002.

Leng, G, KH Kuhn, and H Idel. 1997. *Biological Monitoring of Pyrethroids in Blood and* P*yrethroid Metabolites in Urine: Applications and Limitations*. Sci Total Environ 199:173-181. As cited in ATSDR, 2003.

Leng, G, J Lewalter, B Rohrig, and H Idel. 1999. *The Influence of Individual Susceptibility in Pyrethroid Exposure.* Toxicology Letters 107 (1999) 123-130.

McDaniel, KL and VC Moser. 1993. *Utility of a Neurobehavioral Screening Battery for Differentiating the Effects of Two Pyrethroids, Permethrin and Cypermethrin*. Neurotoxicology and Teratology 15:71-83. As cited in Westchester County, 2002.

Metker, LW, RA Angerhofer, CR Pope, and KC Swentzel. 1977. *Toxicology Evaluation of 3-(Phenoxyphenyl)Methyl(+)-Cis,Trans-3-(2,2-Dichloroethenyl)-2,2-Dimethylcyclopropanecarboxylate (Permethrin*). Environmental Hygiene Agency (Report No. 75-51-0837-78). As cited in WHO, 1990.

Metker, LW. 1978. *Subchronic Inhalation Toxicity of 3-(Phenoxyphenyl)Methyl(+)-Cis,Trans-3-(2,2-Dichloroethenyl)-2,2-Dimethylcyclopropanecarboxylate (Permethrin*). Aberdeen Proving Ground, Maryland, US Army Environmental Hygiene Agency (Report No. 75-51-0026-80. As cited in WHO, 1990.

Meyer, EK. 1999. *Toxicosis in Cats Erroneously Treated With 45 to 65% Permethrin* P*roducts.* J Am Vet Med Assoc 215(2):198:203. As cited in ATSDR, 2003.

Mitchell, JA, MC Wilson, MJ Kallman. 1988. *Behavioral Effects of Pydrin and Ambush in Male Mice*. Neurotoxicol Teratol 10:113-119. As cited in ATSDR, 2003.

Mitsche, T, H Borck, B Horr, N Bayas, HW Hoppe, and F Diel. 2000. *Pyrethroid Syndrome in an Animal Keeper*. Allergy55(1):93-4.

National Library of Medicine (NLM). 2001. *Permethrin.* Hazardous Substance Data Bank Record.

National Pesticide Telecommunications Network (NPTN). 1997. *Permethrin*. NPTN Fact Sheet. Date Reviewed: September 1997.

Prater, MR, RM Gogal, Jr., BL Blaylock, and SD Holladay. 2003. *Cis-Urocanic Acid Increases Immunotoxicity and Lethality of Dermally Administered Permethrin in C57BL/6N Mice.* International Journal of Toxicology, 22:35-42, 2003.

Prater, MR, RM Gogal, Jr., BL Blaylock, and SD Holladay. 2002. *Single-dose Topical Exposure to the Pyrethroid Insecticide, Permethrin in C57BL/6N Mice: Effects on Thymus and Spleen.* Food and Chemical Toxicology 40, 1863-1873, 2002.

Punareewattana, K, BJ Smith, BL Blaylock, J Longstreth, HL Snodgrass, RM Gogal, Jr., RM Prater, and SD Holladay. 2001. *Topical Permethrin Exposure Inhibits Antibody Production and Macrophage Function in C57B1/6N Mice.* Food and Chemical Toxicology 39, 133-139, 2001.

Saito, K, Y Tomigahara, N Ohe, N Isobe, I Nakatsuka, and H Kaneko. 2000. *Lack of Significant Estrogenic or Antiestrogenic Activity of Pyrethroid Insecticides in Three in Vitro Assays Based on Classic Estrogen Receptor Alpha-Mediated Mechanisms.* Toxicological Science; 57(1):54-60. As cited in Westchester County, 2002.

Schultz, MW, M Gomez, RC Hansen, J Mills, A Menter, H Rodgers, FN Judson, G Mertz, and HH Handsfield. 1990. *Comparative Study of 5% Permethrin Cream and 1% Lindane Lotion for the Treatment of Scabies*. Archives of Dermatology 126(7):167-170. As cited in Westchester County, 2002.

Sheets, LP. 2000. *A Consideration of Age-Dependent Differences in Susceptibility to Organophosphorous and Pyrethroid Insecticides*. Neurotoxicology21(1-2):57-63. As cited in Westchester County, 2002.

Soto, J, F Medina, N Dember, and J Berman. 1995. *Efficacy of Permethrin-Impregnated Uniforms in the Prevention of Malaria and Leishmaniasis in Colombian Soldiers.* Clinical Infectious Diseases 21:599-602. As cited in Westchester County, 2002.

Stelzer, KJ and MA Gordon. 1984. *Effects of Pyrethroids on Lymphocyte Mitogenic Responsiveness*. Research Communications in Chemical Pathology and Pharmacology 46(1):137-150. As cited in Westchester County, 2002.

Sumida, K, K Saito, N Ohe, N Isobe, H Kaneko, and I Nakatsuka. 2001. *Evaluation of In Vitro Methods for Detecting the Effects of Various Chemicals on the Human Progesterone Receptor,*

With a Focus on Pyrethroid Insecticides. Toxicology Letters; 118(3):147-55. As cited in Westchester County, 2002.

Tessier, DM and F Matsumura. 2001. *Increased ErB-2 Tyrosine Kinase Activity, MAPK Phosphorylation, and Cell Proliferation in the Prostate Cancer Cell Line LNCaP following Treatment of Select Pestides.* Toxicological Sciences 60, 38-43, 2001.

Tisch, M, P Schmezer, M Faulde, A Groh, and H Maier. 2002. *Genotoxicity Studies on Permethrin, DEET and Diazinon in Primary Human Nasal Mucosal Cells. Photo Journal of* European Archives Otorhinolaryngol. 259:150-153, 2002.

Tyler, CR, N Beresford, M van der Woning, JP Sumpter, and K Thorpe. 2000. *Metabolism and Environmental degradation of Pyrethroid Insecticides Produce* C*ompounds with Endocrine Activities*. Environmental Toxicology and Chemistry 19(4):801-809. As cited in Westchester County, 2002.

United States Environmental Protection Agency (USEPA). 1997. *Permethrin* File. Iris Database On-Line 3/31/1987, updated 4/1/97.

United States Environmental Protection Agency (USEPA). 1992. Integrated Risk Information System (IRIS) Record for Permethrin. Last Revised 1/1/92.

United States Environmental Protection Agency (USEPA). 2000. *Tox Oneliners for Permethrin*. Office of Pesticide Programs. Last Updated May 30, 2000. As cited in Westchester County, 2002.

United States Environmental Protection Agency (USEPA). 2002a. *Chemicals Evaluated for Carcinogenic Potential.* Office of Pesticide Programs, Health Effects Division. Update 5/10/02.

United States Environmental Protection Agency (USEPA), Region IV. 2002b. *Preliminary Remedial Goal Table*. October 1, 2002.

United States Environmental Protection Agency (USEPA), Region III. 2004. *Risk-Based Concentration Table*. April 14, 2004.

van der Rhee, HJ, JA Farquhar, and NPE Vermeulen. 1989. *Efficacy and Transdermal Absorptioin of Permethrin in Scabies Patients.* Acta Derm Venereol (Stockh) 69:170-182. As cited in ATSDR, 2003.

Wauchope, RD, TM Butler, AG Hornsby, PWM Augustijn-Beckers, and JP Burt. 1992. *The SCS/ARS/CES Pesticide Properties Database for Environmental Decision Making.* Springer-Verlag, New York, NY. As cited in Westchester County, 2002.

Westchester County. 2002. *Comprehensive Mosquito-Borne Disease Surveillance and Control Plan*, Generic Environmental Impact Statement. February, 2002.

World Health Organization (WHO). 1990. *Permethrin*. International Programme on Chemical Safety, Environmental Health Criteria 94. Geneva.

World Health Organization (WHO). 1999. Toxicological Evaluations - Permethrin. Joint meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group. Rome. September 20-29, 1999.

World Health Organization (WHO). 2001. *Guidelines for Drinking Water Quality.*

World Health Organization (WHO). http://www.who.int/. April 19, 2001. As cited in ATSDR, 2003.

World Health Organization (WHO). Undated. Fact Sheet, *Permethrin.*

Yamada, T, S Ueda, K Yoshioka, S Kawamura, T Seki, Y Okuno, and N Mikami. 2003. *Lack of Estrogenic or (Anti-)Androgenic Effects of d-Phenothrin in the Uterotrophic and Hershberger Assays*. Science Direct Toxicology 186 (2003) 227-239.

Yang, P, J Lin, AH Hall, TCY Tsao, and M Chern. 2002. *Acute Ingestion Poisoning with Insecticide Formulations Containing the Pyrethroid Permethrin, Xylene, and Surfactant: A Review of 48 Cases.* Clinical Toxicology, 40(2), 107-113, 2002.

4.2.2 Resmethrin

Background

Resmethrin is a Type I pyrethroid and pesticide products containing resmethrin are classified in toxicity class III, requiring a caution label (Westchester County, 2002). Resmethrin products are used for a variety of applications including agricultural as well as residential/household. Resmethrin was introduced commercially in 1969 (WHO/ FAO, 1996). Technical resmethrin is a mixture of four isomers: 1R-cis, 1R-trans,1S-cis and 1S-trans, in a 4:1:4:1 ratio. Typically the cis-isomer is more toxic than the trans-isomer (WHO, 1989). The 1R-trans-isomer is also known as bioresmethrin and the 1R-cis-isomer is known as cismethrin (WHO, 1989).

Absorption/Distribution/Excretion

As with other pyrethroids, resmethrin is absorbed readily following oral and inhalation exposures, and to a lesser degree through the skin (ATSDR, 2003). Oral administration of resmethrin to cows indicated absorption of at least 43 percent, since this amount was excreted as resmethrin metabolites in urine (Ridlen et al., 1984 [as cited in ATSDR, 2003]).

Pyrethrin and pyrethroid compounds are readily metabolized by mammalian systems. This metabolism occurs in the liver and other tissues, rendering pyrethrins and pyrethroids water soluble so that they can be excreted in the urine (ATSDR, 2003).

Resmethrin, as with other pyrethroids, is relatively rapidly eliminated from the body once absorbed. Approximately 50 percent of an administered dose is eliminated by rats within 72 hours (Miyamoto et al., 1971 [as cited in WHO, 1989]). However, it took three weeks for complete elimination to occur. In hens it appears that elimination is even more rapid following oral doses, with 90 percent eliminated within 24 hours. The highest residues were present in the liver and kidney, with residues in eggs reportedly low (Christopher et al., 1985 [as cited in WHO, 1989]) . Similar results were found when lactating cows were given oral doses of 10 mg/kg. Resmethrin was rapidly absorbed, metabolized and excreted. Forty eight hours after treatment, residues were below 1 ug/g in all tissues except the liver and kidney. Very low levels were detected in milk (Ridlen et al., 1984 [as cited in WHO, 1989]).

Toxicity

Neurological Effects

Human Evidence

No information regarding neurological effects in humans was found in this literature search.

Animal Evidence

Symptoms of resmethrin neural toxicity include tremors, hyperactivity and convulsions. As with permethrin, some isomers of resmethrin are more toxic than others. Following acute oral doses, the cis-isomer is approximately 50 times more toxic than the trans-isomer. This appears to be at least partly due to more rapid metabolism of trans-isomer (bioresmethrin) (WHO, 1989).

According to the USEPA, resmethrin has been given to laboratory rats at doses of 62.5 mg/kg-day for 32 weeks, 250 mg/kg-day for 20 days, or 632 mg/kg-day for 7 days without impacting the nervous system (USEPA, 1983 [as cited in Westchester County, 2002]). In another study, resmethrin given to laboratory rats at a dose of 1250 mg/kg-day for 32 weeks did not cause nervous system effects (Cox et al., 1979 [as cited in WHO, 1989]; Schwartz et al., 1979 [as cited in WHO, 1989]). However, doses of 679 mg/kg-day for 90 days did cause tremor in rats exposed via the diet (Swentzel et al., 1977 [as cited in WHO, 1989]). Motor activity was not impaired following a 30 day exposure to 6 mg/kg-day of cis-resmethrin, however, effects on the startle response were noted (Crofton and Reiter, 1984 [as cited in WHO, 1989]).

One-hour inhalation studies were performed, exposing rats and rabbits to 12-13.7 mg/L in air. The animals experienced labored breathing and impaired behavioral responses (Macko et al., 1979 [as cited in WHO, 1989]). In another study in which laboratory rats were exposed to levels of 2.9 to 3.2 mg/L in air for five hours each day for five consecutive days, rapid breathing, increased preening and nasal discharge were observed (Macko et al., 1979 [as cited in WHO, 1989]). A similar exposure regimen, but for a longer study duration, was administered to rats and mice. These animals were exposed to 0 to 210 mg/m^3 for 4 hours a day, five days a week for four weeks. No toxic effects were observed (Miyamoto, 1976).

Liver and Kidney Toxicity

Human Evidence

No information regarding liver and kidney toxicity in humans was found in this literature search.

Animal Evidence

The liver has been observed to be a target for toxicity following short-term, high dose exposure. In a study in which rats were exposed to doses of resmethrin ranging from 0 to 5000 mg/kg-day for 14 days, a higher liver-to-body weight ratio was noted at doses of approximately 300 mg/kg-day (Swentzel et al., 1977 [as cited in WHO, 1989]).

Liver, and kidney relative weights (in males only) were also increased in a study exposing rats to 211 mg/kg-day for 90 days (Swentzel et al., 1977 [as cited in WHO, 1989]). Liver dysfunction was noted in a study in which rats were exposed to bioresmethrin at concentrations in the diet of 1200 ppm for 91 days (Wallwork et al., 1971 [as cited in WHO, 1989]). In another study in which cis-resmethrin was administered in the diet of rats for 24 weeks, increased liver and kidney weights were observed. The no observed effect level was 77.7 to 86.6 mg/ kg bw per day (Miyamoto, 1976).

A six month oral study in beagle dogs also found liver toxicity at doses of 300 mg/kg-day (Gephart et al., 1980 [as cited in WHO, 1989]). The no observed effect level was 30 mg/kg-day. Resmethrin fed in the diet of rats for 112 weeks resulted in a no observed effect level of 500 mg/kg (Knickerbocker et al., 1980 [as cited in WHO, 1989]; Hess et al., 1982 [as cited in WHO, 1989]).

Inhalation of resmethrin at levels of 1 g/m^3 for 6 hours a day, 5 days a week for 90 days indicated minimal changes in the liver. These were found to be reversible (Coombs et al., 1985 [as cited in WHO, 1989]).

Skin and Eye Irritation

Human Evidence

A small percentage of people may experience skin irritation after exposure to resmethrin. In a study in which 230 volunteers were exposed via a skin patch test, only 2 experienced skin irritation (Lisi, 1992 [as reported in Westchester County, 2002]).

Animal Evidence

The application of technical grade resmethrin to the ears of laboratory rabbits caused irritation after 72 hours of exposure (Swentzel et al., 1977 [as reported in WHO, 1989]). However, such irritant effects have not been reported in other studies in which resmethrin has been applied to the skin or eyes (Westchester County, 2002).

Rats exposed to resmethrin at levels of 0.3 g/m^3 in air for 6 hours a day, 5 days a week for 90 days showed signs of irritation (Coombs et al., 1985 [as cited in WHO, 1989]).

Respiratory Effects

Human Evidence

No information regarding respiratory effects in humans was found in this literature search.

Animal Evidence

One study investigated the potential for resmethrin exposure to cause effects on respiratory health, however, no such effects were found (Miyamoto, 1976).

Immunologic Response

Human Evidence

No information regarding impairment of the immune system in humans was found in this literature search.

Animal Evidence

In one study, resmethrin was not found to cause allergic reactions in rabbits (Swentzel et al., 1977 [as cited in Westchester County, 2002]). However, oral administration of resmethrin at a single dose approximately half of the LD_{50} was reported to cause an early and marked stimulation of cellular immune response (Danliker et al., 1979 [as cited in WHO, 1989]).

Bioresmethrin was found to have a low potential for sensitization and irritation in a study in which bioresmethrin was applied dermally to guinea pigs at a rate of 0.1 ml of a 1 percent solution for four days. On the seventh day, 0.2 ml was applied and resulted in slight redness (Chesher and Malone, 1970 [as cited in WHO, 1989]).

Developmental and Reproductive Effects

Human Evidence

There was no information regarding developmental or reproductive effects in humans identified in this literature review.

Animal Evidence

Developmental toxicity has not been noted at oral doses of resmethrin below 100 mg/kg-day in rats and mice (Westchester County, 2002). The third generation of rats treated with 25 mg/kg-day of resmethrin was observed to have lower pup weights and greater frequency of pup death than untreated rats (Penwick Corporation, 1979 [as cited in USEPA, 1988]). This study was used by the USEPA as the basis of their oral Rfd of 0.03 mg/kg-day. In other studies, developmental effects were seen in rats at doses above 40 mg/kg (Becci et al., 1979 [as cited in WHO, 1989]; Machi et al., 1979 [as cited in WHO, 1989]; Schwartz et al., 1979 [as cited in WHO, 1989]; Swentzel et al., 1977 [as cited in WHO, 1989]; Waldron, 1969 [as cited in WHO, 1989]).

Cancer

Human Evidence

Human evidence of carcinogenicity of resmethrin was found in this literature search. The USEPA has not classified resmethrin as to its carcinogenicity potential in humans (USEPA, 2004).

Animal Evidence

Available information indicates that resmethrin is not mutagenic in either bacterial or mammalian systems (Westchester County, 2002; ATSDR, 2003; WHO, 1989). One study has reported an increase in malignant and benign liver tumors in rats given 131 mg/kg-day (USEPA, 2000 [as cited in Westchester County, 2002]). However, other studies at even higher concentrations have not found a treatment related increase in tumors (WHO, 1989; USEPA, 2000 [as cited in Westchester County, 2002]). In a 112-week study in which resmethrin was fed to rats at doses ranging from 0 to 5000 mg/kg in the diet, oncogenicity was not observed. At 500 mg/kg, hypertrophy of liver cells was noted (Knickerbocker et al., 1980 [as cited in WHO, 1989]; Hess et al., 1982 [as cited in WHO, 1989]).

Table 11
Toxicity Study Summary Table- Resmethrin

Compound	Purity	Test Species (Strain)	Route	Dose Duration	Study Duration	Endpoint	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Comments	Study	Reference
Resmethrin	90%	rats	diet	25, 40, 62 mg/kg-d	3-generation	developmental	NA	25	fetal death, dec. pup wgt	Penwick Corporation, 1979a	USEPA, 1988
Resmethrin		rats	diet	0 to 250 mg/kg-d	2 years	inc liver cell size, inc liver wgt, liver lesions, inc thyroid wgt	NA	39.5		Penwick Corporation, 1980a	USEPA, 1988
Resmethrin		dog	diet	0 to 30 mg/kg-day	6 month	inc liver wgt	10	30		Penwick Corporation, 1980b	USEPA, 1988
Resmethrin		rats	oral	0 to 80 mg/kg-day		teratology fetotoxicity	80 40	NA 80		Penwick Corporation, 1979b	USEPA, 1988
Resmethrin		rabbit	oral	0 to 100 mg/kg-day		teratology	100	NA		Penwick Corporation, 1979c	USEPA, 1988
Resmethrin		rats	diet	0 to 125 mg/kg-d		developmental	NA	25	fetal death, dec. pup wgt	Penwick Corporation, 1978	USEPA, 1988
Resmethrin		rats	oral	0 to 70.8 mg/kg-d	pre-mating thru lactation	developmental	0.8	70.8	pup death, dec. pup wgt	USEPA, 1994a	ATSDR, 2003
Resmethrin		rats	gavage	0 to 80 mg/kg-d	GD 6-15	teratology	NR	80	slightly increased skeletal abnormalities, delayed ossification	USEPA, 1994a	ATSDR, 2003
Resmethrin		Sprague-Dawley rats	diet	0-5100 mg/kg-d	14 days	neurological effects, dec body wgt gain, inc liver wgt	310 males	310 females 630 males		Swentzel et al., 1977	WHO, 1989
Resmethrin		Long Evans rats	diet	0-2532 mg/kg-d	14 days	neurological effects, dec body wgt gain, inc liver wgt	148 males 180 females	297 males 386 females		Swentzel et al., 1977	WHO, 1989
Resmethrin		Long Evans rats	diet	0-2400 mg/kg-d	90 days	neurological effects, dec body wgt gain, inc liver wgt	67 females 66 males	219 females 211 males		Swentzel et al., 1977	WHO, 1989
Bioresmethrin		rats	diet	0-8000 ppm	91 days	body weight, blood chemistry, liver function	33 males 36 females			Wallwork et al., 1971	WHO, 1989
Bioresmethrin		dogs	gavage	0- 250	90 days	body weight, blood chemistry	80	250	blood chemistry	Noel et al., 1971	WHO, 1989
Resmethrin	technical	Wistar rats	inhalation	0 to 1 g/m^3	6 hr/day, 5 days/wk for 90 days	irritation organ pathology	0.1g/m^3	0.3 g/m^3 1 g/m^3		Coombs et al., 1985	WHO, 1989
Resmethrin		Sprague-Dawley rats ICR mice	inhalation	0 to 210 mg/m^3	4 hr/day, 5 days/wk for 4 weeks	behavior, histopathology clinical chemistry	210 mg/m^3		no effects observed	Miyamoto, 1976	WHO, 1989
cis-Resmethrin		Sprague-Dawley rats	diet	0-5000 mg/kg	24 weeks	liver and kidney toxicity	77.7 males 86.6 females			Miyamoto, 1976	WHO, 1989
Resmethrin		Wistar rats	diet	0-5000 mg/kg	112 weeks	liver and kidney toxicity	500 mg/kg		some hepatocyte hypertrophy noted at this level. No oncogenicity	Knickerbocker et al., 1980, Hess et al., 1982	WHO, 1989
Resmethrin		Beagle Dogs	diet	0-300 mg/kg bw/d	6 months	incr liver wgt	10	30		Gephart et al., 1980	WHO, 1989

Table 12

Noncancer Criteria (Oral/Dermal)- Resmethrin

Chemical of Potential Concern	Exposure Duration	Criteria	Value	Units	Species	Endpoint	NOAEL	LOAEL	Units	Combined Uncertainty/Modifying Factors	Study Source	Reference for Criteria
Resmethrin	acute	RfD-oral	0.1	mg/kg/day	dog	increased liver weight	1.00E+01	NA	mg/kg/day	100	FDA Research Lab, 1980	Westchester County, 2002
Resmethrin	acute	RfD-dermal	10	mg/kg/day	rabbit	no effect	1.00E+03	NA	mg/kg/day	100	USEPA, 2000	Westchester County, 2002
Resmethrin	subchronic	RfD-oral	0.1	mg/kg/day	dog	increased liver weight	1.00E+01	NA	mg/kg/day	100	FDA Research Lab, 1980	Westchester County, 2002
Resmethrin	subchronic	RfD-dermal	10	mg/kg/day	rabbit	no effect	1.00E+03	NA	mg/kg/day	100	USEPA, 2000	Westchester County, 2002
Resmethrin	chronic	RfD	0.03	mg/kg/day	rat	developmental tox		2.50E+01	mg/kg/day	1000	Penwick Corp, 1979a	USEPA, 1988
Resmethrin	chronic	RfD-oral	0.03	mg/kg/day	rat	developmental	NA	2.50E+01	mg/kg/day	1000	USEPA, 1989	Westchester County, 2002
Resmethrin	chronic	RfD-dermal	0.03	mg/kg/day	rat	developmental	NA	2.50E+01	mg/kg/day	1000	USEPA, 1989	Westchester County, 2002

NA: Not Applicable or Not Available.

NOAEL: no observed adverse effect level

LOAEL: lowest observed adverse effect level

RfD: USEPA reference dose

Table 13
Noncancer Criteria (Inhalation)- Resmethrin

Chemical of Potential Concern	Exposure Duration	Criteria	Value	Units	Endpoint	Species	NOAEL	LOAEL	Units	Combined Uncertainty/Modifying Factors	Study Source	Reference for Criteria
Resmethrin	acute	RfC	0.0001	mg/L	rat	behavior, serum	NA	0.1	mg/L	1000	Huntingdon Research	Westchester
						glucose, dec body wgt					Corp, 1985	County, 2002
Resmethrin	subchronic	RfC	0.0001	mg/L	rat	behavior, serum	NA	0.1	mg/L	1000	Huntingdon Research	Westchester
						glucose, dec body wgt					Corp, 1985	County, 2002
Resmethrin	chronic	RfC	0.00001	mg/L	rat	behavior, serum	NA	0.1	mg/L	10000	Huntingdon Research	Westchester
						glucose, dec body wgt					Corp, 1985	County, 2002
Resmethrin	chronic	RBC	110	ug/m^3	NA	NA	NA	NA	NA	NA	NA	USEPA Region III, 2004

NA: Not Applicable or Not Available.
NOAEL: no observed adverse effect level
LOAEL: lowest observed adverse effect level
RfC: Reference Concentration
RBC: USEPA Region III Risk Based Concentration

Table 14
Cancer Criteria (Oral/Dermal)- Resmethrin

Chemical of Potential Concern	Oral Threshold Dose	Margin of Exposure	Units	Weight of Evidence/ Cancer Guideline Description	Source	Reference
Resmethrin	0.025	100	mg/kg-day	non-mutagenic, threshold	USEPA, 2000	Westchester County, 2002

NA: Not Applicable or Not Available.

Reference List

Resmethrin

Agency for Toxic Substances and Disease Registry (ATSDR). 2003. *Toxicological Profile for Pyrethrins and Pyrethroids.* Department of Health and Human Services, September, 2003.

Becci, PJ, M Knickerbocker, and RA Parent. 1979. *Teratological Evaluation of SBP-1382 in Albino Rabbits*, Waverly, New York, Food and Drug Research Laboratories, 17pp (Report No. 6288) (Unpublished proprietary data supplied by Roussel Uclaf). As cited in WHO, 1989.

Chesher, BC and JC Malone. 1970. *Sensitization Study With NRDC 107 on Guinea Pigs* (Report B 198-70) (Unpublished data of Wellcome Foundation Ltd). As cited in WHO, 1989.

Christopher, RJ, CE Munger, and G Wayne Irvie. 1985. *Distribution and Depletion of (14C)-Resmethrin Isomers Administered Orally to Laying Hens.* Pestic. Sci., 16: 378-382. As cited in WHO, 1989.

Coombs, DW, CJ Hardy, GC Clark, AE Street, C Gopinath, DJ Lewis, and RS Rao. 1985. *Resmethrin 90-Day Inhalation Toxicity Study in the Rat*, Huntingdon, United Kingdom, Huntingdon Research Centre, 80 pp (Report No. SBP 6/84997) (Unpublished proprietary data supplied by Roussel Uclaf). As cited in WHO, 1989.

Cox, GE, PJ Becci, and RA Parent. 1979. *Supplementary Report For Evaluation of the Neurotoxic Effects of SBP-1382 in Albino Rats – Phase I*, Waverly, New York Food and Drug Research Laboratories, 7 pp (Report No. 6362) (Unpublished proprietary data supplied by Roussel Uclaf). As cited in WHO, 1989.

Crofton, KM and LW Reiter. 1984. *Effects of Two Pyrethroid Insecticides on Motor Activity and the Acoustic Startle Response in the Rat*. Toxicol. Appl. Pharmacol. 75:318-328. As cited in WHO, 1989.

Danliker, WB, AN Hicks, SA Levison, K Stewart, and RJ Brown. 1979. *Effects of Pesticides on Immune Response*, Washington, DC, US Environmental Protection Agency (EPA-600/1-79-039-PB 80-1308 34). As cited in WHO, 1989.

Gephart, LA, WD Johnson, PJ Becci, and RA Parent. 1980. *180-Day Subchronic Oral Dosing Study with Resmethrin in Beagle Dogs*, Waverly, New York, Food and Drug Research

Laboratories, 86 pp (Report No. 6289) (Unpublished proprietary data supplied by Roussel Uclaf). As cited in WHO, 1989.

Hess, FG, SW Thompson, and PJ Becci. 1982. *A Lifetime Evaluation of the Dietary Administration of SBP-1382 to Wistar Albino Rats/Amendment*, Waverly, New York, Food and Drug Research Laboratory, 28 pp (Supplemental report No. 5271) (Unpublished proprietary data supplied by Roussel Uclaf). As cited in WHO, 1989.

Knickerbocker, M, PJ Becci, GE Cox, and RA Parent. 1980. *A Lifetime Evaluation of the Dietary Administration of SBP-1382 to Wistar Albino Rats*, Waverly, New York, Food and Drug Research Laboratories, 110 pp (Report No. 5271) (Unpublished proprietary data supplied by Roussel Uclaf). As cited in WHO, 1989.

Lisi, P. 1992. *Sensitization Risk of Pyrethroid Insecticides.* Contact Dermatitis 26(5):349-350. As cited in Westchester County, 2002.

Machi, RA, C Kam, MA Gallo, KR Stevens, and JJ Gagliardi. 1979. *Teratological Evaluation of SBP-1382Technical in the Albino Rat*, Florham Park, New Jersey, Booz, Allen & Hamilton, Inc., 44 pp (Report No. 2054-066) (Unpublished proprietary data supplied by Roussel Uclaf). As cited in WHO, 1989.

Macko, JA, JG Harvey, CR Pope, and AH McCreesh. 1979. *Hazard Evaluation of Aerosol Formulations Containing the Synthetic Pyrethroid Insecticide Resmethrin, (5-Benzyl-1,3-Furl) Methyl-2,2-Dimethyl-3-(2-Methlpropenyl) Cyclopropane-Carboxylate (SBP-1382).* Aberdeen Proving Ground, US Army Environmental Hygiene Agency, 38 pp (Report No. 75-51-0848-79). As cited in WHO, 1989.

Miyamoto, J, T Nishida, and K Ueda. 1971. *Metabolic Fate of Resmethrin, 5-Benzyl-3-Furylmethyl d1-Trans-Chrysanthemate in the Rat*. Pestic. Biochem. Physiol., 1: 293-306. As cited in WHO, 1989.

Miyamoto, J. 1976. *Degradation, Metabolism and Toxicity of Synthetic Pyrethroids*. Environ. Health Perspect., 14: 15-28.

National Library of Medicine (NLM). 2004. *Hazardous Substance Data Bank (HSDB) for Resmethrin.* URL http://toxnet.nlm.nig.gov.

Ridlen, RL, RJ Christopher, GW Ivie, et al. 1984. *Distribution and Metabolism of Cis- and Trans-Resmethrin in Lactating Jersey Cows.* J Agric Food Chem 32:1211-1217. As cited in ATSDR, 2003 and in WHO, 1989.

Schwartz, CS, L Gephart, PJ Becci, and RA Parent. 1979. *The Evaluation of the* E*ffects of SBP-1382 Following Dietary Administration Through Three Generations in Sprague-Dawley Rats,* Waverly, New York, Food and Drug Research Laboratories, 70 pp (Report No. 5739) (Unpublished proprietary data supplied by Roussel Uclaf). As cited in WHO, 1989.

Swentzel, KL, RA Angehofer, and EA Haight. 1977. *Toxicological Evaluation of* Pyrethroid *Insecticide (5-Benzyl-1,3-Furyl) Methyl-2,2Dimethyl-3-(2Methylpropenyl)* Cyclopropane-*Carboxylate (Resmethrin).* Aberdeen Proving Ground. US Army Environmental Hygiene Agency (Report No. 51-0830-77). As cited in WHO, 1989.

United States Environmental Protection Agency (USEPA). 1983. *Tolerances for Pesticides in Food Administered by EPA:Resmethrin.* Federal Register 48:36246-36247, August 10. As cited in Westchester County, 2002.

United States Environmental Protection Agency (USEPA). 1988. Integrated Risk Information System (IRIS) Record for Resmethrin. Last Updated 9/26/88.

United States Environmental Protection Agency (USEPA). 2000. *Tox Oneliners for Resmethrin.* Office of Pesticide Programs. Last updated May 26, 2000. As cited in

Westchester County, 2002.

United States Environmental Protection Agency (USEPA), Region III. 2004. *Risk-Based Concentration Table*. April 14, 2004.

Waldron, MM. 1969. *Foetal Toxicity Study – 95 H 56 – Given Orally in the Rabbit* (Report Path 51) (Unpublished data of Wellcome Foundation Ltd). As cited in WHO, 1989.

Wallwork, LM, RB Clampitt, and JC Malone. 1971. *NRDC 107, Rat Oral 91 Day Toxicity Study* (Report No. B 199-71) (Unpublished data of Wellcome Foundation Ltd). As cited in WHO, 1989.

Westchester County. 2002. *Comprehensive Mosquito-Borne Disease Surveillance and Control Plan*, Generic Environmental Impact Statement. February, 2002.

World Health Organization (WHO) and the Food and Agriculture Organization (FAO). 1996. *WHO/FAO Data Sheets on Pesticides, No. 83 Resmethrin.* WHO/PCS/DS/96.83, July 1996.

World Health Organization (WHO). 1989. *Environmental Health Criteria 92 Resmethrins-Resmethrin, Bioresmethrin, Cisresmethrin.* International Programme on Chemical Safety, World Health Organization, Geneva.

4.2.3 Sumithrin

Background

Sumithrin is a Type I pyrethroid, similar to resmethrin and permethrin. It is also known as d-phenothrin. Sumithrin has been used as a pesticide since 1977. It is found in products used for mosquito control and in shampoo for the treatment of head lice. As with resmethrin, there are four stereoisomers; 1R, trans, 1R, cis, 1S, trans and 1S, cis. Commercial products that are currently available are comprised of a 1:4 ratio of 1R, cis and 1R, trans isomers. The 1R, trans isomer has more potent insecticidal activity than the other isomers (WHO, 1990).

Absorption/Distribution/Excretion

The absorption of sumithrin is similar to other pyrethroids. After oral exposure, almost the entire dose is excreted in the urine and feces within three to seven days (WHO, 1990). The trans-isomer is more readily excreted in the urine (approximately 75 percent), whereas the cis-isomer is more readily excreted in the feces (approximately 74 percent following oral exposure (Kaneko et al., 1981 [as cited in WHO, 1990]). Tissue residues are reported to be low seven days after a single oral dose of 10 mg/kg, with slightly higher levels in fat tissue (1-2.5 mg/kg) (Kaneko et al., 1981 [as cited in WHO, 1990]). Higher concentrations were found in fat tissue (up to 23 mg/kg) when a higher dose, 200 mg/kg, was administered (Isobe et al., 1987 [as cited in WHO, 1990]).

Dermal exposure in rats at a dose of 10 mg/kg resulted in approximately 3-17 percent absorption, depending upon the formulation being applied (Kaneko et al., 1981 [as cited in WHO, 1990]; Isobe et al., 1987 [as cited in WHO, 1990]).

The primary urinary metabolites of sumithrin are 3-phenoxybenzoic acid, its glycine conjugate and 3-(4'-hydroxyphenoxy)benzoic acid. Following oral exposure, these metabolites are excreted in the urine within a few days following exposure (Menzie, 1978). Monitoring for urinary metabolites was conducted by the CDC in September of 2003 to evaluate the potential human exposure from mosquito control spray activities. Pre- and post-spray urine samples were obtained from volunteers in Virginia. The CDC concluded that ULV surface spraying with sumithrin did not increase urinary metabolites of sumithrin (Duprey et al., 2003).

Toxicity

Compared to resmethrin and permethrin, there is much less toxicity information on sumithrin. It does, however, appear to be less acutely toxic than these other pyrethroids (WHO, 1990). In a review conducted by WHO, no human cases of poisonings had been reported during the 10 years that sumithrin had been in use (WHO, 1990).

Neurological Effects

Human Evidence

No information was found regarding neurological effects in humans.

Animal Evidence

Though sumithrin would be expected to have similar neurological effects as other pyrethroids, it apparently requires much higher doses to elicit effects. When sumithrin is injected, signs of toxicity appear rapidly and include tremors, cardiac fibrillation, slow respiration, salivation, lacrimation, difficulty in walking and paralysis. LD_{50} doses range from 265 to 315 mg/kg for male and female rats respectively (Hiromori et al., 1984 [as cited in WHO, 1990]). Rats exposed to 5000 mg/kg-day of sumithrin for five days did not experience leg weakness or impaired voluntary muscle movements (Okuno et al., 1978 [as cited in WHO, 1990]). Neurotoxic effects were also not observed after a four hour inhalation exposure to 3760 mg/m^3, nor 210 mg/m^3 for four hours a day for five days a week for four weeks (Kohda et al., 1979 [as cited in WHO, 1990]).

In a study that exposed neuroblastoma cells from mice to various pyrethroids, sumithrin was the least effective at prolonging the opening of the sodium channel (Ruigt et al., 1987).

Skin and Eye Irritation

Human Evidence

In a study using sumithrin shampoo for the control of head lice, doses between 0.44 and 0.67 mg/kg-day did not produce skin irritation in a small group of volunteers when applied three times with three day intervals (Hashimoto et al., 1980 [as cited in WHO, 1990]). The material was left on the skin for one hour and then washed off. No clinical signs or blood biochemical

abnormalities were observed. Sumithrin was not detected in the blood at a detection limit of 0.006 mg/kg.

Animal Evidence

No information was found on skin or eye irritation in animals.

Gastrointestinal Effects

No information was found on GI effects in animals or humans.

Respiratory Effects

Human Evidence

Emergency room visits for asthma and chronic obstructive pulmonary exposure were evaluated before and after sumithrin spray activities in New York City during the summer and fall of 2000. No increase in the number of emergency room visits were noted even in children (Karpati et al., 2004).

Animal Evidence

Respiratory effects were investigated in one study reviewed for the Westchester County GEIS. Though details of this study were not provided, it was reported that respiratory effects in exposed animals were not observed (Miyamoto, 1976 [as cited in Westchester County, 2002]).

Immunologic Response

No information was found on effects on the immune system in animals or humans.

Endocrine Disruption

Human Evidence

Using cultures of human breast cancer cells, researchers tested the ability of various pyrethroids to mimic estrogen and increase cell proliferation. Sumithrin was found to increase cell proliferation and the expression of a gene known to be responsive to estrogen. Sumithrin was found to be more potent in its estrogenic potential than permethrin, which was also tested in this study (Go et al., 1999).

Sumithrin has been implicated as a cause of a condition among Haitian refugees in 1981 in which men developed unusually large mammary glands (gynecomastia). The clothing and bedding used by these men had been treated with sumithrin (Yamada et al., 2003).

Animal Evidence

Short-term *in vivo* studies have looked into the potential for sumithrin to cause estrogenic or androgenic effects. A study in which female rats were given oral doses of sumithrin (0 to 1000 mg/kg per day) for three days did not detect any changes in the weight of the uterus. The same researchers also evaluated the potential for sumithrin to cause androgenic or anti-androgenic effects. After giving oral doses of 0 to 1000 mg/kg per day to male rats for ten days, no effects were noted in any of the sex accessory glands or tissues. The authors conclude that their results do not indicate that sumithrin has estrogenic or androgenic properties (Yamada et al., 2003).

Similar results have been obtained using *in vitro* animal cell cultures in which sumithrin was not found to cause endocrine disruption effects (Saito et al., 2000 [as cited in Westchester County, 2002]; Sumida et al., 2001 [as cited in Westchester County, 2002]).

Developmental and Reproductive Effects

Human Evidence

No information was found on human developmental or reproductive effects.

Animal Evidence

Three studies evaluated by the WHO, conducted in pregnant rabbits and mice, did not show developmental nor teratogenic effects. Two studies exposed pregnant rabbits to oral doses up to 1000 mg/kg bw per day which did not result in developmental effects, though maternal body weight was decreased at the higher doses (Ladd et al., 1976 [as cited in WHO, 1990]; Rutter, 1974 [as cited in WHO, 1990]). Pregnant mice receiving oral doses up to 3000 mg/kg bw per day. No adverse effects on the dams nor offspring were noted (Nakamoto et al., 1973 [as cited in WHO, 1990]). Developmental effects were observed in another study in which rabbits were exposed to 300 mg/kg-day of sumithrin. Since food consumption and body weight were also decreased in the dams at 100 mg/kg-day, it was concluded that these developmental effects were only seen at maternally toxic doses (Westchester County, 2002). The USEPA considers

sumithrin to have a low potential for reproductive and developmental toxicity due to its rapid metabolism and excretion (USEPA, 2000 [as cited in Westchester County, 2002]).

In a three-generation study, rats were fed up to 2000 mg/kg in the diet. No effect on reproduction was observed (Takatsuka et al., 1980 [as cited in WHO, 1990]). In another study, rats were given up to 3000 mg/kg in the diet. At the highest dose level, a slight increase in relative liver weight was observed in treated animals and their offspring (Tesh et al., 1978 [as cited in WHO, 1990]).

Cancer

Human Evidence

Sumithrin was found to enhance the expression of the Wnt10B proto-oncogene in MCF-7 human breast carcinoma cells (Kasat et al., 2002). These genes increase the level of mitotic activity in a cell and lead to cell proliferation. The Wnt10B gene has been found to be expressed in some human breast adenocarcinomas. Sumithrin at micromolar concentrations increased the expression of this gene four to seven times the expression in controls.

Animal Evidence

There have been some long-term toxicity studies conducted with laboratory animals, including rats, mice and dogs. However, no oncogenicity has been noted in these studies. These studies are discussed in further detail under liver toxicity.

There have been several studies investigating the mutagenic potential of sumithrin. None of these studies indicated that sumithrin is mutagenic (Kishida and Suzuki, 1981a [as cited in WHO, 1990]; Kishida and Suzuki, 1981b [as cited in WHO, 1990]; Kishida and Suzuki, 1981c [as cited in WHO, 1990]; Suzuki et al., 1981a & b [as cited in WHO, 1990]; Kogiso et al., 1986 [as cited in WHO, 1990]; Suzuki and Miyamoto, 1981a [as cited in WHO, 1990]; and Foster et al., 1984 [as cited in WHO, 1990]).

Other Toxicity

Liver Toxicity

Liver toxicity has been noted in some of the long-term oral exposure studies. In one study in which rats were given sumithrin through the diet for six months, effects on the liver were noted.

The no observed effect levels were 55.4 mg/kg bw per day and 63.3 mg/kg bw per day for males and females respectively (Murakami et al., 1981 [as cited in WHO, 1990]). In a similar dietary study in which rats were exposed through the diet for 105 weeks for males and 118 weeks for females, some liver toxicity was noted at the highest dose (3,000 mg/kg in diet) tested. Decreased body weight gain in females was also noted at this dose level (Martin et al., 1987 [as cited in WHO, 1990]). Body weight gain was also decreased in another study in rats given sumithrin in the diet at a concentration of 6,000 mg/kg for 2 years (Hiromori et al., 1980 [as cited in WHO, 1990]).

Similar results have been observed in mice, with increased liver weight and decreased body weight gain the primary effects (WHO, 1990). In beagles, exposed through the diet for 26 weeks, liver enzymes levels were elevated at doses of 300 mg/kg for males and 1000 mg/kg for females (Pence et al., 1981 [as cited in WHO, 1990]). In a similar dietary study with a 52 week duration, relative liver weights were increased and alterations in the adrenal glands were noted at 3000 mg/kg. Effects on the blood, such as reduced red blood cell counts, decreased hemoglobin concentration and total blood protein were observed (Cox et al., 1987 [as cited in WHO, 1990]).

Table 15
Toxicity Study Summary Table- Sumithrin

Compound	Purity	Test Species (Strain)	Test Species Age/Lifecycle	Route	Dose Duration	Study Duration	Endpoint	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	LOAEL Statistical Significance	Comments	Reference
Sumithrin		dog		diet	1 year	1 year	hepatocellular enlargement, focal degeneration in adrenal cortex	7.1	26.8			USEPA, 2002
Sumithrin		rats		diet	13 weeks	13 weeks	increased liver wgt and decreased cholesterol	70	216			Westchester County, 2002
Sumithrin		rats		skin	0-1000 mg/kg day	3 week	no effects noted	1000	NA			Westchester County, 2002
Sumithrin		rats		inhalation	0-1.1 mg/L aerosol 6 hr/day, 5 days/wk	13 weeks	incr liver and thyroid wgt, change in thyroid, incr adrenal gland wgt, adrenal lesions	0.291mg/L	1.1 mg/L			Westchester County, 2002
Sumithrin	96%	rats	females, 20 days	gavage	0,100,300, 1000 daily for 3 days	4 days	incr uterine wgt incr liver wgt	1000 300	 1000	 P<0.01	no estrogenic effects noted, but effects on liver weight were	Yamada et al., 2003
Sumithrin	96%	rats	males, 5 weeks	gavage	0,100,300, 1000 daily for 10 days	11 days	wgt of male sex accessory tissues/organs incr liver wgt	1000 100	 300	 P<0.01	no androgenic effects noted, but effects on liver weight were	Yamada et al., 2003
Sumithrin		rats		diet	0,200,600, 2000 mg/kg diet	3 generation	reproduction	2000				Takatsuka et al., 1980; as cited in WHO, 1990
Sumithrin		Charles River CD rats		diet	0,300,1000 3000 mg/kg diet	2-generation	reproduction	1000	3000		Reproduction not affected, but some animals had increased liver weight	Tesh et al., 1978; as cited in WHO, 1990
Sumithrin		ICR mice		oral	0,30,300,3000 mg/kg-day	gestation days 7 to 12	embryotoxicity teratogenicity	3000			no effects noted	Nakamoto et al., 1973; as cited in WHO, 1990
Sumithrin		Sprague Dawley rats		inhalation	3760 mg/m3	4 hours	neurotoxicity	3760 mg/m3			no histopathological abnormalities on sciatic nerve	Kohda et al., 1977: as cited in WHO, 1990
Sumithrin		ICR mice		ip	2500, 5000 10,000 mg/kg once	48 hours	mutagenicity in bone marrow cells	10,000				Suzuki et al., 1981b; as cited in WHO, 1990
Sumithrin		Sprague Dawley rats		diet	0,1,3,10 g/kg diet	6 months	long term toxicity	55.4 males 63.3 females			serum albumin elevated at 3g/kg increased liver weight	Murakami et al., 1981; as cited in WHO, 1990
Sumithrin		Fisher-344 rats		diet	0,300,1000, 3000 mg/kg diet	105 weeks 115 wks females	oncogenicity	47 males 56 females			no oncogenicity, but decr body wgt gain, incr liver wgt	Martin et al., 1987; as cited in WHO, 1990
Sumithrin		Sprague Dawley rats	racemic phenothrin	diet	0,200,600,2000 6000 mg/kg diet	2 yr	oncogenicity	2000 mg/kg diet	6000 mg/kg diet		no oncogenicity, incr serum glutamine-pyruvate aminotransferase	Hiromori et al., 1980; as cited in WHO, 1990
Sumithrin		B6C3F1 mice		diet	0,300,1000, 3000 mg/kg diet	104 weeks	oncogenicity	40 males 164 females			body wgt decr, liver wgt incr, non-significant incr liver tumors	Amyes et al., 1987; as cited in WHO, 1990
Sumithrin		Beagle dogs		diet	0,100, 300,1000, mg/kg diet	26 weeks		300 mg/kg diet	1000 mg/kg diet		incr liver wgt, alkaline phosphatase activity	Pence et al., 1981; as cited in WHO, 1990
Sumithrin		Beagle dogs		diet	0,100, 300,1000, 3000 mg/kg diet	52 weeks		8.24 males 26.77 females			blood effects, incr liver wgt, histo path in liver and adrenal gland	Cox et al., 1987; as cited in WHO, 1990
Sumithrin		Sprague Dawley rats	racemic phenothrin	inhalation	0,43,220 mg/m3	4 hr/day 5 days a wk 4 weeks		220 mg/m3			no effects noted	Kohda et al., 1979b; as cited in WHO, 1990

Table 16

Noncancer Criteria (Oral/Dermal)- Sumithrin

Chemical of Potential Concern	Exposure Duration	Criteria	Value	Units	Species	Endpoint	NOAEL	LOAEL	Units	Combined Uncertainty/Modifying Factors	Study Source	Reference for Criteria
Sumithrin	acute-dermal	RfD	1.00E+01	mg/kg/day	rat	no effect	1.00E+03		mg/kg/day	100		Westchester County, 2002
Sumithrin	acute-ingestion	RfD	7.00E-01	mg/kg/day	rat	Inc liver wgt, dec chol	70	2.16E+02	mg/kg/day	100	Life Science Research, 1983	Westchester County, 2002
Sumithrin	subchronic-dermal	RfD	1.00E+01	mg/kg/day	rat	no effect	1.00E+03		mg/kg/day	100		Westchester County, 2002
Sumithrin	subchronic-ingestion	RfD	7.00E-01	mg/kg/day	rat	Inc liver wgt, dec chol	7.00E-01	2.16E+02	mg/kg/day	100	Life Science Research, 1983	Westchester County, 2002
Sumithrin	chronic-ingestion dermal*	OPP ADI	7.10E-02	mg/kg/day	dog	Liver effects	7.10E+00	2.68E+01	mg/kg/day	100		USEPA, 2002

* Westchester assumed 70% absorption for dermal to oral

NA: Not Applicable or Not Available.

NOAEL: no observed adverse effect level

LOAEL: lowest observed adverse effect level

RfD: USEPA reference dose

ADI: Acceptable Daily Intake

OPP: Office of Pesticide Programs, USEPA

Table 17
Noncancer Criteria (Inhalation)- Sumithrin

Chemical of Potential Concern	Exposure Duration	Criteria	Value	Units	Species	Endpoint	NOAEL/ LOAEL	Units	Combined Uncertainty/Modifying Factors	Study Source	Reference for Criteria
Sumithrin	acute-inhalation	RfC	2.91E-03	mg/L	rat	Incr liver and thyroid	0.291/1.1	mg/L	100	Huntington Corp	Westchester County
						wgt, adrenal and thyroid lesions				1989	2002
Sumithrin	subchronic-	RfC	2.91E-03	mg/L	rat	Incr liver and thyroid	0.291/1.1	mg/L	100	Huntington Corp	Westchester County
	inhalation					wgt, adrenal and thyroid lesions				1989	2002
Sumithrin	chronic-inhalation	RfC	2.91E-04	mg/L	rat	Incr liver and thyroid	0.291/1.1	mg/L	1000	Huntington Corp	Westchester County
						wgt, adrenal and thyroid lesions				1989	2002

NA: Not Applicable or Not Available.
RfC: USEPA Reference Concentration
NOAEL: no observed adverse effect level
LOAEL: lowest observed adverse effect level

Table 18
Cancer Criteria (Oral/Dermal)- Sumithrin

Chemical of Potential Concern	Margin of Exposure	Threshold Dose	Units	Weight of Evidence/ Cancer Guideline Description	Source	Reference
Sumithrin	10	0.071	mg/kgbw-day	NA	USEPA, 1998e	Westchester County, 2002

NA: not applicable or not available

Reference List

Sumithrin

Amyes, S.J., Martin, P.A., Ashby, R., Lee, P., Brown, P.M., Fowler, J.S.L., & Finn, J.P., 1987. Sumithrin: Oncogenicity and toxicity study report in mice. Life Science Research (Unpublished report submitted to WHO by Sumitomo Chemical Co., Ltd.). As cited in WHO, 1990.

Cox, RH, JD Sutherland, RW Voelker, RD Alsaker, KJ Vargas, SA Lewis, and WJ Hagen. 1987. *Chronic toxicity study in dogs with Sumithrin technical grade.* Hazleton Laboratories Inc. (Unpublished report submitted to WHO by Sumitomo Chemical Co., Ltd.). As cited in WHO, 1990.

Duprey, Z, DVM, MPH, Baumgartner, EA, MD, MPH, Barr, D, PhD, Kieszak, S, MA, Luber, G, PhD, MA, Backer, L, PhD, MPH, Belson, M, MD, Rubin, C, DVM, MPH. 2003. Large Scale Mosquito Control and Human Exposure to Pesticides, Virginia 2003. National Center for Environmental Health, Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry.

Foster, R, RS Tipins, V De Venezia, and A Nunziata. 1984. *Unscheduled DNA synthesis in human cells, cell line: Hela S3, test substance: Sumithrin.* Life Science Research (Unpublished report submitted to WHO by Sumitomo Chemical Co., Ltd.). As cited in WHO, 1990.

Go, V, J Garey, MS Wolff, and BGT Pog. 1999. *Estrogenic Potential of Certain Pyrethroid Compounds on the MCF-7 Human Breast Carcinoma Cell Line.* Environmental Health Perspectives, Volume 107 Number 3 March 1999.

Hashimoto, S, S Okano, H Yamada, and J Miyamoto. 1980. *Human volunteer study with phenothrin and d-phenothrin powder*. (Unpublished report submitted to WHO by Sumitomo Chemical Co., Ltd.). As cited in WHO, 1990.

Hiromori, T, Y Koyama, Y Okuno, M Arai, N Ito, and J Miyamoto. 1980. *Two-year chronic toxicity study of S-2539 in rat.* Industrial Bio-Test Laboratories Inc. (Unpublished report submitted to WHO by Sumitomo Chemical Co., Ltd.). As cited in WHO, 1990.

Hiromori, T, S Hara, T Kadota, and J Miyamoto. 1984. *Acute intravenous toxicity of phenothrin and d-phenothrin in mice.* (Unpublished report submitted to WHO by Sumitomo Chemical Co., Ltd). As cited in WHO, 1990.

Isobe, N, H Matsunaga, I Nakatsuka, and A Yositake. 1987. *Metabolism of [1R, trans] – and [1R, cis] – isomers of phenothrin in rats. (*Unpublished report submitted to WHO by Sumitomo Chemical Co., Ltd). As cited in WHO, 1990.

Kaneko, H, H Ohakawa, and J Miyamoto. 1981. *Absorption and metabolism of dermally applied phenothrin in rats.* J. Pestic. Sci, 6: 169-182. As cited in WHO, 1990.

Karpati, AM, Perrin, MC, Matte, T, Leighton, J, Schwartz, J, Barr, RG. 2004. Pesticide Spraying for West Nile Virus Control and Emergency Department Asthma Visits in New York City, 2000. *Environmental Health Perspectives*, Volume 112, No. 11, pages 1183-1187. August.

Kasat, K, V Go, BGT Pogo. 2002. *Effects of Pyrethroid Insecticides and Estrogen on WNT10B Proto-Oncogene Expression.* Environment International 28 (2002)429-432.

Kishida, F and H Suzuki. 1981a. *Studies on DNA-damaging capacity of Sumithrin with Bacillus subtilis.* (Unpublished Technical Report No. ET-10-0069 submitted to WHO by Sumitomo Chemical Co., Ltd.) As cited in WHO, 1990.

Kishida, F and H Suzuki. 1981b. *Gene mutation test of Sumithrin in bacterial system* (Unpublished Technical Report No. ET-10-0068 submitted to WHO by Sumitomo Chemical Co., Ltd.). As cited in WHO, 1990.

Kishida, F and H Suzuki. 1981c. *Mutagenicity test of Sumithrin in host-mediated assay.* (Unpublished Technical Report No. ET-10-0067 submitted to WHO by Sumitomo Chemical Co., Ltd.). As cited in WHO, 1990.

Kogiso, S, M Hara, K Ito, H Iwawaki, and A Yoshitake. 1986. *In vitro chromosomal aberration test of S-2539F in Chinese hamster ovary cells (CHO-K1).* Unpublished report submitted to WHO by Sumitomo Chemical Co., Ltd.). As cited in WHO, 1990.

Kohda, H, Y Okuno, T Kadota, and J Miyamoto. 1977. *Acute inhalation toxicity of d-phenothrin (S-2539 Forte) in rats.* Unpublished report submitted to WHO by Sumitomo Chemical Co., Ltd.). As cited in WHO, 1990.

Kohda, H, K Nishimoto, K Kadota, and J Miyamoto. 1979b. *Acute and subacute inhalation toxicity studies of S-2539 Forte in rats and mice.* (Unpublished report submitted to WHO by Sumitomo Chemical Co., Ltd.). As cited in WHO, 1990.

Ladd, R, PS Smith, DH Jenkins, GL Kennedy Jr, FK Kinoshita, and ML Keplinger. 1976. *Teratogenic study with S-2539 in albino rabbits.* Industrial Bio-Test Laboratories (Unpublished report submitted to WHO by Sumitomo Chemical Co., Ltd.). As cited in WHO, 1990.

Martin, PA, SJ Amyes, R Ashby, P Lee, PM Brown, JSL Fowler, and JP Finn. 1987. *Sumithrin: Combined toxicity and oncogenicity study in rats.* Life Science Research (Unpublished report submitted to WHO by Sumitomo Chemical Co., Ltd.). As cited in WHO, 1990.

Menzie, CM. 1978. Metabolism of Pesticides, Update II. US Department of the Interior, Fish and Wildlife Service, Special Scientific Report-Wildlife No. 212. Washington, DC: U.S. Government Printing Office, 1978. 238. As cited in National Library of Medicine, 2004.

Miyamoto, J. 1976. Degradation, metabolism and toxicity of synthetic pyrethroids. *Environmental Health Perspectives.* Vol 14, pages 15-28. As cited in Westchester County, 2002.

Murakami, M, T Hiromori, S Ito, and S Hosokawa. 1981. *Six month oral toxicity study of S2539 Forte (Sumithrin R) in rats.* (Unpublished report submitted to WHO by Sumitomo Chemical Co., Ltd.). As cited in WHO, 1990.

Nakamoto, N, T Kato, and J Miyamoto. 1973. *Teratogenicity study of S-2539 Forte in mice.* (Unpublished reported submitted to WHO by Sumitomo Chemical Co., Ltd.). As cited in WHO, 1990.

Okuno, Y, T Kadota, and J Miyamoto. 1978. *Neurotoxicity study of d-phenothrin (S-2539 Forte (R)) in rats by repeated oral administration.* (Unpublished report submitted to WHO by Sumitomo Chemical Co., Ltd.). As cited in WHO, 1990.

Pence, DH, WH Hagen, RD Wlsaker, TF Hastings, BG Dawkins, RL Tacey, and PM Marshall. 1981. *Subchronic toxicity study of S2539-F in dogs,* Vienna, Virginia, Hazleton Laboratories Inc. (Unpublished report submitted to WHO by Sumitomo Chemical Co., Ltd.). As cited in WHO, 1990.

Ruigt, GS, HC Neyt, JM Van der Zalm, and J Van den Bercken. 1987. *Increase of Sodium Current After Pyrethroid Insecticides in Mouse Neuroblastoma Cells.* Brain Research, 437 (1987) 309-322.

Rutter, HA. 1974. *Teratogenicity study in rabbits: S-2539 Forte.* Hazleton Laboratories Inc. (Unpublished report submitted to WHO by Sumitomo Chemical Co., Ltd.) As cited in WHO, 1990.

Saito K, Y Tomigahara, N Isobe, I Nakatsuka, H Kaneko. 2000. *Lack of significant estrogenic or antiestrogenic activity of pyrethroid insecticides in three in vitro assays based on classic estrogen receptor alpha-mediated mechanisms.* Toxicological Science;57(1):54-60. As cited in Westchester County, 2002.

Sumida, K, K Saito, N Ohe, N Isobe, H Kaneko, and I Nakatsuka. 2001. Evaluation of in vitro methods for detecting the effects of various chemicals on the human progesterone receptor, with a focus on pyrethroid insecticides. Toxicology Letters;118(3):147-55. As cited in Westchester County, 2002.

Suzuki, H and J Miyamoto. 1981a. *In vitro sister chromatid exchanges of Sumithrin* (Unpublished Technical Report No. ET-10-0071 submitted to WHO by Sumitomo Chemical Co., Ltd.). As cited in WHO, 1990.

Suzuki H, M Hara, and J Miyamoto. 1981b. *In vivo chromosomal aberration test of Sumithrin on bone marrow cells of mice.* (Unpublished Technical Report No. ET-10-0072 submitted to WHO by Sumitomo Chemical Co., Ltd.). As cited in WHO, 1990.

Takatsuka, M, Y Okuno, T Suzuki, T Kadota, M Yasuda, and J Miyamoto. 1980. *Three-generation reproduction study of S-2539 in rats.* Industrial Bio-Test Laboratories Inc. (Unpublished report submitted to WHO by Sumitomo Chemical Co., Ltd.). As cited in WHO, 1990.

Tesh, JM, CR Willoughby, and JSL Fowler. 1978. *Sumithrin: Effects upon reproductive performance of rats treated continuously throughout two successive generations.* Life Science Research (Unpublished report submitted to WHO by Sumitomo Chemical Co., Ltd.). As cited in WHO, 1990.

United States Environmental Protection Agency (USEPA). 2000. Tox Oneliners for Sumithrin (Phenothrin). Office of Pesticide Programs. Last updated May 26, 2000. As cited in Westchester County, 2002.

United States Environmental Protection Agency (USEPA). 2002. Office of Pesticide Programs Reference Dose Tracking Report. August 15, 2002.

Westchester County. 2002. *Comprehensive Mosquito-Borne Disease Surveillance and Control Plan*, Generic Environmental Impact Statement. February, 2002.

World Health Organization (WHO). 1990. *Environmental Health Criteria 96, d-Phenothrin.* International Programme on Chemical Safety. Geneva.

Yamada, T, S Ueda, K Yoshioka, S Kawamura, T Seki, Y Okuno, and N Mikami. 2003. *Lack of Estrogenic or (Anti-)Androgenic Effects of d-Phenothrin in the Uterotrophic and Hershberger Assays*. Science Direct Toxicology 186 (2003) 227-239.

4.3 Organophosphate Adulticides

Malathion is the only organophosphate that is included in the primary list of vector control agents.

4.3.1 *Malathion*

Background

Malathion is a dimethoxy organophosphate insecticide. Formulations of malathion have been classified as Class II pesticides. This classification is for pesticide formulations which have a moderate acute toxicity and their products carry a warning label: Impurities that are present in malathion commercial products (e.g., isomalathion) may be more toxic than malathion itself (Westchester County, 2002 and New York City, 2001). Therefore, when reviewing toxicity studies it is important to note the purity of the material administered.

Human Exposure

Malathion was one of six organophosphate pesticides detected in the diets of pre-school children from Washington State (Fenske, et. al., 2002). In this sample of 13 children, malathion was detected in processed food from the diets of four children only, at concentrations ranging from 4.3 to 21 ng/g. The concentrations of pesticides detected in this study may have been underestimated because there were analytical difficulties with recovery. However, concentrations detected were in general higher than reported in similar studies (Fenske, et. al., 2002).

Absorption/Distribution/Excretion

Malathion is readily absorbed via inhalation, ingestion and through dermal contact. Oral exposure has been found to produce rapid absorption as evidenced in human poisonings and in animal studies in which 89 percent of an administered dose is absorbed within one hour (Ahdaya et al., 1981 [as cited in ATSDR, 2003]). The half-time for absorption was estimated to be 34 minutes.

A recent study measured the absorption in laboratory rats following dermal exposure and found that approximately 6 percent of the applied dose is absorbed after one hour of exposure (Dary et al., 2001). Longer periods of exposure result in greater absorption; 10 percent after 1 day, 15

percent after 3 days and 30 percent after 7 days. Similar degree of absorption has been found in other animals, including dogs, pigs and human skin grafts (Dary et al., 2001). In another study in mice, approximately 98 percent of the applied dose was absorbed after 48 hours, with an estimated absorption half-life of 130 minutes (Shah et al., 1981 [as cited in ATSDR, 2003]). After treating human volunteers with malathion head lice preparations, only 0.2 to 3.2 percent of the dose was excreted in the urine after 96 hours (Dennis and Lee, 1999 [as cited in ATSDR, 2003]).

In a recent study using laboratory rats, oral exposure was compared to dermal exposure. Though the resulting toxicity was comparable, the oral route of exposure was found to be the more toxic route of exposure (Tos-Luty et al., 2003).

Once absorbed malathion may be transformed into malaoxon, which is the active intermediate compound with the greatest anti-AChE activity. Malathion and its metabolite, malaoxon, can also be detoxified by liver enzymes (carboxylesterases). This detoxification mechanism is much greater in mammals than it is in insects, which leads to greater toxicity in insects than mammals (ATSDR, 2003).

Malathion is fairly rapidly excreted, primarily in the urine. After oral exposure, approximately 90 percent is excreted in the urine within 24 hours (ATSDR, 2003). An elimination half-life has been estimated to be 6.2 hours (Vasilic et al., 1999 [as cited in ATSDR, 2003]). Less than 1 percent is recovered in tissues after 72 hours (WHO, 1997).

Exposure to malathion can be measured by determining the levels of some of its metabolites in urine or measuring the cholinesterase activity in the blood (ATSDR, 2003). Analysis of the urine is only beneficial in recent exposures. Cholinesterase activity in the blood may be depressed for several weeks, but is not specific for malathion since all organophosphates are cholinesterase inhibitors.

Toxicity

Some of the information described below has been obtained from population surveillance efforts that were put in place during ground and aerial applications of malathion to control the Mediterranean fruit fly (Medfly). It should be pointed out that though these studies offer relevant information about possible health impacts in humans exposed to malathion, it is not

directly comparable since the application method, product characteristics and dosage rates differ. The surveillance systems described in these studies is passive and relies upon self-reported cases (Shafey et al., 1999). It is also likely that some of these reports are biased due to anxiety over the spraying event. In addition, very often little data was available on background rates of the reported symptoms (e.g., incidence of effects when spraying does not occur) (Blondell and Spann, 1998). A brief description of some of these surveillance efforts in response to the Medfly applications is first provided since information from these programs is offered in many locations throughout this toxicity summary.

Florida

During the spring and summer of 1998, applications occurred in five counties in Florida. Approximately 2.4 ounces of Fyfanon was applied per acre per week during the treatment period (Shafey et al., 1999). Approximately 230 reports of illnesses were received from residents and physicians through telephone hotlines maintained by the Florida Poison Information Network and county health departments (Shafey et al., 1999). This was out of a population of 132,000 potentially exposed. Of these reported illnesses, 123 (54 percent) were considered to be probably or possibly related to pesticide exposure.

Los Angeles County, California

Los Angeles County had set up a hotline during malathion spraying for Medfly, which was advertised to physicians, the general public, emergency rooms and health clinics. Approximately 1,900 calls were received and conclusions were summarized in an USEPA Incident Data System (USEPA, 1990 [as cited in Westchester County, 2002]; Blondell and Spann, 1998).

Santa Clara County, California

Following aerial applications of malathion bait in 1981, a survey was completed to investigate the number of visits to the emergency room before and during spray events. This study also conducted personal interviews to assess self-reported symptoms before and after spraying (Kahn et al., 1992)

Neurological Effects

Human Evidence

As with other organophosphate pesticides, malathion is a neurotoxin which interferes with the normal transmission of nerve impulses. Malathion inhibits the activity of AChE, which is an enzyme that releases the neurotransmitter acetylcholine from the nerve cell once a signal has been received. This inhibition of AChE leads to an accumulation of acetylcholine at nerve endings, which initially causes excess stimulation of affected nerves. This progresses to an eventual ineffective transmission of nerve impulses if exposures are severe enough. Exposure to malathion will cause various symptoms because acetylcholine is a neurotransmitter in many tissues (e.g., muscles, glands, central nervous system). For example, accumulation of acetylcholine in nerves that activate muscles will cause muscle twitching and contraction. Excess glandular secretions will result from accumulation of acetylcholine in nerves that affect glands. Impacts to nerves in the central nervous system cause behavioral, sensory and cognitive symptoms (USEPA, 1998). Typical symptoms from acute poisoning by malathion include:

- tightness in the chest
- wheezing
- tearing of the eyes
- salivation
- nausea
- diarrhea
- dizziness
- pinpoint pupils
- blurred vision
- muscle weakness and
- difficulty in breathing

The severity of symptoms is dependent upon the dose, with the more serious symptoms, such as respiratory depression and coma, occurring only in acute poisonings to relatively high doses. Lethal doses have been estimated to be between 50 and 2000 mg ingested/kg bw (USEPA, 1998).

Isomalathion, an impurity in commercial malathion products, is also a cholinesterase inhibitor and appears to have a greater than additive effect when administered with malathion (WHO, 1997). Similarly, malaoxon, a metabolite of malathion, is also a more potent cholinesterase inhibitor (WHO, 1997). In cultures of rat neural cells, malaoxon was more than four times as potent at inhibition of cholinesterase (Segal and Federoff, 1989 [as cited in WHO, 1997]). Cholinesterase is rapidly reactivated after inhibition by malaoxon (Abraham and Edery, 1976 [as cited in WHO, 1997]).

Human cases of neurological effects have been reported following acute, short-term exposures to malathion (Westchester County, 2002). One episode involved 60 men who had become ill after eating food prepared by a community kitchen that had recently been treated with malathion. Reported symptoms included tremors and headache, which were temporary (Chaudhry, et al., 1998 [as cited in Westchester County, 2002]). Another study, which evaluated hospital admissions in Singapore, noted the most common sign of poisoning was excessive salivation and bronchial secretions, respiratory paralysis and impaired consciousness. A relapse of symptoms was sometimes noted and is believed to result from the conversion of malathion to malaoxon within 72 hours of exposure and redistribution of malathion from fat stores (Lee and Tai, 2001). Some patients experienced an intermediate syndrome (IMS), which occurred one to four days after poisoning. This syndrome is thought to be caused by persistent cholinesterase inhibition of the neuromuscular system (De Bleeker, 1995 [as cited in ATSDR, 2003]; De Bleeker et al., 1992 [as cited in ATSDR, 2003]). Symptoms of IMS included muscle weakness of the limbs and neck and palsies (paralysis) of the facial nerves and respiratory depression (Lee and Tai, 2001).

ATSDR (2003) has developed an intermediate duration Minimal Risk Level (MRL) for malathion of 0.02 mg/kg bw per day, based upon a study which exposed human volunteers to low doses of malathion. Volunteers had been exposed to 0.11 mg/kg bw per day for 32 days. This was then followed 3 weeks later by a 47 day exposure to 0.23 mg/kg bw per day dose

(Moeller and Rider, 1962 [as cited in ATSDR, 2003]). No effects were seen on cholinesterase activity.

Two occupational studies were reported in the Westchester GEIS. One study involved grain storage workers who were exposed to malathion as well as carbon disulfide. Neurological symptoms included loss of consciousness, rigidity of muscles, tremors, loss of sensation in limbs, impairment of motor function, and respiratory depression (Peters et al., 1982 [as cited in Westchester County, 2002]). Because exposure to carbon disulfide, which is also known to cause neurological toxicity, also occurred in this study, it is difficult to determine what role malathion had in the resulting symptoms. Long-term occupational exposure to crop dusters and harvesters was evaluated in another study. No neurological effects were observed (Krieger and Dinoff, 2000 [as cited in Westchester County, 2002]).

Three studies that reviewed reports of symptoms in areas treated during Medfly control programs had mixed conclusions. No increase in neurological problems was observed in Santa Clara (Kahn et al., 1992). Some symptoms were reported in this study more frequently before spraying and were attributed to anxiety over the pending spraying. Increased reports of dizziness, and headache were observed in Southern California (USEPA, 1990 [as cited in Westchester County, 2002]). Sixty percent of the cases reported during the Florida Medfly surveillance program involved neurological symptoms including headache, vertigo, difficulty in walking, numbness in extremities, disorientation and confusion (Shafey et al., 1999).

Another study looked at symptoms in a population exposed to 3 percent malathion during multiple aerial applications each year for three to five years. This study reported neurological impairment of the eyes, including retinal degeneration, myopia, inability to track an object and spasms (Ishikawa et al., 1993).

Animal Evidence

Cholinesterase inhibition has been noted in laboratory animal studies following single doses of 500-1000 mg/kg bw (Lamb, 1994a [as cited in ATSDR, 2003]). A chronic oral ingestion study found cholinesterase inhibition at 29 mg/kg bw per day for 24 months, but no effect at 2 mg/kg bw (Daly, 1996a [as cited in ATSDR, 2003]). This study was used by the ATSDR (2003) to develop a chronic oral MRL of 0.02 mg/kg bw per day.

Central nervous system effects have been noted in laboratory animal studies. Altered brain and skeletal electrical activity were observed in rats that had been treated with 38 or 75 mg/kg bw per day for 90 days (Desi et al., 1975 [as cited in Westchester County, 2002]). Changes in neurochemicals were observed in rats that were injected with 170 mg/kg bw per day (Cabello et al., 2001). Malathion was found not to cause delayed neurotoxicity in hens (susceptible species) or neuropathological changes in acute and subchronic studies in rats (USEPA, 1998).

Decreased cholinesterase activity was noted in laboratory rats exposed to malathion in air at concentrations beginning at 450 mg/m^3 for 6 hours a day, 5 days a week over a period of 13 weeks (Beattie, 1994 [as cited in ATSDR, 2003]).

Increased excitability has been noted in acute as well as subchronic exposure studies. Ehrich et al., (1993) found increased excitability 21 days after a single oral dose of 600 mg/kg bw of 88 percent pure malathion administered to rats. However, no such effects were observed in another study using 500-2000 mg/kg bw of 96.4 percent malathion (Lamb, 1994a [as cited in ATSDR, 2003]). A 90-day feeding study also did not find behavioral effects, nor effects on motor activity at doses of 4 and 1,575 mg/kg bw per day of malathion (96.4 percent pure) (Lamb, 1994b [as cited in ATSDR, 2003]). However, in another 90-day study a dose of 75 mg/kg bw per day (95 percent pure) did increase excitability whereas 38 mg/kg bw did not show such effects (Desi, et al., 1976 [as cited in ATSDR, 2003]).

Skin and Eye Irritation

Human Evidence

Dermal exposure to malathion has the ability to produce mild reactions. Sweating and twitching in the area exposed may occur. In addition, exposure to malathion may produce a skin rash, which was reported to be a non-specific sensitization reaction occurring in individuals previously exposed to either malathion or other pesticides (Sharma and Kaur, 1990 [as cited in Westchester County, 2002]).

Eye irritation in humans has been reported after repeated one-hour exposures to concentrations of 85 mg/m^3 (Golz, 1959 [as cited in ATSDR, 2003]). Skin and eye irritation has also been reported in pesticide applicators in Pakistan. These effects were believed to be due to poor work practices which lead to excessive skin contact and contamination with the degradation product,

isomalathion. Constriction of pupils, aching in and behind the eyes, blurred vision and tearing were reported (Baker et al., 1978 [as cited in Westchester County, 2002]). Visual impairment in Japanese children living in agricultural areas where malathion is used extensively has also been reported (Ishikawa et al., 1993).

Studies in California during the Medfly spraying programs have produced mixed results. Based on calls received by a hotline set up in Los Angeles County, it was suggested that some individuals may have experienced allergic or irritative symptoms such as headache, eye irritation and skin rash, which may have been caused by malathion itself or odors associated with the pesticide (Blondell and Spann, 1998). Some of these surveillance programs following Medfly spraying have shown an increase in the number of visits to the emergency room, while others have not observed such an increase (Kahn et al., 1992; USEPA, 1990 [as cited in Westchester County, 2002]). Of the 123 relevant self-reported cases in Florida, 28 (23 percent) involved irritation of the skin (redness, burning and/or blistering) and 23 (19 percent) involved irritation of the eyes (e.g., tearing, conjunctivitis, irritation of the eyelids and blurred vision) (Shafey et al., 1999).

Animal Evidence

The ATSDR Toxicological Profile for Malathion describes an experiment in which laboratory mice were submerged in an eight percent solution of malathion. Mild dermatitis and conjunctivitis was observed (Relford et al., 1989 [as cited in ATSDR, 2003]). Skin growths were observed in guinea pigs treated with repeated applications of 200 mg/kg per day of malathion over a 30-day study period (Dikshith et al., 1987 [as cited in ATSDR, 2003]). In another experiment in which 1000 mg/kg /day was applied to the skin of rabbits in an intermediate-duration study, no observable effects were noted (Moreno, 1989 [as cited in ATSDR, 2003]).

Malathion applied directly to the eyes of laboratory rats at a dose of 100 mg per eye for five days a week for four weeks produced only mild irritation, though animals were not examined immediately following the application and some recovery may have occurred (Boyes et al., 1999 [as cited in ATSDR, 2003]).

Gastrointestinal Effects

Human Evidence

Exposure to organophosphates is known to produce GI symptoms such as loss of appetite, nausea, vomiting, abdominal pain, cramps and diarrhea. Such symptoms may result following oral ingestion or inhalation and occur relatively quickly following exposure. Examination of hospital emergency room visits in California during aerial spraying for the control of Medflies have found some reports of increased emergency room visits for GI problems (USEPA, 1990 [as cited in Westchester County, 2002]; Kahn et al., 1992; Thomas et al., 1992 [as cited in ATSDR, 2003]). Of the cases reported in the Florida Medfly program which were deemed probable or possibly related to the pesticide spraying, approximately 63 percent were related to GI symptoms (e.g., nausea, vomiting, diarrhea, and cramps) (Shafey et al., 1999).

Respiratory Effects

Human Evidence

Severe respiratory effects, such as pulmonary edema, lung congestion and emphysema, may result from acute poisonings following exposure to organophosphates in general. Based upon human poisoning episodes, the ATSDR estimates that respiratory effects can be expected from oral exposures ranging from 214 to 1071 mg/kg bw (ATSDR, 2003). In studies using male volunteers, exposure to 85 mg/m^3 resulted in mild nasal irritation (Golz, 1959 [as cited in ATSDR, 2003]).

Some studies have observed an increase in the number of self-reported cases of shortness of breath and upper respiratory irritation especially, in children, following the Medfly control program in California and Florida (USEPA, 1990 [as cited in Westchester County, 2002]; Shafey et al., 1999; Blondell and Spann, 1998). Of the cases reported during the Florida Medfly program that were classified as probably or possibly related to pesticides, respiratory symptoms were the most common complaint received. Difficulty in breathing, coughing, wheezing and upper respiratory pain and irritation were among the symptoms recorded (Shafey et al., 1999). In another study an increased response in these symptoms, as well as asthma, was not observed (Kahn et al., 1992). However, according to an USEPA review, this study "…was based on indirect assessments and surveys that were relatively insensitive to rare effects that might occur

in especially susceptible individuals" (Blondell and Spann, 1998). Of the 539 cases submitted to the California Pesticide Illness Surveillance Program during 1982 through 1995, four were reports of asthma due to malathion exposure (Blondell and Spann, 1998). One individual reported two asthma attacks during Medfly applications, one of which required medical attention at a hospital.

In a study that looked at pesticide exposures and wheeze among a cohort of farmers in North Carolina and Iowa, malathion was found to be associated with wheeze in a dose-dependent manner (Hoppin et al., 2002).

Animal Evidence

Excessive cell growth was noted in the upper respiratory system following exposure of laboratory rats to an aerosol containing 100 mg/m^3 of malathion for six hours a day, five days a week over a period of thirteen weeks (Beattie, 1994 [as cited in ATSDR, 2003]). This study was used by the ATSDR in developing a MRL of 0.02 mg/m^3 for intermediate exposures (ATSDR, 2003). Oral exposure to doses of 411 mg/kg bw per day in a seven day dietary study found severe respiratory distress (Ojha et al., 1992 [as cited in ATSDR, 2003]). A single dose of 1,950 mg/kg bw administered to rats resulted in hemorrhage and congestion of blood in the lungs (Piramanayagam et al., 1996 [as cited in ATDSR, 2003]). Other longer term studies in rats using doses of approximately 300 to 600 mg/kg bw malathion did not find effects to the respiratory system (NCI, 1978 [as cited in ATSDR, 2003]; NCI, 1979a). Nasal lesions were observed in mice exposed orally to 167 mg/kg bw (males) and 1,500 mg/kg bw (females) for 18 months (Slauter, 1994 [as cited in ATSDR, 2003]).

Rabbits exposed to a malathion aerosol at concentrations of 123 mg/m3 for six hours experienced cholinesterase inhibition (Weeks et al., 1977). In a 90-day rat inhalation study, nasal lesions were also observed at concentrations of 100 ug/l of air (100 mg/m3) (Beattie, 1994 [as cited in ATSDR, 2003]). These studies were used by ATSDR to develop MRLs.

Immunologic Response

Human Evidence

No increase in emergency room visits for allergic reactions was detected in Santa Clara, California following aerial applications for Medfly control (Kahn et al., 1992). There was one case of an allergic skin reaction following spraying in Southern California (Schanker et al., 1992 [as cited in Westchester County, 2002]). Since this was out of approximately 300 people reporting skin-related problems, it appears that allergic skin reactions are not common. In controlled studies using human volunteers, exposure to 10 percent malathion caused contact sensitization in approximately half of the individuals. In addition, exposure to 0.1 and 0.01 percent solutions to previously sensitized individuals also evoked a response. There is also some evidence that skin sensitization occurs in mosquito control field workers (Milby et al., 1964 [as cited in ATSDR, 2003]).

Animal Evidence

Various effects on immune response have been observed from laboratory animal studies, including a suppressed response of a particular immunoglobulin (IgM) (Casale et al., 1983 [as cited in ATSDR, 2003]), increased macrophage response (Rodgers and Ellefson, 1990 [as cited in ATSDR, 2003]), increased proliferation of T-cells to non-specific stimulation and increased the response of splenocytes to an antigen (Rodgers et al., 1986 [as cited in ATSDR, 2003]). These effects were noted in mice with single oral doses of approximately 700 mg/kg bw. In a recent study, effects on the immune system were investigated using mice that were given malathion orally for 28 days. Spleen cells were then removed and challenged with an antigen (sheep red blood cells). The immune response of the treated cells differed from controls. At the lowest dose (0.018 mg/kg bw) an increased antibody response was observed (Johnson et al., 2002). However, this response was less evident as the dose increased, leading the authors to conclude that at higher doses malathion inhibited the immune system.

Effects on circulating antibody levels and cell-mediated immune response have been found at doses on the order of 10 to 20 mg/kg bw for intermediate exposure durations (8 to 22 weeks) using rats or mice (Banerjee et al., 1998 [as cited in ATSDR, 2003]).

Administration of malathion to the skin of laboratory rats and mice resulted in an increase in the levels of histamine circulating in the blood). This effect was seen following dermal as well as oral exposures. The authors suggest that this may imply that eye irritation and irritation of mucous membranes may actually be a systemic response resulting from increased histamine levels and not necessarily an effect of direct exposure (Rodgers and Xiong, 1997 [as cited in ATSDR, 2003]).

An earlier study conducted by Relford et al. (1989 [as cited in ATSDR, 2003]) did not find consistent immune suppression in mice that were dipped in a 2 and 8 percent solution of malathion. In another study, malathion at concentrations ranging from 17.8 to 177.6 mg/l was applied to the shaved abdomen of mice. Hypersensitivity to malathion was not noted in animals that had previously been exposed (Cushman and Street, 1983 [as cited in ATSDR, 2003]).

Endocrine Disruption

Human Evidence

There have been some studies that have demonstrated an ability of organophosphate pesticides to produce some effects on the endocrine system. Organophosphates have been shown to reduce pituitary function, either directly or secondarily to inhibition of nerve function to the pituitary (Guven et al., 1999 [as cited in Westchester County, 2002]). This can impact body growth, metabolism, sexual maturation and milk production in women. These effects are generally limited to short term exposure to high levels. A study of pesticide workers suggests that there is a correlation between exposure to organophosphates and decreased testosterone levels (Padungtod, 1998 [as cited in Westchester County, 2002]). However, there were no studies which evaluated endocrine disruption by malathion specifically (Westchester County, 2002).

In an *in vitro* assay malathion was not found to have either estrogenic or androgenic activity using human estrogen and androgen receptors (Kojima et al., 2004).

Animal Evidence

Laboratory animal studies have shown effects of malathion exposure on luteinizing hormone, progesterone and thyroid hormones (Westchester County, 2002). Luteinizing hormone is a reproductive hormone, which in males induces the production of testosterone and in females

regulates the menstrual cycle. Progesterone plays a similar role in females. Therefore, it is possible that interference with these hormones may lead to decreased reproductive capacity. Circulating thyroid hormones (T3 and T4) were decreased and thyroid stimulating hormone levels increased when malathion was given to rats (0.06 mg per rat for 21 days) (Akhtar et al., 1996 [as cited in Westchester County, 2002]). These thyroid hormones play a large role in regulating many functions in the body, including metabolism and growth.

Chronic exposure studies conducted by the NCI have not shown adverse effects to the adrenal glands, thyroid, or parathyroid glands at doses ranging from 359 to 622 mg/kg bw in rats or approximately 3000 mg/kg bw in mice (NCI, 1978 [as cited in ATSDR, 2003]; NCI, 1979a). In another study, however, increased thyroid and parathyroid gland weights were observed after administration of 415 mg/kg bw malathion for two years to rats (Daly, 1996a [as cited in ATSDR, 2003]).

Developmental and Reproductive Effects

Human Evidence

Two studies have evaluated reproductive outcomes in the San Francisco Bay area, which had been treated as part of the Medfly control program. One study evaluated over 22,000 discharge records between July 1981 through August 1982 and found that living in an area that was treated was not associated with an increase in low birth weight or birth defects (Grether et al., 1987 [as cited in Westchester County, 2002]). In another study in the San Francisco Bay area, over 7000 pregnancies were evaluated. Healthy births were compared to stillbirths and births of children with physical deformities. No significant association was found between living in a treated area and the occurrence of a negative birth outcome (Thomas et al., 1992 [as cited in Westchester County, 2002]). However, this same study was cited by the ATSDR (2003) as finding a moderate association between stillbirths and exposure accumulated up to one month prior to fetal death. This study was also reported to find a statistically significant association between GI anomalies in offspring and exposure to malathion during the second trimester (Thomas et al., 1992 [as cited in ATSDR, 2003]).

Pre-natal exposure to malathion and other organophosphates was evaluated in a group of low-income women in an agricultural area in California. Metabolites were analyzed in maternal

urine samples and cholinesterase was measured in maternal and cord blood. The only effects observed to be related to organophosphate exposure were increased body length and head circumference and a decreased duration of pregnancy (Eskenazi et al., 2004). However, no statistical differences were found to be associated with concentrations of a urinary metabolite of malathion specifically.

Malathion levels were measured in breast milk in a study of nursing mothers in Bhopal India (Sanghi et al., 2003). Breast milk samples were obtained from 12 women and eight samples were found to contain malathion at an average concentration of 43 parts per billion (ppb). The authors estimated that, based on the assumption that infants consumed 500 ml per day, the acceptable daily intake (ADI) set by the WHO would be exceeded by 40 percent. There was a correlation of increasing malathion concentrations with increasing maternal age (Sanghi et al., 2003).

In Manila, Philippines, exposure to various environmental chemicals was evaluated via analysis of meconium (the first stool of newborns). The meconium from 426 infants was analyzed for heavy metals (mercury, lead, cadmium and arsenic). Pesticides (chlordane, chlorpyrifos, diazinon, DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane], lindane, pentachlorophenol, malathion and parathion) were analyzed in 200 infants . Malathion was detected in 53 percent of the samples at an average concentration (in the samples with detections) of 71.69 ppm. In addition, significant intercorrelation was found between malathion and parathion, chlordane, DDT, chlorpyrifos and cadmium. The applicability of these results for the United States may be limited since Manila was chosen by the authors because of its "…high environmental pollution index." (Ostrea et al., 2002). However, it does demonstrate that exposure can occur in utero.

Animal Evidence

High doses of exposure have produced some reproductive toxicity. Many studies have investigated the potential for malathion to affect the male reproductive system. In an acute study in which 240 mg/kg bw was injected into male mice, malathion was found to impair testicular function. Decreased testosterone levels and sperm counts were observed, as well as depletion of the seminiferous epithelium. The shape of the sperm was also altered, indicating a potential for teratogenic effects (Bustos-Obregn, 2003). Decreased sperm counts and impaired ability to

produce healthy sperm were observed in a study in which a dose of 23 mg/kg bw was injected into male rats (Prakash and Verikatesh, 1996 [as cited in Westchester County, 2002]; Akbarsha et al., 2000 [as cited in Westchester County, 2002]). Oral doses ranging from 20-40 mg/kg bw produced effects such as reduced number of Sertoli and Leydig cells, reduced spermatogonia and increased serum follicle stimulating hormone in rats. Some of these effects appear to be at least partially reversible (Krause et al., 1976 [as cited in ATSDR, 2003]; Krause, 1977 [as cited in ATSDR, 2003]; Ojha et al., 1992 [as cited in ATSDR, 2003]).

Effects on female reproductive organs have been noted in laboratory rats given163 mg/kg bw per day for seven days in the diet (Ojha et al., 1992 [as cited in ATSDR, 2003]). A dose of 10 mg/kg bw per day administered orally in rats for 15 weeks did not produce alterations in the ovaries (Ozman and Akay, 1993 [as cited in ATSDR, 2003]). A chronic study conducted in rats that were given 332 mg/kg bw per day in the diet for 103 weeks did not show any effects in mammary glands, uteri or ovaries (NCI, 1978 [as cited in ATSDR, 2003]). Cystic endometrial hyperplasia was observed in mice that received a dose of 1,490 mg/kg bw per day for 80 weeks (NCI, 1978 [as cited in ATSDR, 2003]). Reduced conception rates were observed in cattle exposed to malathion as a result of decreased levels of progesterone (Prakash et al., 1992 [as cited in Westchester County, 2002]).

Studies have been conducted in laboratory rats and rabbits to investigate the potential for adverse effects in the offspring of animals exposed while pregnant. Increased rate of abortions, decreased number of fetal implants and reduced fetal weight have been noted in rats given 500 to 827 mg/kg bw per day. However, this may have been confounded by maternal toxicity, which was also noted in these studies (Mathews and Devi, 1994 [as cited in ATSDR, 2003]; Prabhakaran et al., 1993 [as cited in ATSDR, 2003]). Treatment of female rats with 50 mg/kg bw for three months before breeding did not affect reproductive capacity. Similarly, administration of 612 and 703 mg/kg bw in a two generation reproductive study did not affect reproductive capacity, though a slight decrease in body weight gain in offspring was noted (Lechner and Abdel-Rahman, 1984 [as cited in ATSDR, 2003]; Schroeder, 1990 [as cited in ATSDR, 2003]). The NOAEL for reduced fetal weight gain was 131 and 153 mg/kg bw for males and females, respectively. Embryo toxicity was not observed in offspring of female rats treated with 800 mg/kg bw, nor in rabbits given oral doses of 100 mg/kg bw per day during

gestation (Lochry, 1989, Machin and McBride, 1989a [as cited in ATSDR, 2003]). However, in another study, a slight increase in resorption sites was noted in pregnant rabbits exposed to 50 mg/kg/day for gestation day six to 18 (USEPA, 2000e). This same study was evaluated by ATSDR and determined to not indicate developmental toxicity because some maternal toxicity was noted (Siglin, 1985 [as cited in ATSDR, 2003]). Decreases in fetal cholinesterase activity were noted in pregnant rabbits administered 126 mg/kg/day (Machin and McBride, 1989b [as cited in ATSDR, 2003]).

Cancer

The ability of malathion to produce cancer has been reviewed by the WHO's IARC, as well as the USEPA. IARC considers malathion to be not classifiable as to its carcinogenicity in humans, because the evidence is inadequate to assess human carcinogenicity (IARC, 1983). Prior to 1997 the USEPA had considered malathion to be a likely carcinogen (Westchester County, 2002). However, in 1997 the USEPA began a re-evaluation of the carcinogenicity of malathion (USEPA, 2000c). After Science Advisory Panel review, the weight of evidence was downgraded (SAP, 2000). Malathion is now considered to have suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential (USEPA, 2002b). The studies that were the basis of this weight of evidence evaluation are included in the discussion below.

At the time of the Westchester and New York City Environmental Impact Statements, the USEPA had not yet finalized its evaluation of malathion's carcinogenicity. For the purposes of those risk assessments, malathion was evaluated as a carcinogen utilizing the cancer slope factor previously calculated by USEPA (1.52×10^{-3} $(mg/kg\text{-}d)^{-1}$) (Westchester County, 2002; New York City, 2001).

ATSDR considers the evidence of carcinogenicity from human studies to provide insufficient evidence). Studies which do provide some evidence of carcinogenicity are limited in that the exposure estimates are unreliable and confounded by co-exposure to more than one pesticide. In addition, the excess cancer that has been noted in some of these studies of human populations has only been a small increase (ATSDR, 2003).

Human Evidence

A Canadian epidemiological study evaluated Non-Hodgkin's Lymphoma (NHL) and exposure to various pesticides. This case control study included a total of 517 cases of NHL diagnosed between 1991 and 1994. Malathion was among the pesticides evaluated. A statistically significant association between exposure to organophosphates in general and NHL was observed. More specifically, malathion had an adjusted odds ratio of 1.83 which represented a statistically significant association (McDuffie et al., 2001). Similarly, NHL was found to be significantly associated with malathion exposure in a group of farmers in Iowa and Minnesota (odds ratio of 2.9) (Cantor et al., 1992 [as cited in ATSDR, 2003]). A statistically non-significant increase in NHL was noted in a population-based case-control study in women in Nebraska (Zahm et al., 1993 [as cited in ATSDR, 2003]). In another population-based case-control study, the risk of leukemia was not significantly elevated in farmers who applied, mixed or handled malathion. However, a slightly elevated risk of leukemia was found when malathion was used as an animal insecticide (Brown et al., 1990 [as cited in ATSDR, 2003]). The risk of multiple myeloma was found to be slightly elevated (non-significantly) in Iowa farmers exposed to malathion (odds ration 1.9) (Brown et al., 1993 [as cited in ATSDR, 2003]).

An increase in childhood cancer was observed in a recent study which evaluated children of pesticide applicators in Iowa (Flower et al., 2004). Several specific cancer types were increased including lymphomas and brain cancer. When the use of malathion by either parent was evaluated, no significant increase in childhood cancer was observed.

An evaluation of farm workers exposed to malathion did not detect any genetic damage to lymphocytes (van Bao et al., 1974 [as cited in ATSDR, 2003]).

Animal Evidence

Animal studies have been conducted to evaluate the mutagenic potential of malathion. Westchester County (2002) suggested that these studies do not indicate that malathion is mutagenic. This is consistent with a review by the USEPA. Cancer Assessment Review Committee (CARC), which concluded that the weight of evidence does not support a mutagenic hazard from malathion (USEPA, 2000c). ATSDR (2003) concluded that there is evidence that technical grade malathion is a weak genotoxic agent. WHO (1997) concluded that most of the

evidence indicates that malathion is not genotoxic, Though there is some evidence that malathion can produce chromosomal aberrations *in vitro*, but not *in vivo.*

Only one recent study on mutagenicity was found in the literature. In this study, malathion was injected (intraperitoneal) as well as administered by gavage and found to produce chromosomal aberrations, sister chromatid exchange and abnormal sperm cells in acute and sub-acute treatment of mice. The lowest dose tested, 2.5 mg/kg bw, produced a significant increase in these three endpoints (Giri et al., 2002).

Malaoxon, a metabolite of malathion, did not produce mutation in bacteria but did cause sister chromatid exchange in mammalian cells (WHO, 1997). Malaoxon did cause mutations in mammalian cells in an *in vitro* study (USEPA, 2000b).

In a cancer bioassay conducted by Slauter (1994), malathion was administered in the diet to mice at doses of 17.4 to 3,448 mg/kg bw per day. Liver adenomas and carcinomas were observed, however, statistical significance and a dose-response trend was only apparent at the two highest doses. These doses were determined to be excessive, based on cholinesterase inhibition. Therefore, the significance of these results in questionable. however, the USEPA CARC concluded that this study provided evidence of carcinogenicity (USEPA, 2000c).

In another study evaluated by the USEPA and ATSDR, malathion was administered to rats in their diet at doses between 2-868 mg/kg bw per day for two years. In females, liver tumors were observed to be statistically increased at the highest dose tested (Daly, 1996a [as cited in USEPA, 2000c and ATSDR, 2003]). The concurrent toxicity observed at this dose limits its usefulness as evidence of carcinogenicity (USEPA, 2000c). No liver tumors were observed in males, though excessive toxicity observed at the two highest doses may have precluded the development of tumors (USEPA, 2000c). Nasal and oral tumors were also observed, however, their occurrence was infrequent. It could not be determined whether these nasal tumors were treatment related. However, because these tumors were observed at doses without other forms of toxicity, there were no similar tumors in controls and the spontaneous occurrence of these tumors is relatively infrequent, their occurrence is potentially significant (Westchester County, 2002). Thyroid tumors were observed but determined to not be treatment related (USEPA, 2000c).

A similar study in rats was conducted with malaoxon, using doses of 1-141 mg/kg bw per day for 103 weeks (Daly, 1996b [as cited in USEPA, 2000c and ATSDR, 2003]). Mononuclear cell leukemia was observed in males at the highest dose tested. However, this dose had significant mortality and was considered excessive (USEPA, 2000c). In addition, the incidence of mononuclear cell leukemia in historical controls is comparable to the incidence in this treatment group. These results are consistent with a NCI study which did not provide evidence of carcinogenicity (NCI, 1979b). The NCI study was re-evaluated in 1985. This re-evaluation confirmed earlier conclusions, except that this re-evaluation found equivocal evidence of C-cell neoplasms of the thyroid (Huff et al., 1985 [as cited in WHO, 1997]).

The NCI has conducted two 80-week dietary studies, one in rats and the other in mice. Rats were treated with 359 and 622 mg/kg bw per day. Proliferative lesions were found in the thyroid, though not statistically dose-related (NCI, 1978 [as cited in ATSDR, 2003]). In the mouse study, daily doses of either 1490 or 2980 mg/kg bw were administered. Hepatocellular tumors were increased but were not statistically significant (NCI, 1978 [as cited in ATSDR, 2003]).

In a recent study conducted by Cabello et al. (2001), young rats were exposed to malathion (Fyfanon^{TM}) at a dose of 17mg/100 g bw via subcutaneous injection twice a day for five days. Treatment with malathion lead to an increase in the density of terminal end buds in the mammary gland. These terminal end buds are highly proliferative tissues and are susceptible to transformation to tumors (Cabello et al., 2001). As the mammary gland develops, the terminal end buds differentiate into alveolar buds. As treated animals were followed, an increased incidence of mammary tumors was noted. Twenty-eight months after treatment, 17 of the 70 animals (24 percent) treated with malathion developed mammary tumors, whereas there were no tumors observed in the control animals. This study also treated animals with parathion, a related organophosphate, and similar results were observed. The authors of this study suggest that the development of mammary tumors was a result of cholinergic stimulation, which lead to proliferation of mammary tissues (Cabello et al., 2001).

In an *in vitro* study using rat epithelial cell cultures, investigators found that treatment with malathion inhibited gap junction intercellular communication (GJIC). This inhibition was 95 percent reversible 250 minutes after treatment with malathion. The authors concluded that this

demonstrated malathion's epigenetic toxicity (i.e., it may cause tumors via disruption of communication between cells, leading to uncontrolled growth) (Masten et al., 2001).

Other Toxicity

Hematological Effects

Hemoglobin levels were measured in workers employed in the manufacturing of malathion. Sixty individuals were evaluated and hemoglobin levels were found to decrease with length of employment (Reeves et al., 1981 [as cited in ATSDR, 2003]).

Lysyl Hydroxylase

Lysyl hydroxylase is an enzyme that is involved in building collagen, a structural protein found in all tissues. According to Samimi et al. (2001b) reports in the literature describe abnormal feathering and beak deformities in birds and gill abnormalities in bluegills exposed to malathion and malaoxon (Samimi et al., 2001b). These authors conducted *in vitro* studies using fetal rat fibroblasts (Samimi et al., 2001b) as well as an enzyme lysyl hydroxylase assay (Samimi et al., 2001a) and showed that malathion and malaoxon directly inhibited this enzyme. Malaoxon was more potent at this inhibition.

Oxidative Stress

The ability of malathion to induce oxidative stress in laboratory rats has been investigated. A dose of 20 ppm in food was given to rats for four weeks (Ahmed et al., 2000). An increase in lipid peroxidation and antioxidant enzyme (catalase, superoxide dismutase) activity was noted, indicative of oxidative stress leading to the formation of free radicals. In addition, glutathione levels were decreased, indicating utilization of this protective detoxification pathway.

Table 19
Toxicity Study Summary Table- Malathion

Compound	Purity	Test Species (Strain)	Test Species Age/Lifecyle	Route	Dose Duration	Study Duration	Endpoint	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	LOAEL Statistical Significance	Comments	Reference
Malathion	technical grade	Swiss Albino Mice	10-12 wk	i.p. i.p. gavage i.p.	5 days; 2 mg/kg 24 hr; 2.5, 5, 10 mg/kg 24 hr; 2.5, 5 mg/kg 5 days; 2.5, 5, 10 mg/kg	6 days 1 day 1 day 35 days	chromosome aberration & sister chromatid exchange " " sperm head abnormality		2.5 2.5 2.5	P<0.001 P<0.001 P<0.02	Authors conclude tech. Grade malathion is a mutagen & can cause germ cell mutations. May be related to malaoxon or isomalathion.	Giri, et al., 2002
Malathion	99.80%	WB-F344 rat epithelial cell line			 0.5mM 50mM	30 min incub 0 to 100 min	gap junction intercellular comminication (GJIC)				dose-response eval time-dependent eval dose-response ozonation	Masten et al., 2001
Malathion	99%	Wistar Rats	200-230 g	dermal oral	8, 16 mg 4hr/day 11.2 mg/day	28 days 28 days	degeneration of hepatocytes, damage to intracelluar struct. in liver, lung, kidney and heart degeneration of hepatocytes, damage to intracelluar struct. in liver, lung, kidney and heart	8 mg	16 mg 8 mg 11.2 mg		 oral more toxic than dermal, histo changes similar	Tos-Luty et al., 2003
Malathion	tech grade 94.60%	male albino rats 215 g avg	adult	dermal	1-50 % sol	30 min to 1 hr	measured absorption				6% absorbed dose after .5-1 hr greatest absorption from aqueous solution	Dary et al., 2001
Malathion	commercial 95.20%	male CF1 mice	10-12 wks	i.p.	250 mg/kg bw once	1-40 days	spermatogenesis		250 mg/kg bw		depletion of seminiferous epi-thelium, decrease plasma test	Bustos-Obreg et al., 2003
Malathion	commercial prod (500 EC)	SJL/J mice female	5-6 wks	gavage	0.018-180 mg/kg; altern days for 28 days	30 days	Immune response. Incr IgM antibody response		0.018 mg/kg bw	P<0.05	stimulat was higher at the lowest dose; higher conc may inhibit immune response	Johnson et al., 2002
Malathion	Technical grade 96%	Male albino Wistar rats	200-250 g	oral in food	200 ppm 4 weeks	4 weeks	enhanced lipid perox. Incr anti-oxid enzyme activity, red GSH, enhanced GPx and GR activity		200 ppm 200 ppm 200 ppm	P<0.001 P<0.001 P<0.001	Each endpoint is indicative of oxidative stress caused by malathion	Ahmed, et al., 2000
Malathion	Technical grade 96.40%	B6C3F1 mice male, female		diet	0, 100, 800, 8000, 16000 ppm or 17,140,1500,3000 m 21,170,1700,3500 f	18 mnths	oncogenicity dec. plasma cholinesterase	800 ppm or 140 mg/kg	8000 ppm 100, 8000 800	p=0.01	sign incid of liver adenomas & carcinomas @ 8000 & 16000 ppm. Determined to be excess doses based on dec. cholinester. signif incr hepatocellular carcinoma no dose trend	Slauter, RW, 1994 (as cited in USEPA, 2000c and ATSDR, 2003)
Malathion	Technical grade 97.00%	Fischer 344 rats		diet	0, 50, 500 6000, 12000	24 mnths	oncogenicity	 500 ppm or 29 mg/kg bw	6000 ppm	p<0.05	incr female liver tumors, dose excessive nasal tumors could not be determ whether treatment related or not. Thyroid tumors not treatment related. dec survival, brain AChE, bw gain	Daly, 1996a (as cited in USEPA, 2000c and ATSDR, 2003)
Malathion	95%	Fischer 344 rats		diet	0,2000, 4000 or 100, 200 mg/kg	103 weeks	oncogenicity				not carcinogenic	NCI, 1979a
Malathion	92.10%	Sprague-Dawley rats		diet	0,100,1000,5000 or 5,50,250 mg/kg	103 weeks	RBC acetylcholinesterase	100 ppm 5 mg/kg bw	1000 ppm		no evidence of carcinogenicity	Rucci et al., 1980 (as cited in WHO, 1997)
Malathion	94.00%	Crl:CD:(SD)BR rats		gavage	0,200,400,800 mg/kg bw	GD 6-15	developmental	400 mg/kg	800 mg/kg		LOAEL based on maternal tox	Lochry, 1989 (as cited in ATSDR, 2003)
Malathion	92.40%	New Zealand White Rabbits		gavage	25,50,100 mg/kg	GD 6-18	developmental	25 mg/kg	100 mg/kg		maternal toxicity fetal toxicity	Siglin, 1985 (as cited in ATSDR, 2003)
Malathion	not reported	humans, male		capsules	0.11to 0.34 mg/kgbw/day	subchronic	RBC cholinesterase inhib	0.23 mg/kgbw/day	0.34 mg/kgbw/day		RBC cholinesterase inhib	Moeller and Rider, 1962 (as cited in ATSDR, 2003)
Malathion	96.40%	rats		aerosol	100 mg/m3	6 hr/day 5 day/wk 13 weeks	respiratory lesions		100 mg/m3		hyperplasia of larynx and olfactory epithelia	Beattie, 1994; as cited in ATSDR, 2003
Malathion	95.00%	rabbits		aerosol	65, 123 mg/m3	6 hours	cholinesterase inhib	65 mg/m3	123 mg/m3		decrease plasma and RBC cholinesterase activity	Weeks et al., 1977; as cited in ATSDR, 2003
Malathion	99.0%	Sprague-Dawley male rats	225-250 g	dermal ethanol	44.4mg/kg/day alone & w/ 0.13 permethrin, 40 DEET & all 3	30 days	neurobehavioral effects acetylcholine esterase activity		44.4 44.4			Abdel-Rahman et al., 2004
Malathion	94.00%	Sprague-Dawley rats		diet	0,550,1700,5000 7500 or 43, 130, 390 600 m & 50,150, 440 660 f mg/kg	2-generation	reproductive developmental	7500 or 600 mg/kg 1700 or 130 mg/kg			 reduced fetal weight	Schroeder, 1990 (as cited in ATSDR, 2003)
Malaoxon	Technical grade 96.40%	Fischer 344 rats		diet	0,20,1000,2000 or 1,57,110mg/kg m 1,68,140 mg/kg f	24 mnths	oncogenicity	 20 ppm, 1 mg/kg	2000 ppm	p<0.05	Mortality excessive (50%) mononuclear cell leukemia in male rats. Determined not treatment related since only at excess. Dose, no trend, w/I historical control. Dec Dec food consump, brain AChE	Daly, 1996b (as cited in USEPA, 2000c and WHO, 1997)
Malaoxon	>95 %	Fischer 344 rats		diet	0,500, 1000 ppm	103 weeks	oncogenicity				NCI concluded noncarcinogenic re-eval by Huff, 1985, who found equivocal evid of C-cell neoplasm of thyroid	NCI, 1979b

Table 20

Noncancer Criteria (Oral/Dermal)- Malathion

Chemical of Potential Concern	Exposure Duration	Criteria	Value	Units	Species	Endpoint	NOAEL	LOAEL	Units	Combined Uncertainty/Modifying Factors	Study Source	Reference for Criteria
malathion	acute	RfD	5.00E-01	mg/kg/day	rabbit	developmental	5.00E+01		mg/kg/day	100	Siglin, 1985	USEPA, 2000e
malathion-dermal	acute	RfD	5.00E-01	mg/kg/day	rabbit	RBC chol inhib	5.00E+01		mg/kg/day	100		Westchester, 2002
malathion	intermed	MRL	2.00E-02	mg/kg/day	human	RBC chol inhib	2.3E-01		mg/kg/day	10	Moeller and Rider, 1962	ATSDR, 2003
malathion-dermal	intermed	RfD	5.00E-01	mg/kg/day	rabbit	RBC chol inhib	5.00E+01		mg/kg/day	10		Westchester, 2002
malathion	chronic	MRL	2.00E-02	mg/kg/day	rat	RBC chol inhib	2.0E+00		mg/kg/day	100	Daly, 1996a	ATSDR, 2003
malathion	chronic	RfD	2.00E-02	mg/kg/day	human	RBC chol inhib	2.30E-01		mg/kg/day	10	Moeller and Rider, 1962	USEPA, 2002d; IRIS
malathion	chronic	RfD	2.40E-02	mg/kg/day	rat	RBC chol inhib	2.40E+00		mg/kg/day	100	Daly, 1996a	USEPA, 2000e
malathion	chronic	ADI	3.00E-01	mg/kg/day	rat	brain chol inhib, dec survival, body-wgt gain, hematological effects	2.90E+01		mg/kg/day	100	Daly, 1996a	WHO, 1997
malathion-dermal	chronic	RfD	2.40E-02	mg/kg/day	rat	RBC chol inhib	2.40E+00		mg/kg/day	100		Westchester, 2002

NOAEL: no observed adverse effect level

LOAEL: lowest observed adverse effect level

RfD: USEPA oral reference dose

ADI: World Health Organization Acceptable Daily Intake

MRL: Agency for Toxic Substances and Disease Registry Minimum Risk Level

Table 21
Noncancer Criteria (Inhalation)- Malathion

Chemical of Potential Concern	Exposure Duration	Criteria	Value	Units	Endpoint	Species	NOAEL	LOAEL	Units	Combined Uncertainty/Modifying Factors	Study Source	Reference for Criteria
Malathion	acute	MRL	2.00E-01	mg/m^3	RBC chol inhib	rabbit	65		mg/m^3	100	Weeks et al., 1977	ATSDR, 2003
Malathion	acute	RfC	1.00E-01	mg/m^3	RBC chol inhib	rat		100	mg/m^3	1000		Westchester Cnty, 2002
					respir tract lesions							
Malathion	intermediate	EPA MOE endpt			respir tract lesions	rat	25.8		mg/kg/day		Beattie, 1994	USEPA, 2000e
Malathion	intermediate	RfC	1.00E-01	mg/m^3	RBC chol inhib respir tract lesions	rat		100	mg/m^3	1000		Westchester Cnty, 2002
Malathion	intermediate	MRL	2.00E-02	mg/m^3	respir tract lesions	rat	100		mg/m^3	1000	Beattie, 1994	ATSDR, 2003
Malathion	chronic	RfD	2.00E-02	mg/kg/day								USEPA Region 3, 2004
Malathion	chronic	PRG	7.30E+01	ug/m^3								USEPA Region 9, 2002
Malathion	chronic	RBC	7.30E+01	ug/m^3								USEPA Region 3, 2004
Malathion	chronic	RfC	1.00E-01	mg/m^3	RBC chol inhib	rat		100	mg/m^3	1000		Westchester Cnty, 2002
					respir tract lesions							

NOAEL: no observed adverse effect level
LOAEL: lowest observed adverse effect level
MRL: Minimal Risk Level, ATSDR
EPA MOE endpoint: endpoint used by USEPA in their margin of exposure risk evaluation (USEPA, 2000)
PRG: USEPA Region 9 Preliminary Remediation Goal, http://www.epa.gov/Region9/waste/sfund/prg/files/02table.pdf
RBC: USEPA Region 3 Risk Based Concentrationhttp://www.epa.gov/reg3hwmd/risk/human/rbc/rbc1003.pdf

Table 22
Cancer Criteria (Oral/Dermal)- Malathion

Chemical of Potential Concern	Oral Cancer Slope Factor $(mg/kg\text{-}day)^{-1}$	Weight of Evidence/ Cancer Guideline Description	Source	Date
Malathion	0.00152		Westchester	2002
Malathion		suggestive	USEPA	2002
		evidence of		
		carcinogenicity		

Reference List

Malathion

Abdel-Rahman, A, AM Dechkovskaia, LB Goldstein, SH Bullman, W Khan, EM El-Masry, and MB Abou-Donia. 2004. *Neurological Deficits Induced by Malathion, DEET, and Permethrin, Alone or in Combination in Adult Rats.* Journal of Toxicology and Environmental Health, Part A, 67:331-356, 2004.

Abraham, S and H Edery. 1976. *Rapid spontaneous reactivaton of cholinesterase inhibited by malaoxon.* Israel J. Med. Sci., 12, 1524. As cited in WHO, 1997.

Agency for Toxic Substances and Disease Registry (ATSDR). 2003. Toxicological Profile for Malathion. Department of Health and Human Services. September.

Ahdaya, SM, RJ Monroe, and FE Guthrie. 1981. *Absorption and distribution of intubated insecticides in fasted mice*. Pestic Biochem Physiol 16:38-46. As cited in ATSDR, 2003.

Ahmed, RS, V Seth, ST Pasha, and BD Banerjee. 2000. *Influence of Dietary Ginger (Zingiber Officinales Rosc) on Oxidative Stress Induced by Malathion in Rats.* Food Chem Toxicol. 2000 May;38(5):443-50.

Akbarsha, MA, PN Latha, and P Murugaian. 2000. *Retention of cytoplasmic droplet by rat cauda epididymal permatozoa after treatment with cytotoxic and xenobiotic agents.* Journal of Reproduction and Fertility 120(2):385-90. As cited in Westchester County, 2002.

Akhtar, N, SA Kayani, MM Ahmad, and M Shahab. 1966. *Insecticide-induced changes in secretory activity of the thyroid gland in rats.* Journal of Applied ogy 16(5):397-400. As cited in Westchester County, 2002.

Baker, EL, M Zack, JW Miles, L Alderman, M Warren, RD Dobbin, S Miller, and WR Teeters. 1978. *Epidemic Malathion Poisoning in Pakistan Malaria Workers*. The Lancet January 7:31-33. As cited in Westchester County, 2002.

Banerjee, BD, ST Pasha, QZ Hussain, et al. 1998. *A comparative evaluation of immunotoxicity of Malathion after subchronic exposure in experimental animals*. Ind J Exp Biol 36:273-282. As cited in ATSDR, 2003.

Beattie, G. 1994. *A 13-week toxicity study of aerolized Malathion administered by whole body inhalation exposure to the albino rat*: Lab project No: 90729. Unpublished study prepared by Product Safety Assessment, Bio-Research Labs, Ltd. MRID 43266601. As cited in ATSDR, 2003.

Blondell, J. 1994. *Review of adverse health effects attributed to Medfly spraying with malathion.* Memorandum to Larry Dorsey, Office of Prevention, Pesticides and Toxic Substances, USEPA, April 22, 1994.

Blondell, J and MF Spann. 1998. *Review of Malathion Incident Reports, DP Barcode D247492, Chemical #057701, Reregistration Case #0248.* Memorandum to Paula Deschamp, Office of Prevention, Pesticides and Toxic Substances, USEPA, August 18, 1998.

Blondell, J. 1999. *Review of Poison Control Center Data for Residential Exposures to Organophosphate Pesticides, 1993-1996 DP Barcode 253361, Reregistration Case #0100.* Memorandum to Dennis Utterback, Office of Prevention, Pesticides and Toxic Substances, USEPA, February 11, 1999.

Blondell, J and MF Spann. 2000. *Updated Review of Malathion Incident Reports DP Barcode D268749, Chemical #057701, Reregistration Case #0248.* Memorandum to Paula Deschamp, Office of Prevention, Pesticides and Toxic Substances, USEPA, September 11, 2000.

Boutsiouki, P, JP Thompson, and GF Clough. 2001. *Effects of Local Blood Flow on the Percutaneous Absorption of the Organophosphorus Compound Malathion: a microdialysis study in man.* Arch Toxicology (2001) 75: 321-328.

Boyes, WK, E Hunter, C Gary, et al. 1999. *Topical exposure of the eye to the organophosphorus insecticide Malathion: lack of visual effects.* J Appl Toxicol 19:473-483. As cited in ATSDR, 2003.

Brown, LM, A Blair, R Gibson, et al. 1990. *Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota.* Can Res 50:6585-6591. As cited in ATSDR, 2003.

Brown, LM, LF Burmeister, GD Everett, et al. 1993. *Pesticide exposures and multiple myeloma in Iowa men.* Cancer Causes Control 4:153-156. As cited in ATSDR, 2003.

Busto-Obregn, E and P Gonzlez-Hormazabal. 2003. Effect of a Single Dose of Malathion on Spermatogenesis in Mice. *Asian J. Androl* June 5: 105-107.

Cabello, G, M Valenzuela, A Viaxa, V Duran, I Ruldolph, N Hrepic, and G Calaf. 2001. *A Rat Mammary Tumor Model Induced by the Organophosphorous Pesticides Parathion and Malathion, Possibly Through Acetylcholinesterase Inhibition*. Environmental Health Perspectives. 2001 May; 109(5):471-9.

Cantor, KP, A Blair, G Everett, et al. 1992. *Pesticides and other agricultural risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota.* Cancer Res 52:2447-2455. As cited in ATSDR, 2003.

Casale, GP, SD Cohen, and RA DiCapua. 1983. *The effects of organophosphate-induced cholinergic stimulation on the antibody response to sheep erythrocytes in inbred mice*. Toxicol Appl Pharmacol 68:198-205. As cited in ATSDR, 2003.

Chaudhry, R, SB Lall, B Mishra, and B Dhawan. 1998. *A foodborne outbreak of organophosphate poisoning.* British Medical Journal 317:268-269. As cited in Westchester County, 2002.

Cushman, JR and JC Street. 1983. *Allergic hypersensitivity to the insecticide Malathion in BALB/c mice*. Toxicol Appl Pharmacol 70:29-42. As cited in ATSDR, 2003.

Daly, I. 1996a. *A 24-month oral toxicity/oncogenicity study of Malathion in the rat via dietary administration*. Final report: Lab project No: 90-3641: J-11 90-3641. Unpublished study prepared by Huntington Life Sciences. MRID 43942901. As cited in ATSDR, 2003 and USEPA, 2000c.

Daly, I. 1996b. *A 24-month oral toxicity/oncogenicity study of malaoxon in the rat via dietary administration*. Lab study number 93-2224. Unpublished study prepared by Huntington Life Sciences. MRID43975201. As cited in ATSDR, 2003, USEPA, 2000c and in WHO, 1997.

Dary, CC, JN Blancato, and MA Saleh. 2001. *Chemomorphic Analysis of Malathion in Skin Layers of the Rat: Implications for the Use of Dermatopharmacokinectic Tape Stripping in Exposure Assessment to Pesticides.* Regulatory Toxicology and Pharmacology, 2001 Dec; 34, 234-248.

De Bleecker, J, K VanDenNeucker, and J Willems. 1992. *The intermediate syndrome in organophosphate poisoning: Presentation of a case and review of the literature*. Clin Toxicol 30(3):321-329. As cited in ATSDR, 2003.

De Bleecker, JL. 1995. *The intermediate syndrome in organophosphate poisoning: An overview of experimental and clinical observations.* Clin Toxicol 33(6):683-686. As cited in ATSDR, 2003.

Dennis, GA and PN Lee. 1999. *A phase I volunteer study to establish the degree of absorption and effect on cholinesterase activity of four head lice preparations containing Malathion.* Clin Drug Invet 18(2):105-115. As cited in ATSDR, 2003.

Desi, I, G Dura, L Gonczi, Z Kneffel, A Strohmayer, and S Szabo. 1975. *Toxicity of Malathion to mammals, aquatic organisms and tissue culture cells.* Archives of Environmental Contamination and Toxicology 3:410-425. As cited in Westchester County, 2002.

Desi, I, G Dura, L Gonczi, et al. 1976. *Toxicity of Malathion to mammals, aquatic organisms and tissue culture cells.* Arch Environ Contam Toxicol 3:410-425. As cited in ATSDR, 2003.

Dierickx, PJ. 2000. *Cytotoxicity of the MEIC Reference Chemicals in Rat Hepatoma-derived Fa32 Cells.* Toxicology 150 (2000) 159-169.

Dikshith, TSS, MK Srivastava, RB Raizada, et al. 1987. *Interaction of Hexachlorocyclohexane and Malathion in male guinea pigs after repeated dermal application.* Vet Hum Toxicol 29(2):138-143. As cited in ATSDR, 2003.

Ehrich, M, L Shell, M Rozum, et al. 1993. *Short-term clinical and neuropathologic effects of cholinesterase inhibitors in rats.* J Am Coll Toxicol 12(1):55-68. As cited in ATSDR, 2003.

Eskenazi, B, K Harley, A Bradman, E Weltzien, NP Jewell, DB Barr, CE Furlong, and NT Holland. 2004. Association of in Utero Organophosphate Pesticide Exposure and Fetal Growth and Length of Gestation in an Agricultural Population. *Environmental Health Perspectives,* Volume 112, No. 10, pages 1116-1124. July.

Fenske, RA, G Kedan, C Lu, JA Fisker-Andersen, CL Curl. 2002. *Assessment of Organophosphorous Pesticide Exposures in the Diets of Preschool Children in Washington State*. Expo Anal Environ Epidemiol. 2002 Jan-Feb; 12(1):21-8.

Flower, KB, JA Hoppin, CF Lynch, A Blair, C Knott, DL Shore, and DP Sandler. 2004. Cancer Risk and Parental Pesticide Application in Children of Agricultural Health Study Participants. *Environmental Health Perspectives*, Volume 112, No. 5, pages 631-635. April.

Giri, S, SB Presad, A Giri, and GD Sharma. 2002. *Genotoxic Effects of Malathion: An Organophosphorus Insecticide, Using Three Mammalian Bioassays In Vivo.* Mutation Research 514 (2002) 223-231.

Golz, HH. 1959. *Controlled human exposures to Malathion aerosols.* AMA Arch Ind Health 19:53-59. As cited in ATSDR, 2003.

Grether, JK, JA Harris, R Neutra, and KW Kizer. 1987. *Exposure to aerial Malathion application and the occurrence of congenital anomalies and low birthweight*. American Journal of Public Health 77(8):1009-1010. As cited in Westchester County, 2002.

Guven, M, F Bayram, K Unluhizarci, and F Kelestrimur. 1999. *Endocrine changes in patients with acute organophosphate poisoning*. Human and Experimental Toxicology 18(10):598-601. As cited in Westchester County, 2002.

Hoppin, JA, DM Umbach, SJ London, MCR Alavanja, and DP Sandler. 2002. *Chemical Predictors of Wheeze Among Farmer Pesticide Applicators in the Agricultural Health Study.* Am J Respir Crit Care Med, Vol. 165. pp 683-689, 2002.

Huff, JE, R Bates, SL Eustis, JK Haseman, and EE McConnell. 1985. *Malathion and malaoxon: Histopathology reexamination of the National Cancer Institute carcinogenesis studies*. Environ. Res., 37, 154-173. As cited in WHO, 1997.

International Agency for Research on Cancer (IARC). 1983. *IARC Monographs Malathion*. Vol. 30 (1983) (p. 103).

Ishikawa, S, M Miyata, S Aoki, and Y Hanai. 1993. *Chronic intoxication of organophosphorous pesticide and its treatment.* Folia Medica Cracovensia 34:139-151.

Johnson, VJ, AM Rosenberg, K Lee, and BR Blakeley. 2002. *Increased T-lymphocyte dependent antibody production in female SJL/J mice following exposure to commercial grade malathion.* Toxicology 170 (2002) 119-129.

Kahn, E, M Berlin, M Deane, R Jackson, and J Stratton. 1992. *Assessment of Acute Health Effects from the Medfly Eradication Project in Santa Clara County, California.* Archives of Environmental Health Vol. 47 (No. 4) July/August.

Kojima, H, E Katsura, S Takeuchi, K Niyama, and K Kobayashi. 2004. Screening for Estrogen and Androgen Receptor Activities in 200 Pesticides by In Vitro Reporter Gene Assays Using Chinese Hamster Ovary *Cells. Environmental Health Perspectives*, Volume 112, No. 5, pages 524-531. April.

Krause, W, K Hamm, and J Weissmuller. 1976. *Damage to spermatogenesis in juvenile rat treated with DDVP and Malathion.* Bull Environ Contam Toxicol 15(4):458-462. As cited in ATSDR, 2003.

Krause, W. 1977. *Influence of DDT, DDVP and Malathion on FSH, LH and Testosterone Concentration in Testis*. Bull Environ Contam Toxicol 18(2):231-242. As cited in ATSDR, 2003.

Krieger, RI and TM Dinoff. 2000. *Malathion deposition, metabolite clearance, and cholinesterase status of date dusters and harvesters in California*. Archives of Environmental Contamination and Toxicology;38(4):546-53. As cited in Westchester County, 2002.

Lamb, I. 1994a. *An acute neurotoxicity study of Malathion in rats.* Final report: Lab project No. WIL/206005. Unpublished study prepared by WIL Research Labs., Inc. MRID 43146701. As cited in ATSDR, 2003.

Lamb, I. 1994b. *A subchronic (13-week) neurotoxicity study of Malathion in rats: Final Report: Lab project No. WIL-206006*. Unpublished study prepared by WIL Research Labs., Inc. MRID 43269501. As cited in ATSDR, 2003.

Lechner, DW and MS Abdel-Rahman. 1984. *A teratology study of carbaryl and malathion mixtures in rat*. J Toxicol Environ Health 14:267-278. As cited in ATSDR, 2003.

Lee, P and DY Tai. 2001. *Clinical Features of Patients with Acute Human Organophosphate Poisoning Requiring Intensive Care.* Intensive Care Medicine 2001 Apr; 27(4):694-699.

Lochry, E. 1989. *A development toxicity study with CA 6,601 in rats.* Argus Research Laboratories Protocol 101-005. Unpublished study prepared by Argus Research Laboratories, Inc. MRID 41160901. As cited in ATSDR, 2003.

Machin, MGA and WG McBride. 1989a. *Teratological study of Malathion in the rabbit*. J Toxicol Environ Health 26:249-253. As cited in ATSDR, 2003.

Machin, MGA and WG McBride. 1989b. *Placental transfer of Malathion in the rabbit*. Med Sci Res 17:743-744. As cited in ATSDR, 2003.

MacIntosh, DL, CW Kabiru, and PB Ryan. 2001. *Longitudinal Investigation of Dietary Exposure to Selected Pesticides*. Environmental Health Perspectives, Feb 2001; 109(5):145-150.

Masten, SJ, M Tian, BL Upham, and JE Trosko. 2001. *Effect of selected pesticides and their ozononation by-products on gap junctional intercellar communication using rat liver epithelial cell lines.* Chemosphere 44 (2002) 457-465.

Mathews, MS and KS Devi. 1994. *Effect of chronic exposure of pregnant rats to Malathion and/or estrogen and/or progesterone on xenobiotic metabolizing enzymes*. Pestic Biochem Physiol 48:110-122. As cited in ATSDR, 2003.

McDuffie, HH, P Pahwa, JR McLaughlin, JJ Spinelli, S Fincham, JA Dosman, D Robson, LF Skinnider, and NW Choi. 2001. *Non-Hodgkin's Lymphoma and Specific Pesticide Exposures in Men.* Cancer Epidemiology Biomarkers & Prevention. Vol. 10, 1155-1163, November 2001.

Milby TH, Epstein WL, 1964. *Allergic contact sensitivity to Malathion*. Arch Environ Health 9:434-437. As cited in ATSDR, 2003.

Moeller, HC and JA Rider. 1962. *Plasma and red blood cell cholinesterase activity as indications of the threshold of incipient toxicity of ethyl-p-nitrophenyl thionobenzenephosphonate (EPN) and Malathion in human beings.* Toxicol Appl Pharmacol 4:123-130. As cited in ATSDR, 2003.

Moreno, O. 1989. *21-Day dermal toxicity study with AC 6,601 in rabbits: Laboratory report No MB880101*. Unpublished study prepared by MB Laboratories, Inc. MRID 41054201. As cited in ATSDR, 2003.

National Cancer Institute (NCI). 1978. *Bioassay of Malathion for possible carcinogenicity.* Technical Report Series No. 24. U.S. Department of Health, Education and Welfare. Public Health Service. National Institute of Health. National Cancer Institute. As cited in ATSDR, 2003.

National Cancer Institute (NCI). 1979a. *Bioassay of Malathion for Possible Carcinogenicity.* Carcinogenesis Testing Program Division of Cancer Cause and Prevention. Carcinogenesis Technical Report Series No. 192, 1979.

National Cancer Institute (NCI). 1979b. *Bioassay of Malaoxon for Possible Carcinogenicity.* Carcinogenesis Testing Program Division of Cancer Cause and Prevention, NIH Publication No. 79-1390.

National Library of Medicine (NLM). 2003. *Hazardous Substance Data Bank (HSDB) for Malathion*. URL http://toxnet.nlm.nih.gov.

New York City. 2001. Adult Mosquito Control Programs Final Environmental Impact Statement. Prepared by Allee King Rosen and Fleming, Inc., Gradient Corporation, PBS & J and Environmental Focus. July.

Ojha, S, SP Norton, N Shrivastava, et al. 1992. *Toxic effect of Malathion on the reproductive system of albino rats.* Environ Ecol 10(4):833-836. As cited in ATSDR, 2003.

Ostrea Jr., EM, V Morales, E Ngoumgna, R Prescilla, E Tan, E Hernandez, GB Ramirez, H Cifra, and ML Manlapaz. 2002. *Prevalence of Fetal Exposure to Environmental Toxins as Determined by Meconium Analysis.* NeuroToxicology 23 (2002) 329-339.

Ozmen, G and MT Akay. 1993. *The effects of Malathion on some hormone levels and tissues secreting these hormones in rats.* Vet Hum Toxicol 35(1):22-24. As cited in ATSDR, 2003.

Padungtod, C, BL Lasley, DC Christiani, LM Ryan, and X Xu. 1998. *Reproductive hormone profile among pesticide factory workers*. Journal of Occupational and Environmental Medicine 40(12):1038-1047. As cited in Westchester County, 2002.

Peters, HA, RL Levine, CG Matthews, SL Sauter, and JH Rankin. 1982. *Carbon disulfide-induced neuropsychiatric changes in grain storage workers*. American Journal of Industrial Medicine 3:373-391. As cited in Westchester County, 2002.

Piramanayagam, S, BM Manohar, and A Sundararaj. 1996. *Pathology of Malathion toxicity in rats.* Indian Vet J 73(7):734-737. As cited in ATSDR, 2003.

Prabhakaran, S and KS Devi. 1993. *Impact of protein deficiency and exposure to Hexachlorocyclohexane or Malathion on lipid metabolism in pregnant rats.* Ind J Biochem Biophys 30:234-238. As cited in ATSDR, 2003.

Prabhakaran, S, F Shameem, and KS Devi. 1993. *Influence of protein deficiency on Hexachlorocyclohexane and Malathion toxicity in pregnant rats.* Vet Hum Toxicol 35(5):429-433. As cited in ATSDR, 2003.

Prakash, N, K Narayana, GS Murthy, NR Moudgal, and Honnegowda. 1992. *The effect of malathion, an organophosphate, on the plasma FSH, 17B-estradiol and progesterone concentrations and acetylcholinesterase activity and conception in dairy cattle.* Veterinary and Human Toxicology 34(2):116-119. As cited in Westchester County, 2002.

Prakash, N and U Verikatesh. 1996. *Human chorionic gonadotrophin (hcG) protects Malathion induced plasma luteinizing hormone and testosterone changes in rats.* Indian Journal of Pharmacology 28(4):257-260. As cited in Westchester County, 2002.

Reeves, JD, DA Driggers, and VA Kiley. 1981. *Household insecticides associated aplastic anaemia and acute leukaemia in children.* Lancet 11:300-301. As cited in ATSDR, 2003.

Relford, RL, AJ Ainsworth, and JE Harkness. 1989. *Effects of a commercial Malathion dip preparation on the cellular and humoral immune response of BALB/c mice*. Lab Anim Sci 39(1):56-59. As cited in ATSDR, 2003.

Rodgers, KE, N Leung, CF Ware, et al. 1986. *Lack of immunosuppressive effects of acute and subacute administration of Malathion on murine cellular and humoral immune responses.* Pestic Biochem Physiol 25:358-365. As cited in ATSDR, 2003.

Rodgers, KE and DD Ellefson. 1990. *Modulation of respiratory burst activity and mitogenic response of human peripheral blood mononuclear cels and murine splenocytes and peritoneal cells by Malathion*. Fundam Appl Toxicol 14:309-317. As cited in ATSDR, 2003.

Rodgers, K and S Xiong. 1997. *Effect of acute administration of Malathion by oral and dermal routes on serum histamine levels.* Int J Immunopharmacol 19(8):437-441. As cited in ATSDR, 2003.

Rucci, G, PJ Becci, and RA Parent. 1980. *The Evaluation of the Chronic Toxicity Effects of Cythion Administered in the Diet to Sprague-Dawley Rats for 24 Consecutive Months.* Unpublished report (Project No. 5436) from Food and Drug Research Laboratories Inc., Waverly, New York, USA. Submitted to WHO by Cheminova, Lemvig, Denmark. As cited in WHO, 1997.

Samimi, A and JA Last. 2001a. *Inhibition of Lysyl Hydroxylase by Malathion and Malaoxon*. Toxicology and Applied Pharmacology. 172, 203-209 (2001).

Samimi, A and JA Last. 2001b. *Mechanism of Inhibition of Lysyl Hydroxylase Activity by the Organophosphates Malathion and Malaoxon.* Toxicology and Applied Pharmacology. 176, 181-186 (2001).

Sanghi, R, MK Pillai, TR Jayalekshmi, and A Nair. 2003. *Organochlorine and organophosphorus pesticide residues in breast milk from Bhopal, Madhya Pradesh, India*. Hum Exp Toicol. 2003 Feb; 22(2):73-6.

Schanker, HM, G Rachelefsky, S Siegel, R Katz, S Spector, A Rohr, C Rodriquiz, K Woloshin, and PJ Papanek, Jr. 1992. *Immediate and delayed type hypersensitivity to malathion*. Annals of Allergy 69:526-528. As cited in Westchester County, 2002.

Schroeder, R. 1990. *A two generation (two litters) reproduction study with AC 6,601 to rats. Study No. 87-3243*. Unpublished prepared by Bio/Dynamics, Inc. MRID 41583401. As cited in ATSDR, 2003.

Scientific Advisory Panel (SAP). 2000. *Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding: A Consultation on the EPA Health Effect Division's Proposed Classification of the Human Carcinogenic Potential of Malathion*. Report No. 2000-04. FIFRA Scientific Advisory Panel.

Segal, LM and S Fedoroff. 1989. *The acute and subchronic effects of organophosphorous and carbamate pesticides on cholinesterase activity in aggregate cultures of neural cells from the foetal rat brain*. Toxicol. in vitro, 3, 111-122. As cited in WHO, 1997.

Shafey, O, HJ Sekereke, Jr., BJ Hughes, S Heber, RG Hunter, RG Brooks. 1999. *Surveillance for Acute Pesticide-Related Illness During the Medfly Eradication Program—Florida, 1998.* MMWR, Vol. 48, No. 44, P. 8, 1015-1027.

Shah, PV, RJ Monroe, and FE Guthrie. 1981. *Comparative rates of dermal penetration of insecticides in mice*. Toxicol Appl Pharmacol 59:414-423. As cited in ATSDR, 2003.

Sharma, VK and S Kaur. 1990. *Contact sensitization by pesticides in farmers*. Contact Dermatitis 23:77-80. As cited in Westchester County, 2002.

Siglin, J. 1985. *A teratology study with AC 6,601 in rabbits: FDRL Study No. 8171*. Unpublished study prepared by Food and Drug Research Laboratories (Incorporates a range-finding study). MRID 00152569. As cited in ATSDR, 2003.

Slauter, R. 1994. *18-Month oral (dietary) oncogenicity study in mice: Malathion*. Lab project No 668001. Unpublished study prepared by International Research and Development Corp. MRID 43407201. As cited in ATSDR, 2003.

Thomas, DC, DB Petitti, M Goldhaber, SH Swan, EB Rappaport, and I Hertz-Picciotto. 1992. *Reproductive outcomes, in relation to Malathion spraying in the San Francisco Bay area, 1981-1982*. Epidemiology 3:32-39. As cited in ATSDR, 2003 and Westchester County, 2002.

Tos-Luty, S, D Obuchowska-Przebirowska, J Latuszynska, M Tokarska-Rodak, and A Haratym-Maj. 2003. Dermal and Oral Toxicity of Malathion in Rats. Ann Agric Environ Med 2003, 10, 101-106.

United States Environmental Protection Agency (USEPA). 1990. Memorandum from J. Blondell to P. Fenner-Crisp re: *Malathion Incidents Related to Medfly Spraying in 1981*. Office of Pesticides and Toxic Substances, Washington, DC. March 16. As cited in Westchester County, 2002.

United States Environmental Protection Agency (USEPA). 1997. *MALATHION: - Report of the Hazard Identification Assessment Review Committee.* Memorandum from Jess Rowland to Al

Nielsen, Health Effects Division, Office of Prevention, Pesticides and Toxic Substances. December 17.

United States Environmental Protection Agency (USEPA). 1998. *Hazard Assessment of the Organophosphates.* Report of the Hazard Identification Assessment Review Committee. July 7.

United States Environmental Protection Agency (USEPA). 2000a. *Malathion Reregistration EligibilityDocument Environmental Fate and Effects Chapter.* Office of Prevention, Pesticides and Toxic Substances.

United States Environmental Protection Agency (USEPA). 2000b. Correction to November 1, 1999 MALATHION: *Revised NOAEL for Derivaiton of the Chronic Reference Dose* – Report of the Hazard Identification Assessment Review Committee. Memorandum from Jess Rowland to Paula Deschamp, Office of Pesticide Programs. April 26.

United States Environmental Protection Agency (USEPA). 2000c. *Evaluation of the Carcinogenic Potential of Malathion.* Cancer Assessment Document #2 Report of the 12-April-2000 Meeting. Cancer Assessment Review Committee, Office of Pesticide Programs. April 28.

United States Environmental Protection Agency (USEPA). 2000d. *Malathion: Revised Occupational and Residential Exposure and Risk Assessment for the Reregistration Eligibility Decision (RED) Document.* Office of Prevention, Pesticides and Toxic Substances. September 14, 2000.

United States Environmental Protection Agency (USEPA). 2000e. *Human Health Risk Assessment for the Reregistration Eligibility Decision (RED) Document. Chemical No. 0577701. Case No. 0248. Barcode D269070.* Memorandum from Paula Deschamp to Patricia Moe, Office of Prevention, Pesticides and Toxic Substances. September 22.

United States Environmental Protection Agency (USEPA), Region IV. 2002. *Preliminary Remedial Goal Table*. October 1, 2002.

United States Environmental Protection Agency (USEPA). 2002a. *Summary of Toxicity Information for Chemicals Listed in IRIS.* Screening-Level Review of the IRIS Database Phase II. January 1, 2002.

United States Environmental Protection Agency (USEPA). 2002b. *Chemicals Evaluated for Carcinogenic Potential.* Office of Pesticide Programs, Health Effects Division. Update 5/10/02.

United States Environmental Protection Agency (USEPA). 2002c. *Evaluation of the Recent Literature and Determination of Currency for: Malathion (CAS No. 121-75-5).* Screening-Level Review of the IRIS Database Phase II. September 2002.

United States Environmental Protection Agency (USEPA). 2002d. Integrated Risk Information System (IRIS) Record for *Malathion*. Last Revised 12/3/02.

United States Environmental Protection Agency (USEPA), Region III. 2004. *Risk-Based Concentration Table*. April 14, 2004.

van Bao, T, I Szabo, P Ruzieska, et al. 1974. *Chromosome aberrations in patients suffering acute organic phosphate insecticide intoxication.* Humangenetik 24:33-37. As cited in ATSDR, 2003.

Vasilic, Z, B Stengl, and V Drevenkar. 1999. *Dimethyphosphorous metabolites in serum and urine of persons poisoned by Malathion or thiometon*. Chem Bio Interact 119-120:479-487.

Weeks, MH, MA Lawson, RA Angerhofer, et al. 1977. *Preliminary assessment of the acute toxicity of Malathion in animals.* Arch Environ Contam Toxicol 6:23-31. As cited in ATSDR, 2003.

Westchester County. 2002. *Comprehensive Mosquito-Borne Disease Surveillance and Control Plan*, Generic Environmental Impact Statement. February, 2002.

World Health Organization (WHO). 1997. *Toxicological and Environmental Evaluations 1994, Malathion.* Joint Meeting of the Food and Agriculture Organization (FAO) Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group. September 22 to October 1, 1997.

Zahm, SH, DD Weisenburger, RC Saal, et al. 1993. *The role of agricultural pesticide use in the development of non-Hodgkin's lymphoma in women*. Arch Environ Health 48(5):353-358. As cited in ATSDR, 2003.

4.4 Garlic Oil

Background

Garlic oil is obtained from the cloves of the plant *Allium sativum.* Garlic is classified as an allium vegetable along with onions, leeks, scallions, shallots and chives (NCI, 2002). As a pesticide, garlic can be formulated as a powder, distilled liquid, or emulsifiable oil (USEPA, 1992a). Garlic was first registered as a pesticide in 1983, for use in repelling birds. This product was formulated with red pepper (capsaicin). In 1992, the USEPA issued a reregistration document for garlic, in which it waived most of the data requirements for reregistration. This waiver was granted because garlic is used widely in foods and is "generally recognized as safe" (GRAS). In addition, garlic has a non-toxic mode of action as a repellent, is presumed to be non-persistent since it is an organic material which is known to bio-degrade, and there have not been reports of adverse effects from its use as a pesticide (USEPA, 1992b). However, the USEPA did require product specific data to characterize the product formulation's chemistry, efficacy and acute toxicity, especially in terms of potential effects to applicators (USEPA, 1992a).

Garlic has historically been used as a medicinal herb and much of the toxicological literature on garlic relates to its beneficial effects. Garlic is composed of many sulfur containing organic chemicals, flavonoids and selenium, which may vary depending upon the formulation of the garlic product. For example, raw garlic contains diallyl sulfide (approximately 30-100 mg/g of garlic) (Takahashi et al., 1992), which is responsible in part for garlic's strong taste and odor (Yang et al., 2001). Diallyl disulfide is also found in natural garlic (approximately 530-610 mg/g of garlic) (Takahashi et al., 1992), and is a major component in processed garlic (Knowles and Milner, 2000). Allicin is an unstable component of chopped garlic and water extracts of garlic (Borek, 2001). A derivative of allicin, ajoene, is found in oil-macerated garlic products (Hattori et al., 2001). Aged Garlic Extract (AGE) is prepared from a prolonged extraction of garlic at room temperature and contains S-allylcysteine and S-allylmercaptocysteine (Borek, 2001).

Adverse Effects

Garlic has been found to produce some allergic reactions. It is one of the more frequent causes of contact dermatitis in caterers and other food handlers (Seuri et al., 1993; Anibarro et al., 1997). Occupational asthma has been reported, though relatively infrequently. In a province of

Spain, in which approximately 6,000 families work on garlic farms, seven individuals were found to have a specific allergy to garlic (Anibarro et al., 1997). Asthmatic reactions typically followed allergic rhinitis and appeared to be an immunoglobulin gamma E (IgE)-mediated response. The affected individuals appeared to have a relatively long period of exposure (19 years) and typically also had reactions to pollen (Anibarro et al., 1997).

Researchers at the NIH have found that garlic supplements can impede the effectiveness of a medication (saquinavir) used to treat HIV/AIDS (NIH, 2001). When healthy volunteers were given garlic caplets twice a day for three weeks along with a daily dose of saquinavir, it was found that levels of saquinavir were reduced by 50 percent (NIH, 2001). It was concluded that doctors and patients should be cautious about using garlic supplements during HIV therapy.

Excess intake of garlic can have adverse side effects, such as garlic odor on breath or skin, occasional allergic reactions, stomach disorders and diarrhea, decrease in serum protein and calcium levels, bronchial asthma and contact dermatitis (NCI, 2002).

Beneficial Effects

There are many studies in the literature that report beneficial effects of garlic oil, ranging from cancer prevention to reducing cholesterol. Some of these beneficial effects may be related to the many kinds of amino acids with numerous thiol/allyl sulfur groups present in garlic products (Lee et al., 1999; NCI, 2002).

Cancer Prevention

Allyl sulfur (organosulfur) chemicals are known to slow or prevent the growth of tumor cells and are naturally occurring in garlic and onions (NCI, 2002). As summarized in a NCI Fact Sheet on garlic and cancer prevention, of the 37 studies that have looked into the use of garlic and related allyl sulfur compounds in the diet, 28 have shown a protective effect against cancer. The evidence is particularly strong for prostate and stomach cancers (NCI, 2002).

One recent case-control epidemiologic study reviewed the intake of various allium vegetables by prostate cancer patients and men without prostate cancer in China. This study found that men who consumed more than 10 g of garlic a day had a 50 percent lower risk of prostate cancer than those who consumed less than 2 g per day. Garlic and scallions had the greatest beneficial effect

(Hsing et al., 2002). In another population-based epidemiological study, the reduced risk of gastric cancer and intake of garlic was investigated. This study also compared the occurrence of *H. pylori* infection in two counties in China. The county with a lower gastric cancer rate consumed approximately ten times the quantity of garlic (approximately ten cloves per day as compared to one clove per day). *H. pylori* infection was found to be much higher in the county with a high gastric cancer rate. It was proposed that one mechanism by which garlic reduced the risk of gastric cancer was through inhibiting the growth of *H. pylori* bacteria (You et al., 2001). Similarly, a study of gastric cancer patients in Florence Italy found a protective effect of frequent consumption of garlic in one specific type of gastric tumor (microsatellite negative) (Palli et al., 2001).

Researchers have attempted to determine the mechanism by which garlic inhibits the occurrence of cancer by studying various chemical components of garlic in laboratory studies, either using animals or cultured human cell lines. Milner (2001) summarized various studies that have provided information on the possible mechanisms by which garlic may provide a protective effect against cancer. Because there is evidence that garlic inhibits so many different types of cancer (breast, colon, uterine, skin, esophagus and lung) Milner suggested that garlic must affect several mechanisms that are fundamental to the overall cancer process.

One such mechanism is the inhibition of enzymes that are responsible for activation of chemicals to intermediates that are reactive with DNA. Diallyl sulfide, a component of garlic, has been found to inhibit the activity of cytochrome P450 enzymes, which are often responsible for the activation of chemical carcinogens (Yang et al., 2001). Other enzymes have been implicated, including; cyclooxygenase, lipoxygenase and NAD(P)H quinine oxireductase (Milner, 2001). Conversely, garlic components have been found to activate enzymes responsible for the elimination of many chemicals from the body, including glutathione and glutathione-s-transferase (Milner, 2001; Singh et al., 1996).

Pre-treatment of mice with a garlic extract was shown to decrease chromosomal damage following gamma radiation exposure. The authors suggest that this observation may be due to the ability of garlic components to "scavenge" very reactive free radicals (Singh, et al., 1996). Free radicals are implicated in not only the initiation step in chemical carcinogenesis but also many other diseases such as atherosclerosis, stroke, aging and Alzheimer's disease (Borek, 2001).

Crushed garlic administered orally to mice was found to reduce chromosomal damage induced by arsenic. It was suggested that the sulfur (thiol) groups on garlic constituents bind to arsenic preventing arsenic from binding to enzymes involved in the development of cancer. However, the administration of garlic alone was found to increase the occurrence of chromosomal damage above that observed in untreated animals (Choudhury et al., 1997).

Garlic components have also been found to reduce cell proliferation, particularly in tumor cells (Milner, 2001). Diallyl disulfide, another garlic component, has been found to inhibit an enzyme system involved in the proliferation of tumor cells by blocking the transformation of the enzyme to its active form (Knowles and Milner, 2000). Similarly, S-allylmercaptocysteine has been found to inhibit cell growth and cell division in cultures using human colon cancer cells (Shirin et al., 2001) and diallyl disulfide was found to inhibit the growth of human breast cancer cell lines by decreasing cell proliferation (Nakagawa et al., 2001).

Administration of another garlic component, glutamyl-Se-methylselenocysteine, was also effective in reducing pre-malignant lesions, as well as carcinomas of the breast in rats that had been pre-treated with a known carcinogen (Dong et al., 2001). Mice that had been pre-treated with dimethylbenzanthracene were found to develop fewer skin tumors when also treated with ajoene, a sulfur component of garlic (Nishikawa et al., 2002). A similar study involving pre-treatment with a known carcinogen, N-nitrosodiethylamine, found administration of S-allylcysteine reduced the development of liver tumors by reducing lipid peroxidation (Sundaresan and Subramanian, 2003). In a similar study, another garlic component, diallyl disulfide was found to inhibit the carcinogenic response (Takahashi, et al., 1992).

Other mechanisms for this protective effect that have been proposed include blocking the formation of DNA adducts, increasing DNA repair or cell signaling, induction of cell death (apotosis) and depressing the growth of gastric microbes which may be involved in the formation of nitrosamines (Milner, 2001). Garlic and or its derivatives have been found to increase apotosis in many studies, including human colon cancer cell lines (Shirin et al., 2001), and human breast cancer cell lines (Nakagawa et al., 2001). Another study that investigated the DNA adduct binding in breast tissue following treatment with dimethylbenzanthracene, found that a garlic powder supplemented diet caused a reduction in the formation of DNA adducts in rats (Amagase and Milner, 1993). Garlic was found to enhance the immune system and was found to

be comparable to the immunotherapeutic drug, *bacillus Calmette-Guerin*, in suppressing bladder cancer tumors (Lamm and Riggs, 2001).

There are some studies in the literature that either show garlic does not offer a protective effect or that some garlic components actually enhance the development of tumors. Milner (2001) offers that some of these discrepancies may be due to the dose of garlic administered or other components in the diet, such as fat, that may impair the protective effect of garlic. Some studies on individual components have indicated that garlic components actually enhance the carcinogenic response of chemical carcinogens. For example, diallyl sulfide enhanced the development of liver cancer in animals treated with the carcinogen, diethylnitrosamine (Takada, et al., 1994). This enhancement appeared to be related to a cancer promotion effect of diallyl sulfide, via increased cell proliferation. It does not appear that garlic produces genetic damage (Abraham and Kesavan, 1984).

Chemical Toxicity

The inhibition of liver toxicity by two components of garlic was studied. In each of these studies, acetaminophen was used to induce liver toxicity. In one study, ajoene was administered prior to treatment of mice with acetaminophen. Various enzymatic indicators of liver toxicity were measured and provided evidence of a protective effect by pre-treatment of ajoene (Hattori et al., 2001). Similarly, S-allylmercaptosysteine was given to mice prior to the treatment with acetaminophen and found to inhibit cytochrome P450 activity and decreased lipid peroxidation in the liver (Sumioka et al., 1998).

Reproductive/Developmental Effects

The protective effect of garlic on the embryotoxicity of methylmercury chloride was investigated in a study in which pregnant rats were given 20 mg/kg bw methylmercury chloride alone and with 0.5 and 1.0 g/kg bw garlic. This study showed that co-administration with garlic juice reduced various endpoints of toxicity such as maternal and fetal death rate, decreased maternal and fetal body weight, as well as reducing the body burden of mercury in both the fetuses and mothers (Lee et al., 1999).

Cholesterolemic and Atherogenic Effects

There have been many human trials that have provided evidence that garlic can reduce cholesterol levels. A meta-analysis was conducted by Washafsky et al. (1993) in which the results of 5 human trials were pooled, for a total of 410 people evaluated. This analysis indicated that one half to one clove of garlic per day did in fact reduce cholesterol levels on the order of 9-12 percent. The Agency for Healthcare Research and Quality reviewed the evidence of positive health impacts of garlic and concluded that the 37 randomized human trials on the effect of garlic in reducing cholesterol found small, but statistically significant reductions in the short term (i.e., 3 months). However, no significant reductions were seen beyond that time period (Agency for Healthcare Research and Quality , 2000).

Some proposed mechanisms include reductions in the hepatic enzymes that generate cholesterol and an increase in the excretion of cholesterol in bile. In another study, AGE was found to be effective in reducing total cholesterol by seven percent and LDL cholesterol by ten percent in hypercholesterolemic men (Yeh and Liu, 2001). These men received approximately 2,700 mg/day of the AGE and reductions in cholesterol were significant compared to controls after five months of treatment. The authors concluded the cholesterol-lowering effects of the garlic extract were due to an inhibition of hepatic cholesterol synthesis (Yeh and Liu, 2001).

In an animal study using rabbits, an AGE (Kyolic) was found not to reduce plasma cholesterol, but did reduce lipid-filled lesions in the aorta by 64 percent (Campbell et al., 2001).

In a review article by Borek (2001), it is suggested that these antiatherogenic effects are at least in part due to antioxidant effects of garlic constituents, specifically those found in AGE.

Neurological Effects

The potential protective effect of garlic constituents on Alzheimer's Disease has been investigated. Evidence suggests that neurological damage that leads to Alzheimer's Disease may result from the impacts of reactive oxygen species (e.g., free radicals). In a cell culture study using neurological cells, AGE was found to offer a protective effect against neurological damage caused by oxidative stress (Peng et al., 2002).

Table 23
Toxicity Study SummaryTable - Garlic Oil

Compound	Purity	Test Species (Strain)	Test Species Age/Lifecyle	Route	Dose Duration	Study Duration	Endpoint	NOEL (mg/kg-day)	LOEL (mg/kg-day)	LOEL Statistical Significance	Comments	Reference
Garlic	natural	human	adult male	diet	5 yr prior		inhib prostate cancer		>10 g/day	P_{trend} <0.001	diet history of >10g/day garlic associated with lower risk of prostate cancer (OR=0.51)	Hsing et al., 2002
Garlic	Korean garlic juice methyl chloride	Fischer 344 rats	pregnant rats GD 7	oral	GD 7 20mg/kgbw MMC 0.5, 1mg.kgbw garlic juice	to GD 20	inhib embryotoxicity		1g/kg bw		garlic decreased embryotoxicity of MMC, by increasing maternal bw, fetal survival, fetal bw and litter wgt, and decreasing maternal death, implantation loss and body burden of mercury.	Lee et al., 1999
S-allylmercapto-cysteine		male ddY mice	6 weeks	oral	500mg/kgbw acetaminophen 50,100,200 mg/kg SAMC, 2 and 24hr prior	12 hr post treatment	inhib liver toxicity		50 mg/kg bw	P<0.01	measured various liver enzyme levels and determined an inhib of liver toxicity by acetaminophen	Sumioka et al., 1998
Ajoene	>98%	male ICR mice	6 weeks 28-30 g	oral	20,50,100 mg/kg ajoene, 2 & 24 hr prior 300 mg/kg bw acetaminophen	6 hr post treatment	inhib liver toxicity		50 mg/kg bw	P<0.01	measured various liver enzyme levels and determined an inhib of liver toxicity by acetaminophen	Hattori et al., 2001
Garlic	natural	human case-control		diet	12 mnth prior		inhib gastric cancer tumors, microsatellite instability				Pop. From Florence Italy, found MSI neg tumors sig. reduced by hi consumption of garlic & onions	Palli et al., 2001
Diallyl Sulfide	NR	male F344 rats	5 weeks	intrgastric intub	200 mg/kgbw 3 X per week for 6 weeks	8 weeks	effect on carcinogenic response after nitrosamine treatment		200mg/kg bw	P<0.001	increased GST-P positive foci	Takahashi et al., 1992
Dipropyl trisulfide Dimethyl trisulfide Allyl mercapton Isothiocyanic acid isobutyl ester	NR	male F344 rats	5 weeks	gavage	150, 100, 50, 100 resptiv.	8 weeks	effect on carcinogenic response after diethylnitrosamine treatment		DPT, AM, IAIE doses	P<0.05	increased GST-P positive foci	Takada et al., 1994
garlic	powdered spice	NMRI mice	3-5 mnth	oral	2.5 - 7.5 g/kgbw	30hr post treatment	chromosomal damage	7.5 g/kg bw				Abraham and Kesavan, 1984
garlic cloves	natural	Swiss albino mice	6-8 wk 25-30 g	gavage	100 mg/kgbw per day for 30 or 60 days		chromosomal damage after treatment with arsenic		100 mg/kg bw	P<0.01	increased chromosomal damage from control but decreased chromosomal damage caused by arsenic	Choudhury et al., 1997
Dially disulfide	84.30%	BALB/c mice female	6 week	ip	143 mg/kgbw 3 times/wk	35 days	number of tumors		143 mg/kg	P<0.05		Nakagawa et al., 2001
Ajoene	98%	ICR mice male	7 weeks	dermal	50,100 250 ug	18 weeks	Incidence of tumors		50 ug or 1.56 ug/kg bw	P<0.05	inhibited skin tumor formation	Nishikawa et al., 2002
Aqueous garlic extract		Swiss Albino Mice male 28-32 g	9-11 weeks	gavage	125,250,500 extract mg/kgbw for 5 days	1 week	chromosomal damage following gamma radiation exposure		125 mg extract/ kg bw	P<0.05	reduced in vivo chromosomal damage caused by gamma radiation. Would approximate a human dose of 8 g garlic per day	Singh et al., 1996
S-Allylcysteine		Wistar Rats male	6-8 weeks	gavage	200 mg/kgbw every other day	6 weeks	hepatocarcinoma incid lipid peroxidation		200 mg/kg bw	P<0.05	hepatocarcinoma did not occur in animals treated with NDEA and SAC. Sac found to savange free radicals.	Sundaresan and Subramanian, 2003
Aged Garlic Extract (AGE)		hypercholestero-lemic men	mean age 48 yr	oral capsule	2700 mg/day	5 months	dec serum cholesterol by 7-10%		2700 mg/day	P<0.05	Dec. significant at 5 months	Yeh and Liu, 2001
Aged Garlic Extract (AGE), Kyolic		New Zealand white rabbits		oral syringe	800ul/kgbw each day for 6wk	8 weeks	cholesterol levels, aortic arch cholesterol, thoracic aorta fatty streaks		800ul/kg bw	P<0.05	plasma cholest. Not reduced. thoracic aorta fatty streaks red by 64%	Campbell, et al., 2001

Reference List

Garlic Oil

Abraham, S and PC Kesavan. 1984. *Genotoxicity of Garlic, Turmeric and Asafoetida in Mice.* Mutation Research, 136(1984)85-88.

Agency for Healthcare Research and Quality (AHRQ). 2000. Evidence Report/Technology Assessment: Number 20. *Garlic: Effects on Cardiovascular Risks and Disease, Protective Effects Against Cancer, and Clinical Adverse Effects.* AHRQ Publication Number 01-E022.

Amagase, H and JA Milner. 1993. *Impact of Various Sources of Garlic and Their Constituents on 7,12-dimethylbenz[a]anthracene Binding to Mammary Cell DNA.* Carcinogenesis Vol. 14 No. 8, pp 1627-1631, 1993.

Anibarro MD, B, JL Fontela MD, and F De La Hoz PhD. 1997. *Occupational Asthma Induced by Garlic Dust.* J Allergy Clin Immunol, Volume 100, Number 6, Part 1.

Borek, C. 2001. *Antioxidant Health Effects of Aged Garlic Extract*. American Society for Nutritional Sciences, 0022-3166/01.

Campbell, J, JL Efendy, NJ Smith, and GR Campbell. 2001. *Molecular Basis by Which Garlic Suppresses Atherosclerosis.* American Society for Nutritional Sciences, 0022-3166/01.

Choudhury, AR, T Das, A Sharma, and G Talukder. 1997. *Inhibition of Clastogenic Effects of Arsenic Through Continued Oral Administration of Garlic Extract in Mice In Vivo.* Mutation Research 392 (1997) 237-242.

Dong, Y, D Lisk, E Block, and C Ip. 2001. *Characterization of the Biological Activity of ?-Glutamyl-Se-methylselenocysteine: A Novel, Naturally Occurring Anticancer Agent from Garlic.* Cancer Research 61, 2923-2928, 2001.

Hattori, A, N Yamada, T Nishikawa, H Fukuda, and T Fujino. 2001. *Protective Effect of Ajoene on Acetaminophen-induced Hepatic Injury in Mice.* Biosci, Biotechnol, Biochem, 65 (11), 2555-2557, 2001.

Hsing, AW, AP Chokkalingam, YT Gao, MP Madigan, J Deng, G Gridley, and JF Fraumeni, Jr. 2002. *Allium Vegetables and Risk of Prostate Cancer: A Population-Based Study.* Journal of the National Cancer Institute, Vol. 94, No. 21, November 6, 2002.

Knowles, LM and JA Milner. 2000. *Diallyl Disulfide Inhibits p34^{cdc2} Kinase Activity Through Changes in Complex Formation and Phosphorylation.* Carcinogenesis Vol. 21 No. 6 pp. 1129-1134, 2000.

Lamm, D, and DR Riggs. 2001. *Enhanced Immunocompetence by Garlic: Role in Bladder Cancer and Other Malignancies.* American Society for Nutritional Sciences. 0022-3166/01.

Lee, JH, HS Kang, and J Roh. 1999. *Protective Effects of Garlic Juice Against Embryotoxicity of Methylmercuric Chloride Administered to Pregnant Fischer 344 Rats.* Yonsei Medical Journal, Vol. 40, No. 5, pp. 483-489, 1999.

Milner, JA. 2001. *A Historical Perspective on Garlic and Cancer.* American Society for Nutritional Sciences 0022-3166/01.

Nakagawa, H, K Tsuta, K Kiuchi, H Senzaki, K Tanaka, K Hioki, and A Tsubura. 2001. *Growth Inhibitory Effects of Diallyl Disulfide on Human Breast Cancer Cell Lines.* Carcinogenesis Vol. 22 No. 6, pp. 891-897, 2001.

National Cancer Institute (NCI). 2002. *Garlic and Cancer Prevention*. November 27, 2002. NCI Fact Sheet.

National Institutes of Health (NIH). 2001. *Garlic Supplements Can Impede HIV Medication.* NIH News Release, December 5, 2001.

Nishikawa, T, N Yamada, A Hattori, H Fukuda, and T Fujino. 2002. *Inhibition by Ajoene of Skin-tumor Promotion in Mice.* Biosci, Biotechnol, Biochem, 66 (10), 2221-2223, 2002.

Palli, D, A Russo, L Ottini, G Masala, C Saieva, A Amorosi, A Cama, C D'Amico, M Falchetti, R Palmirotta, A Decarli, RM Costantini, and JF Fraumeni, Jr. 2001. *Red Meat, Family History, and Increased Risk of Gastric Cancer with Microsatellite Instability.* Cancer Research 61, 5415-5419, July 15, 2001.

Peng, Q, AR Buz'Zard, BHS Lau. 2002. *Neuroprotective Effect of Garlic Compounds in Amyloid-B Peptide-Induced Apoptosis In Vitro.* Med Sci Monit, 2002; 8(8): BR328-337.

Seuri, M, A Taivanen, P Ruoppi, and H Tukiainen. 1993. *Three Cases of Occupational Asthma and Rhinitis Caused by Garlic.* Clinical and Experimental Allergy, 1993, Volume 23, pages 1011-1014.

Shirin, H, JT Pinto, Y Kawabata, JW Soh, T Delohery, SF Moss, V Murty, RS Rivin, PR Holt, and IB Weinstein. 2001. *Antiproliferative Effects of S-Allylmercaptocysteine on Colon Cancer Cells When Tested Alone or in Combination with Sulindac Sulfide.* Cancer Research 61, 725-731, January 15, 2001.

Singh, SP, SK Abraham, and PC Kesavan. 1996. *Radioprotection of Mice Following Garlic Pretreatment.* Br J Cancer Suppl 1996 Jul;27:S102-4.

Sumioka, I, T Matsura, S Kasuga, Y Itakura, and K Yamada. 1998. *Mechanisms of Protection by S-Allylmercaptocysteine Against Acetaminophen-Induced Liver Injury in Mice*. Jpn. J. Pharmacol. 78,199-207.

Sundaresan, S and P Subramanian. 2003. *S-Allylcysteine Inhibits Circulatory Lipid Peroxidation and Promotes Antioxidants in N-Nitrosodiethylamine-Induced Carcinogenesis.* Pol. J Pharacol., 2003, 55, 37-42.

Takada, N, M Kitano, T Chen, Y Yano, S Otani, and S Fukushima. 1994. *Enhancing Effects of Organosulfur Compounds from Garlic and Onions on Hepatocarcinogenesis in Rats: Association with Increased Cell Proliferation and Elevated Ornithine Decarboxylase Activity.* Jpn. J. Cancer Res. 85, 1067-1072, November 1994.

Takahashi, S, K Hakoi, H Yada, M Hirose, N Ito, and S Fukushima. 1992. *Enhancing Effects of Diallyl Sulfide on Hepatocarcinogenesis and Inhibitory Actions of the Related Diallyl Disulfide on Colon and Renal Carcinogenesis in Rats.* Carcinogenesis Vol. 13 No. 9, pp.1513-1518, 1992.

United States Environmental Protection Agency (USEPA). 1992a. Reregistration Eligibility Document. *Allium Sativum (Garlic)* List D, Case 4007. Office of Pesticide Programs.

United Stated Environmental Protection Agency (USEPA). 1992b. *Allium Sativum (Garlic).* Office of Prevention, Pesticides and Toxic Substances. 738-F-92-015 June 1992.

Warshafsky MD, S, R Kamer MD, and S Sivak MD. 1993. *Effect of Garlic on Total Serum Cholesterol – A Meta-analysis.* American College of Physicians 1993.

Yang, C, S Saranjit, K Chabra, JY Hong, and TJ Smith. 2001. *Mechanisms of Inhibition of Chemical Toxicity and Carcinogenesis by Diallyl Sulfide (DAS) and Related Compounds from Garlic.* American Society for Nutritional Sciences, 0022-3166/01.

Yeh, Y and L Liu. 2001. *Cholesterol-Lowering Effect of Garlic Extracts and Organosulfur Compounds: Human and Animal Studies.* American Society for Nutritional Sciences, 0022-3166/01.

You, WC, L Zhang, KF Pan, J Jiang, PhD, YS Chang, GI Perez-Perez, WD Liu, JL Ma, MH Gail, MJ Blaser, JF Fraumeni, Jr., and GW Xu. 2001. *Helicobacter Pylori Prevalence and CagA Status Among Children in Two Counties in China with High and Low Risks of Gastric Cancer.* Ann Epidemiol 2001; 11:543-546.

4.5 Synergists

Synergists are included in pesticide products to enhance their efficacy of the product. PBO is the only synergist included in the primary list of vector control agents

4.5.1 Piperonyl Butoxide (PBO)

Background

PBO was developed in 1947 and is used in agricultural and nonagricultural formulations (IARC, 1983). PBO is a chemical that is added to certain pesticide products as a synergist. PBO inhibits cytochrome P450 enzymes, which are responsible for the metabolism of many compounds, including pesticides. When added to products such as pyrethroid pesticides, PBO enhances pesticidal activity by inhibiting the ability of an organism to detoxify the pyrethroid. This effectively decreases the dose of the pyrethroid product needed to be effective (NTP, 1979). PBO also inhibits this detoxification mechanism in mammals though higher doses are required relative to insects (Westchester County, 2002).

Human Exposure

A recent study that monitored air exposures to pregnant minority women that lived in urban settings found concentrations of various pesticides, including PBO. The highest concentrations were found when can sprays or bombs had been used for pest control (Whyatt et al., 2002).

Absorption/Distribution/Excretion

The dermal absorption of PBO has been studied in human volunteers. In one study, dermal absorption was measured following a single application to the forearm. Radiolabeled PBO was left on the arm for eight hours, after which it was washed off. The majority of the applied PBO remained on the skin unabsorbed. Approximately 1.8 percent of the applied dose was excreted in the urine, when unformulated PBO was applied to the skin. Absorption was lower (0.47 percent) with formulated PBO. Trace amounts of the dermally applied PBO was detected in the feces. Three primary metabolites were detected in the urine and though they were not identified, they were different than the metabolites found in mice after exposure to PBO (Selim et al., 1999). In a similar study with human volunteers, 2 percent of the dose applied to the arm was absorbed (Wester et al., 1994 [as cited in WHO, 1995]).

Two days after oral administration of PBO to mice at doses of 2- 3.4 mg/kg bw, 72-97 percent was recovered; 76 percent in expired air, 6 percent in urine, 4 percent in feces and 7 percent remained in the carcass (Kamienski and Casida, 1970 [as cited in WHO, 1995]). It appears from animal studies that when higher doses are administered (e.g., 500 mg/kg bw) more PBO is excreted in the urine and feces (Selim, 1985 [as cited in WHO, 1995]). Peak concentrations in the blood were reached three to twelve hours after an oral administration (Fishbein et al., 1969 [as cited in WHO, 1995]).

PBO undergoes metabolic transformation once absorbed, with over 10 metabolites observed, though not identified. Un-metabolized PBO was only detected in fat and lung tissue (Fishbein et al., 1969 [as cited in WHO, 1995]).

Toxicity

Metabolic Inhibition

The synergistic properties of PBO arise from its ability to inhibit microsomal mixed function oxidase enzymes (WHO, 1995). PBO exerts this inhibitory effect through a metabolite that binds to the heme (iron) portion of microsomal enzymes (Liu et al., 2001). This group of enzymes is important in the metabolism of many compounds. In some cases, this group of enzymes may transform the compound into an inactive metabolite, as is the case with pyrethroids. PBO increases the potency of pyrethroids by decreasing metabolic degradation of the parent compounds. Sometimes, metabolic transformation leads to a more potent compound. This occurs with some chemical carcinogens and drugs. Co-administration with PBO in this case would lead to a decreased potency. For example, acetaminophen can be toxic at sufficient doses due to a toxic metabolite. When mice are treated with toxic doses of acetaminophen along with PBO, the resulting toxicity is reduced (Brady et al., 1988 [as cited in WHO, 1995]). Similarly, airway resistance that results from parathion exposure is blocked when PBO is also administered (Segura et al., 1999). Therefore, it can be expected that co-exposure to PBO may effect the toxicity of other chemicals, by either enhancing or reducing toxicity.

When PBO is administered daily for more than a week, enlargement of the liver is observed, along with an increase in activity of some liver enzymes. For example, in a study in which rats were fed PBO in the diet at concentrations ranging from 5,000 to 10,000 ppm, enzyme activity

was increased two to four times. However, dietary concentrations of 1,000 ppm did not cause an increase in the size of the liver, nor enzymatic activity (Goldstein et al., 1973 [as cited in WHO, 1995]). In an *in vitro* study using rat liver cells, PBO was also found to increase the activity of a specific form of a liver enzyme, while other forms were inhibited (Heder et al., 2001).

Neurological Effects

No information on neurological effects from PBO exposure was found in the literature.

Skin and Eye Irritation

Human Evidence

There have been case reports of workers who use flea dip products containing PBO and pyrethrin developing symptoms, such as skin and eye irritation, when adequate personal protection was not used (Mehler et al., 1999). During the time period 1989 and 1997, 16 cases of pesticide illness were reported in California, Washington and Texas. Two of these reports involved systemic symptoms with the use of pyrethrin/PBO products and seven involved localized symptoms such as chemical conjunctivitis. A Toxic Exposure Surveillance System (TESS) maintained by the American Association of Poison Control Centers was also reviewed and found 20 similar reports during the years 1993 and 1996. Five of the cases involved pyrethrin and PBO. It is difficult to distinguish what contribution PBO may have had in these exposures (Mehler et al., 1999).

Animal Evidence

No ocular effects were seen when rats were exposed to air concentrations ranging from 15 to 512 mg/m^3 for six hours a day, five days a week for thirteen weeks (Newton, 1992 [as cited in WHO, 1995]). When PBO was applied directly to the eyes of laboratory rabbits, irritation was observed, but recovery was complete after 72 hours (Romanelli, 1991b [as cited in WHO, 1995]).

PBO applied to the clipped skin of rabbits produced very mild irritation (Romanelli, 1991a [as cited in WHO, 1995]).

Gastrointestinal Effects

Human Evidence

No information was found on GI effects in humans.

Animal Evidence

In a two-year study in which rats were given diets containing PBO at concentrations of 5,000 or 10,000 ppm, GI lesions were noted (Maekawa et al., 1985 [as cited in WHO, 1995]). A dose-related increase in ulcers and hemorrhage of the colon was also observed in this study. In another study in which even higher doses were used, hemorrhaging of the upper colon lead to a high rate of mortality in rats at does of 24,000 ppm in the diet (or approximately 2,000 mg/kg bw per day) . Liver weights in females were also increased at doses of 6,000 ppm (537 mg/kg bw per day) (Takahashi et al., 1994b [as cited in WHO, 1995]).

Respiratory Effects

Human Evidence

No information on respiratory effects in human was found.

Animal Evidence

Rats exposed to air concentrations of 5.9 g/L for four hours experienced tearing, salivation, nasal discharge and difficulty in breathing (Hoffman, 1991 [as cited in WHO, 1995]). In a longer exposure study, rats were exposed to concentrations ranging from 15 to 512 mg/m^3 for six hours a day, five days a week for thirteen weeks. Irritant effects were seen in the larynx at the highest dose tested (512 mg/m^3), as well as nasal discharge at 155 and 512 mg/m^3 (Newton, 1992 [as cited in WHO, 1995]).

Immunologic Response

Human Evidence

An animal keeper who had developed neurological symptoms after working with animals for 13 years was tested for immunologic symptoms. She had an allergic response to pyrethroid pesticides, but this response was not affected by co-exposure to PBO. In addition in a

lymphocyte-stimulating test, pyrethroids caused a significant decrease in lymphocyte proliferation in this individual, though PBO did not affect this response (Mitsche et al., 2000).

Immunologic responses were measured in blood taken from volunteers, some who tend to have allergies (atopic) and others who do not. After exposure to PBO alone, histamine release was significantly increased in the blood from atopic (allergic) individuals (Diel et al., 1999a; Diel et al., 1999b).

Animal Evidence

In a skin sensitization study, guinea pigs were treated with PBO three times a week for three weeks. No contact sensitization was observed after a subsequent challenge with PBO (Romanelli, 1991b [as cited in WHO, 1995]).

Some studies indicate that PBO depletes immune system T-cells in the spleen and thymus (Mitsumori et al., 1996 [as cited in Emerson et al., 2001]). These immune system cells have been implicated in some autoimmune diseases, such as multiple sclerosis. In an experiment in which an animal model of multiple sclerosis can be induced, administration of PBO before symptoms result was shown to decrease the severity of symptoms (Emerson et al., 2001). In this study, 600mg/kg bw was administered to mice through injections under the skin. The authors of this study suggest that PBO reduces the activation and proliferation of T-cells so that the immune response, and resulting multiple sclerosis syndrome, is inhibited.

Endocrine Disruption

No information was found regarding effects of PBO on the endocrine system.

Developmental and Reproductive Effects

Human Evidence

No information on developmental or reproductive effects in humans was found.

Animal Evidence

Treatment of pregnant rats during the period of organogenesis did not affect the developing fetus. However, three-generation reproductive studies have noted a reduction in the number of pregnancies and offspring (IARC, 1983).

Since that review by IARC, additional studies have been conducted. Mice that were fed diets containing 1,500, 3,000 or 6,000 ppm of PBO before mating, during gestation and until pups were eight weeks old, had lower pup weights at birth for all dose levels (Tanaka, 1992 [as cited in WHO, 1995]). Results of behavioral testing indicated effects in pups exposed to the high dose.

In a two-generation study in which mice were provided diets containing 100 to 8,000 ppm PBO, litter weight was significantly reduced at the highest dose tested in the first generation and significantly reduced at all dose levels in the second generation. The survival index was also reduced significantly in pups in both generations at the highest dose. In the second generation, the litter size was significantly reduced at 4,000 and 8,000 ppm (Tanaka et al., 1992 [as cited in WHO, 1995]).

A two-generation study in rats had similar results in that pup body weight was reduced at a dietary concentration of 5,000 ppm (350 mg/kg bw per day). However, survival was not affected by the treatment. The NOAEL for this study was determined to be 1,000 ppm or 68 mg/kg bw per day (Robinson et al., 1986 [as cited in WHO, 1995]).

Embryo and fetotoxicity has been observed in mice, rats and rabbits exposed to PBO. Mice receiving a single dose of 1,065 mg/kg bw during gestation had offspring with reduced body weight at birth and other fetotoxic effects (Tanaka et al., 1994 [as cited in WHO, 1995]).

In some studies, the NOAEL for fetotoxicity is above the NOAEL for maternal toxicity, as noted by decreases in maternal body weight gain. A study in which pregnant rats were given 300 or 1,000 mg/kg bw per day during gestation, did not observe reproductive toxicity, nor fetotoxic effects, but a decrease in maternal body weight gain at both doses was noted (Kennedy et al., 1977 [as cited in WHO, 1995]). Similarly, in another study the maternal toxicity NOAEL was 200 mg/kg bw, whereas the fetotoxicity NOAEL was 1,000 mg/kg bw (the highest dose tested) (Chun and Neeper-Bradley, 1991 [as cited in WHO, 1995]). Teratogenicity was not noted in a study of rabbits in which doses of 50, 100 and 200 mg/kg bw were administered by gavage. Maternal toxicity, however, was noted at the 100 mg/kg bw dose (Leng et al., 1986 [as cited in WHO, 1995]).

In another study, pregnant rats received doses between 62.5 and 500 mg/kg bw during gestation. No evidence of maternal toxicity or fetotoxicity was observed (Khera et al., 1979 [as cited in WHO, 1995]).

Cancer

The carcinogenicity of PBO has been evaluated by the USEPA and the IARC. IARC evaluated the carcinogenic potential of PBO in 1983 and concluded that PBO was "Not classifiable as to carcinogenicity to humans" due to inadequate evidence (IARC, 1983). A subsequent evaluation was conducted in 1987 and updated in 1998, with no change to this classification (IARC, 1998). The USEPA Office of Pesticide Programs has classified PBO as a Possible Carcinogen (Group C), based upon an increase in liver tumors in mice at high doses (USEPA, 2002). As reported in the Westchester County GEIS, the USEPA recommends a MOE approach to evaluating carcinogenic risks from exposure to PBO (Westchester County, 2002).

Human Evidence

No case reports or epidemiological studies on the carcinogenic potential of PBO in humans were found.

Animal Evidence

Mice that were given PBO in the diet at dosages of 30, 100 or 300 mg/kg bw per day for 78 weeks had a higher incidence of liver adenomas, which is a nonmalignant tumor. At the highest dose, an increase incidence in hepatocellular carcinoma was also observed in males only, though not statistically significant from controls. Liver weights were also increased at 100 and 300 mg/kg bw dose levels. A NOAEL based on the effects to the liver was reported to be 30 mg/kg bw per day (Hermanski and Wagner, 1993 [as cited in WHO, 1995]).

In another 12-month dietary study, mice were given diets containing 6,000 or 12,000 ppm PBO. Hepatocellular adenomas and carcinomas increased in a dose-dependent manner (Takahashi et al., 1994a [as cited in WHO, 1995]). Hepatocellular adenomas and carcinomas were also increased in rats fed diets containing 12,000 and 24,000 ppm of PBO (Takahashi et al., 1994b [as cited in WHO, 1995]).

Other studies have been conducted that have not observed a carcinogenic response. In a two-year study in which rats were given diets containing PBO at concentrations of 5,000 or 10,000 ppm, PBO was not found to be carcinogenic (Maekawa et al., 1985 [as cited in WHO, 1995]). However, GI lesions were noted as discussed in the above section. In a study in which rats were fed diets containing PBO at doses of 30, 100 or 500 mg/kg bw per day for 104-105 weeks an increased incidence of tumors was not observed (Graham, 1987 [as cited in WHO, 1995]). The NOAEL from this study was determined to be 30 mg/kg bw per day, based upon liver effects (WHO, 1995).

NCI conducted a study in which rats and mice were given diets containing PBO. Male and female rats were fed diets containing 5,000 or 10,000 ppm of PBO for 107 weeks. Mice were given diets with a time weighted average dose of 1,036 or 2,804 ppm for 112 weeks. In rats, an increase in lymphomas was observed. However, the incidence was within the historical range for this strain and therefore, could not be specifically attributed to treatment with PBO (NCI, 1979). In mice, adenomas of the lacrimal (tear) gland were observed, but not significantly different than controls (NCI, 1979). NCI concluded that under the conditions of this study, PBO was not carcinogenic to the rat and mouse strains tested.

PBO has been tested in various genotoxicity assays. The overall evidence from mutagenicity studies indicates that PBO is not mutagenic (IARC, 1983). Negative results have been found in bacterial reverse mutation tests, chromosomal aberrations tests, Drosophila wing spot test and sister chromatid exchange tests. It was, however positive in a mouse lymphoma mutation assay (Osaba et al., 1999).

Studies have also been completed that expose animals to PBO along with another chemical. One such study exposed mice to 5 mg PBO via subcutaneous injection alone and in combination with Freon 113 or Freon 112. After 52 weeks, no tumors were found in animals treated with PBO alone, but the incidence of hepatomas was significantly increased in male mice exposed to Freon and PBO as compared to Freon or PBO alone (Epstein et al., 1967 [as cited in WHO, 1995]). In another study, rats were given PBO and pyrethrins in the diet at concentrations of 2,000 and 400 ppm, respectively. No treatment related effects, including neoplasms, were noted (Hunter et al., 1977 [as cited in WHO, 1995]).

Other Toxicity

Liver Toxicity

There have been several short-term exposure studies in many animal species that indicate the liver to be the primary target organ for PBO toxicity. This is consistent with PBO's ability to increase the activity of liver enzymes. Mice exposed via the diet to concentrations ranging from 3,000 to 9,000 ppm for 20 days showed a dose-related increase in liver, as well as kidney weights. Food intake and body weight were found to be decreased. Serum levels of cholesterol, phospholipids and proteins were increased. Of note was a 235 percent increase in the enzyme gamma-glutamyl transpeptidase in females exposed to the highest dose. The NOAEL was 1,000 ppm, corresponding to a dose of 150 mg/kg bw per day (Fujitani et al., 1993 [as cited in WHO, 1995]).

In rats, a four-week exposure study was conducted in which animals were exposed to PBO in the diet at doses ranging from 62.5 to 2,000 mg/kg bw per day. Body weight gain was reduced in females and males at the 500 and 1,000 mg/kg bw per day doses, respectively. Liver weight was increased at doses of 250 mg/kg bw per day and higher. The relative weight of the adrenal glands, kidneys and brain were also increased. The NOAEL reported in this study was 125 mg/kg bw per day, based upon the effects on the liver (Modeweg-Hansen et al., 1984 [as cited in WHO, 1995]).

Liver effects were also observed when laboratory animals were exposed via inhalation. In one study, rats were exposed to air concentrations of 15 to 512 mg/m^3 for six hours a day, five days a week for thirteen weeks. Increase in liver weight was observed at 155 and 512 mg/m^3 concentrations. Microscopic changes in hepatocytes (liver cells) were observed as well. An increase in kidney weight was seen at the highest dose tested (512 mg/m^3) (Newton, 1992 [as cited in WHO, 1995]).

Effects on the liver were also observed in dogs exposed via the diet for eight weeks at concentrations of 500 to 3,000 ppm. Treatment-related microscopic changes in the liver were observed at all concentrations so that a NOAEL could not be determined (Goldenthal, 1993a [as cited in WHO, 1995]).

Longer-term exposures have resulted in similar observations. Dogs receiving PBO in capsule form at doses between 3 and 320 mg/kg bw per day for one year had a dose-related increase in liver weight. Increased kidney and adrenal gland weights were also observed at doses of 100 and 320 mg/kg bw per day (Sarles and Vandergrift, 1952 [as cited in WHO, 1995]). A NOAEL of 16 mg/kg bw per day was observed in dogs receiving PBO in the diet at concentrations of 100 to 2,000 ppm for one year. This NOAEL was based on the observation of effects on the liver including hypertrophy and histological effects (Goldenthal, 1993b [as cited in WHO, 1995]).

In a study in which rats were fed diets containing PBO at doses of 30, 100 or 500 mg/kg bw per day for 104-105 weeks, increased liver weights were observed at the mid and high dose levels. The NOAEL for this study was 30 mg/kg bw per day. Thyroid, kidney, adrenal and ovarian enlargement was also noted (Graham, 1987 [as cited in WHO, 1995]).

Table 24
Toxicity Study Summary Table - Piperonyl Butoxide (PBO)

Compound	Purity	Test Species (Strain)	Test Species Age/Lifecycle	Route	Dose	Study Duration	Endpoint	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	LOAEL Statistical Significance	Comments	Reference
piperonyl butoxide	88.40%	Fischer 344 rats		diet	5,000, 10,000 ppm	107 weeks	lymphomas dec body wgt		5000 ppm 5000 ppm	P<0.007 for dose trend dose response trend	concl within historical incidence and not clearly treatment related	NCI, 1979
piperonyl butoxide	88.40%	B6C3F1 mice		diet	1036, 2804 ppm	112 weeks	lacrimal gland adenoma dec body wgt		1036 ppm 1036 ppm	P<0.023 for dose trend dose response trend	dose trend significant but each dose group not signif different than controls	NCI, 1979
piperonyl butoxide	not specified	ICR (Crj:CD-1 mice		diet	1000, 3000, 9000 ppm	20 days	incr liver wgt, hypertrophy	150	3000 ppm	dose response trend		Fujitani et al., 1993; as cited in WHO, 1995
piperonyl butoxide	not specified	Sprague-Dawley rats		diet	62.5, 125, 250, 500, 1000, 2000 mg/kg bw day	4 weeks	incr liver wgt, hypertrophy	125	250		incr in adrenals, kidney, and brain also noted.	Modeweg-Hansen et al., 1984; as cited in WHO, 1995
piperonyl butoxide	91%	Charles River CD rats		inhalation	15, 74, 155, 512 mg/m3	6 h/day, 5d/wk 13 weeks	incr liver wgt, hypertrophy nasal discharge	74 74	155 155			Newton, 1992; as cited in WHO, 1995
piperonyl butoxide	90.78%	dogs		diet	100, 600, 2000 ppm	1 year	incr liver wgt	16	960		alkaline phosphatase also incr at highest dose, cholesterol levels decr. Gall bladder wgt incr also	Goldenthal, 1993b; as cited in WHO, 1995
piperonyl butoxide	90.78%	dogs		diet	500, 1000, 2000 3000 ppm	8 weeks	incr liver and gall bladder wgt		500 ppm		Reduced body weight gain at 1000 ppm	Goldenthal, 1993a; as cited in WHO, 1995
piperonyl butoxide	90.78	CD-1 mice		diet	30, 100, 3000 mg/kg bw day	78 weeks	incr liver wgt hepatocellular adenomas	30 30	100 100			Hermanski and Wagner, 1993; as cited in WHO, 1995
piperonyl butoxide	94.30%	Crj:CD-1 mice		diet	6000, 12000 ppm	12 months	body weight hepatocellular adenomas and carcinomas		6000 ppm 6000 ppm			Takahashi et al., 1994a; as cited in WHO, 1995
piperonyl butoxide	89%	Sprague-Dawley Crl:CDR (SD)BR rats		diet	30,100,500 mg/kg bw day	104-105 weeks	incr liver and kidney wgt thyroid, adrenal and ovarian enlargement	30 100	100 500		No tumors noted	Graham, 1987; as cited in WHO, 1995
piperonyl butoxide	89%	Fischer 344/Du Crj rats		diet	5000, 10000 ppm	2 years	neoplasms GI effects, ulcers, inflammatory cell infiltration	10000 ppm	 5000 ppm		Not found to be carcinogenic	Maekawa et al., 1985; as cited in WHO, 1995
piperonyl butoxide	94%	Fischer 344/Du Crj rats		diet	6000, 12000 24000 ppm	104 weeks	incr liver wgt hepatocellular adenomas and carcinomas	 6000 ppm	6000 ppm 12000 ppm			Takahashi et al., 1994b; as cited in WHO, 1995
piperonyl butoxide	unspecified	Crj:CD-1 mice		diet	1000, 2000, 4000 8000 ppm	2 generation	mean F2 litter weight survival index	 4000 ppm	1000 ppm 8000 ppm	reported to be stat. significant reported to be stat. significant	survival decr at 4000, but not signif.	Tanaka et al., 1992; as cited in WHO, 1995
piperonyl butoxide	unspecified	Crj:CD-1 mice		diet	1500, 3000, 6000 ppm	4 wks prior to mating thru 8 wk post partum	pup body weight reduced		1500 ppm			Tanaka, 1992; as cited in WHO, 1995
piperonyl butoxide	unspecified	Sprague-Dawley rats	7 weeks	diet	300, 1000, 5000 ppm	85 days prior to mating thru 2 matings	parental tox and pup develop	68	350			Robinson et al., 1986; as cited in WHO, 1995
piperonyl butoxide	>95%	Crj:CD-1 mice		gavage	1065, 1385, 1800 mg/kg bw day	gestation day 9	maternal body wgt gain fetal body wgt, exencephaly craniochisis, kinky tail etc	1800	 1065		no maternal tox noted	Tanaka et al., 1995; as cited in WHO, 1995
piperonyl butoxide	tech grade	COBS random-bred albino rats		gavage	300, 1000 mg/kg bw day	gest. day 6-15	maternal body wgt gain fetal body wgt	 1000	1000		no fetotox noted	Kennedy et al., 1977; as cited in WHO, 1995
piperonyl butoxide	80%	Wistar rats		gavage	62.5, 125, 250, 500 mg/kg bw/d	gest. day 6-15	maternal tox, fetotox, embryo tox, tertogenicity	500			no effects noted	Khera et al., 1979; as cited in WHO, 1995
piperonyl butoxide	90.78%	Sprague-Dawley CD rats		gavage	200, 500, 1000 mg/kg bw day	gest. day 6-15	decr maternal body wgt gain fetotox	200 1000	500		no fetotox effects	Chun and Neeper-Bradley, 1991; as cited in WHO, 1995
piperonyl butoxide	100%	New Zealand white rabbits		gavage	50, 100, 200 mg/kg bw day	gest day 7-19	decr maternal body wgt gain fetotox	50 200	100		no fetotox effects	Leng et al., 1986; as cited in WHO, 1995
piperonyl butoxide	not reported	SJL mice		sub cutaneous	600 mg/kg bw day	day 1-9	dec T-cell response		600		inhibited T-cell response to agent which causes allergic encephal omyelitis	Emerson et al., 2001

Table 25

Noncancer Criteria (Oral/Dermal)- Piperonyl Butoxide

Chemical of Potential Concern	Exposure Duration	Criteria	Value	Units	Species	Endpoint	NOAEL/ LOAEL	Units	Combined Uncertainty/Modifying Factors	Study Source	Reference for Criteria
Piperonyl Butoxide	acute	RfD	2.00E+00	mg/kg/day	rats	reduced maternal body wgt gain	2.00E+02	mg/kg/day	100	MRID 42380801	USEPA, 2000; as cited in Westchester, 2002
Piperonyl Butoxide	sub-chronic	RfD	1.75E-01	mg/kg/day	dogs	body and liver wgt	17.5/75	mg/kg/day	100	USEPA, 1996	Westchester, 2002
Piperonyl Butoxide	acute and sub-chronic	RfD-dermal	1.00E+01	mg/kg/day	rabbits	highest dose tested	1.0E+03	mg/kg/day	100	MRID 42218201	USEPA, 2000; as cited in Westchester, 2002
Piperonyl Butoxide	chronic	RfD-dermal	2.00E-01	mg/kg/day	rats	reduced maternal body wgt gain	2.00E+02	mg/kg/day	1000	MRID 42380801	USEPA, 2000; as cited in Westchester, 2002
Piperonyl Butoxide	chronic	RfD	1.75E-02	mg/kg/day	dogs	body and liver wgt	17.5/75	mg/kg/day	1000	USEPA, 1996	Westchester, 2002
Piperonyl Butoxide	chronic	ADI	2.00E-01	mg/kg/day	dogs	incr liver wgt	1.60E+01	mg/kg/day	100	Goldenthal, 1993b	WHO, 1995

NA: Not Applicable or Not Available.

NOAEL: no observed adverse effect level

LOAEL: lowest observed adverse effect level

RfD: USEPA reference dose

ADI: World Health Organization Acceptable Daily Intake

Table 26
Noncancer Criteria (Inhalation)- Piperonyl Butoxide

Chemical of Potential Concern	Exposure Duration	Criteria	Value	Units	Endpoint	Species	NOAEL/ LOAEL	Units	Combined Uncertainty/Modifying Factors	Study Source	Reference for Criteria
Piperonyl Butoxide	acute and	RfC	0.00074	mg/L	Inc liver & kidney wgt	rats	0.074	mg/L	100	USEPA, 2000	Westchester, 2002
Piperonyl Butoxide	chronic	RfC	0.000074	mg/L	Inc liver & kidney wgt	rats	0.074	mg/L	1000	USEPA, 2000	Westchester, 2002

RfC: Reference Concentration
NA: Not Applicable or Not Available.
NOAEL: no observed adverse effect level
LOAEL: lowest observed adverse effect level

Table 27
Cancer Criteria (Oral/Dermal)

Chemical of Potential Concern	Margin of Exposure	Threshold Dose	Units	Weight of Evidence/ Cancer Guideline Description	Source	Reference
Piperonyl Butoxide	10	0.0175	mg/kg-day	Group C, possible	USEPA OPP	USEPA, 2002
				human carcinogen		

OPP: Office of Pesticide Programs, USEPA

Reference List

Piperonyl Butoxide

Brady, JT, DA Montelius, WP Beierschmitt, DS Wyand, EA Khairallah, and SD Cohen. 1988. *Effect of Piperonyl Butoxide Post-Treatment on Acetaminophen Hepatoxicity.* Biochem Pharmacol. 1988 May 15;37(10):2097-9.

Chun, JS and TL Neeper-Bradley. 1991. *Developmental Toxicity Evaluation of Piperonyl Butoxide Administered by Gavage to CD (Sprague-Dawley) Rats.* Unpublished report No. 54-586 from Bushy Run Research Center, Export, Pennsylvania, USA. Submitted to WHO by Endura SA, Bologna, Italy for the Piperonyl Butoxide Task Force, Washington DC, USA. As cited in WHO, 1995.

Diel, F, B Horr, H Borck, H Savtchenko, T Mitsche, and E Diel. 1999a. *Pyrethroids and Piperonyl-Butoxide Affect Human T-Lymphocytes In Vitro.* Toxicology Letters 107 (1999) 65-74.

Diel, F, B Horr, M Detscher, L Sosnovikova, and H Borck. 1999b. *Pyrethroids and the Synergist Piperonyl-Butoxide (PBO) Affect T-Cells and Basophils.* Inflamm. Res. 48. Supplement I (1999) S15-S16.

Emerson, M, S Biswas, and S LeVine S. 2001. Cuprizone and Piperonyl Butoxide, Proposed Inhibitors of T-Cell Function, Attenuate Experimental Allergic Encephalomyelitis in SJL Mice. Journal of Neuroimmunology 119 (2001) 205-213.

Epstein, SS, S Joshi, J Andrea, P Clapp, H Falk, and N Mantel. 1967. *Synergistic Toxicity and Carcinogenicity of 'Freons' and Piperonyl Butoxide.* Nature, 214, 526-528. As cited in WHO, 1995.

Fishbein, L, HL Falk, J Fawker, S Jordan, and B Corbett. 1969. *The Metabolism of Piperonyl Butoxide in the Rat with ^{14}C in the Methylenedioxy or Alpha-Methylene Group.* J. Chromatogr., 41, 61-79. As cited in WHO, 1995.

Fujitani, T, T Tanaka, Y Hashimoto, and M Yoneyama. 1993. *Subacute Toxicity of Piperonyl Butoxide in ICR Mice.* Toxicology, 83, 93-100. As cited in WHO, 1995.

Gold, L, N Manley, T Stone, and L Rohrbach. 1999. *Supplement to the Carcinogenic Potency Database (CPDB): Results of Animal Bioassays Published in the General Literature in 1993 to 1994 and by the National Toxicology Program in 1995 to 1996.* Environmental Health Perspectives Supplements Volume 107, Number S4 August 1999.

Goldenthal, EI. 1993a. *Evaluation of Piperonyl Butoxide in and Eight Week Toxicity Study in Dogs.* Unpublished report No. 542-004 from International Research and Development Corp., Mattawan, Michigan, USA. Submitted to WHO by Endura SA, Bologna, Italy, for the Piperonyl Butoxide Task Force, Washington DC, USA. As cited in WHO, 1995.

Goldenthal, EI. 1993b. *Evaluation of Piperonyl Butoxide in a One Year Chronic Dietary Toxicity Study in Dogs.* Unpublished report No, 542-005 from International Research and Development Corporation, Mattawan, Michigan, USA. Submitted to WHO by Endura SA, Bologna, Italy, for the Piperonyl Butoxide Task Force, Washington DC, USA. As cited in WHO, 1995.

Goldstein, JA, P Hickman, and RD Kimbrough. 1973. *Effects of Purified and Technical Piperonyl Butoxide on Drug Metabolizing Enzymes and Ultrastructure of Rat Liver.* Toxicol. Appl. Pharmacol., 26, 444-458. As cited in WHO, 1995.

Graham, C. 1987. *24-Month Dietary Toxicity and Carcinogenicity Study of Piperonyl Butoxide in the Albino Rat.* Unpublished Report No. 81690 from Bio-Research Ltd Laboratory, Seneville, Quebec, Canada. Submitted to WHO by Endura SA, Bologna, Italy, for the Piperonyl Butoxide Task Force, Washington DC, USA. As cited in WHO, 1995.

Heder, A, K Hirsch-Ernst, D Bauer, G Kahl, and H Desel. 2001. *Induction of Cytochrome P450 2B1 by Pyrethroids in Primary Rat Hepatocyte Cultures.* Biochemical Pharmacology 62 (2001) 71-79.

Hermanski, SJ and CL Wagner. 1993. *Chronic Dietary Oncogenicity Study With Piperonyl Butoxide in CD-1 Mice.* Unpublished report No. 91N0134 from Bushy Run Research Center. Export, Pennsylvania, USA. Submitted to WHO by Endura SA, Bologna, Italy, for the Piperonyl Butoxide Task Force, Washington DC, USA. As cited in WHO, 1995.

Hoffman, GM. 1991. *An Acute Inhalation Toxicity Study of Piperonyl Butoxide in the Rat.* Unpublished report No. 91-8330 from Bio/dynamics, East Millstone, New Jersey, USA. Submitted to WHO by Endura SA, Bologna, Italy, for the Piperonyl Butoxide Task Force, Washington DC, USA. As cited in WHO, 1995.

Hunter, B, JL Bridges, and DE Prentice. 1977. *Long Termyrethri Feeding of Pns and Piperonyl Butoxide to Rats.* Unpublished report from Huntington Research Centre, Huntington, Cambs, United Kingdom. Submitted to WHO by Endura SA, Bologna, Italy, for the Piperonyl Butoxide Task Force, Washington DC, USA. As cited in WHO, 1995.

International Agency for Research on Cancer (IARC). 1983. *Piperonyl Butoxide.* Vol. 30(1983) (p. 183).

Kamienski, FX and JE Casida. 1970. *Importance of Demthylenation in the Metabolism In Vivo and In Vitro of Methylenedioxphenyl Synergists and Related Compounds in Mammals.* Biochem. Pharmacol. 19, 91-112. As cited in WHO, 1995.

Karpati, AM, MC Perrin, T Matte, J Leighton, J Schwartz, and RG Barr. 2004. Pesticide Spraying for West Nile Virus Control and Emergency Department Asthma Visits in New York City, 2000. *Environmental Health Perspectives*, Volume 112, No. 11, pages 1183-1187. August.

Kennedy, GL, SH Smith, FK Kinoshita, ML Keplinger, and JC Calandra. 1977. *Teratogenic Evaluation of Piperonyl Butoxide in the Rat.* Food Cosmet. Toxicol. 15, 337-339. As cited in WHO, 1995.

Khera, KS, C Whalen, G Angers, and G Trivett. 1979. *Assessment of the Teratogenic Potential of Piperonyl Butoxide, Biphenyl and Phosalone in the Rat.* Toxicol. Appl. Pharmacol. 47, 353-358. As cited in WHO, 1995.

Leng, JM, CA Schwartz, and JL Schardein. 1986. *Teratology Study in Rabbits.* Unpublished Report No. 542-002 from International Research and Development Corp., Mattawan, Michigan, USA. Submitted to WHO by Endura SA, Bologna, Italy, for the Piperonyl Butoxide Task Force, Washington DC, USA. As cited in WHO, 1995.

Liu, H, SV Shah, and R Baliga. 2001. *Cytochrome P-450 as a Source of Catalytic Iron in Minimal Change Nephrotic Syndrome in Rats.* Am J Physiol Renal Physiol 280:F88-F94, 2001.

Maekawa, A, H Onodera, K Furuta, H Tanigawa, T Ogiu, and Y Hayashi. 1985. *Lack of Evidence of Carcinogenicity of Technical-Grade Piperonyl Butoxide in F344 Rats: Selective Induction of Ileocaecal Ulcers.* Food Chem. Toxicol. 7, 675-682. As cited in WHO, 1995.

Mehler, L, J Shannon, and L Baum. 1999. *Illness Associated with Occupational Use of Flea-Control Products—California, Texas and Washington, 1989-1998.* Morbidity and Mortality Weekly Report, June 4, 1999/48(21);443-447.

Mitsche, T, H Borck, B Horr, N Bayas, HW Hoppe, and F Diel. 2000. *Pyrethroid Syndrome in an Animal Keeper*. Allergy55(1):93-4.

Mitsumori, K, K Takegawa, T Shimo, H Onodera, K Yasuhara, and M Takahashi. 1996. *Morphometric and Immunohistochemical Studies on Atrophic Changes in Lympho-hematopoietic Organs of Rats Treated with Piperonyl Butoxide or Subjected to DietaryRestriction.* Arch. Toxicol. 70, 809-814. As cited in Emerson et al., 2001.

Modeweg-Hansen, L, M Lalande, C Bier, G Losos, and BE Osborne. 1984. *A Dietary Dose Range-Finding Study of Piperonyl Butoxide in the Albino Rat.* Unpublished report No. 81802B from Bio-Research Laboratories, Edgewater, Maryland, USA. Submitted to WHO by Endura SA, Bologna, Italy, for the Piperonyl Butoxide Task Force, Washington DC, USA. As cited in WHO, 1995.

National Cancer Institute (NCI). 1979. *Bioassay of Piperonyl Butoxide For Possible Carcinogenicity*. U.S. Department of Health, Education and Welfare.

National Library of Medicine (NLM). 2004. *Hazardous Substance Data Bank (HSDB) for Piperonyl Butoxide*. URL http://toxnet.nlm.nih.gov/cgi-bin/sis/search.

National Toxicology Program (NTP). 1979. *TR-120 Bioassay of Piperonyl Butoxide for Possible Carcinogenicity (CAS No. 51-03-6).* National Institute of Environmental Health Sciences, National Institutes of Health.

Newton PE. 1992. *A Subchronic (3 month) Inhalation Toxicity Study of Piperonyl Butoxide in the Rat Via Whole-Body Exposures.* Unpublished Report No. 91-8333 from Bio/dynamics, Eat

Millstone, New Jersey, USA. Submitted to WHO by Endura SA, Bologna, Italy, for the Piperonyl Butoxide Task Force, Washington DC, USA. As cited in WHO, 1995.

Osaba, L, A Aguirre, A Alonso, and U Graf. 1999. *Genotoxicity Testing of Six Insecticides in Two Crosses of the Drosophila Wing Spot Test.* Mutation Research 439 (1999) 49-61.

Robinson, K, L Pinsonneault, and BG Procter. 1986. *A Two-Generation (Two Litter) Reproduction Study of Piperonyl Butoxide Administered in the Diet to the Rat.* Unpublished report No. 81689 from Bio-Research Laboratories Ltd, Montreal, Quebec, Canada. Submitted to WHO by Endura SA, Bologna, Italy, for the Piperonyl Butoxide Task Force, Washington DC, USA. As cited in WHO, 1995.

Romanelli, P. 1991a. *Primary Skin Irritation-Rabbits.* Unpublished Report No. 91-7317A from Biosearch Inc., Philadelphia, Pennsynvania, USA. Submitted to WHO by Endura SA, Bologna, Italy, for the Piperonyl Butoxide Task Force, Washington DC, USA. As cited in WHO, 1995.

Romanelli, P. 1991b. *Primary Eye Irritation-Rabbits.* Unpublished Report No. 91-7317A from Biosearch Inc., Philadelphia, Pennsylvania, USA. Submitted to WHO by Endura SA, Bologna, Italy, for the Piperonyl Butoxide Task Force, Washington DC, USA. As cited in WHO, 1995.

Sarles, MP and WB Vandegrift. 1952. *Chronic Oral Toxicity and Related Studies on Animals with the Insecticide and Pyrethrum Synergist, Piperonyl Butoxide.* Am. J. Trop. Med. Hyg., 1, 862-883. As cited in WHO, 1995.

Segura, P, J Chavez, LM Montano, MH Vargas, A Delaunois, V Carbajal, and P Gustin. 1999. *Identification of Mechanisms Involved in the Acute Airway Toxicity Induced by Parathion.* Naunyn Schmiedebergs Arch Pharmacol. 1999 Dec; 360(6):699-710.

Selim, S. 1985. *Single and Repeated Oral Dose Pharmacokinetic and Distribution Studies of Piperonyl Butoxide.* Unpublished report No. WP509070/1 from Biological Test Center, Irvine, California, USA. Submitted to WHO by Endura SA. Bologna, Italy for the Piperonyl Butoxide Task Force, Washington DC, USA. As cited in WHO, 1995.

Selim, S, F Preiss, K Gabriel, J Jonkman, and T Osimitz. 1999. *Absorption and Mass Balance of Piperonyl Butoxide Following an 8-h Dermal Exposure in Human Volunteers.* Toxicology Letters 107 (1999) 207-217.

Takahashi, O, T Oishi, T Fujitani, T Tanaka, and M Yoneyama. 1994a. *Piperonyl Butoxide Induces Hepatocellular Carcinoma in Male CD-1 Mice.* Arch. Toxicol. 68, 467-469. As cited in WHO, 1995.

Takahashi, O, T Oishi, T Fujitani, T Tanaka, and M Yoneyama. 1994b. *Chronic Toxicity Studies of Piperonyl Butoxide in F344 Rats: Induction of Hepatocellular Carcinoma.* Fundam. Appl. Toxicol. 22, 293-303. As cited in WHO, 1995.

Tanaka, T. 1992. *Effects of Piperonyl Butoxide on F1 Generation Mice.* Toxicol. Lett., 60, 83-90. As cited in WHO, 1995.

Tanaka, T, O Takahashi, and S Oishi. 1992. *Reproduction and Neurobehavioural Effects in Three-Generation Toxicity Study of Piperonyl Butoxide Administered to Mice.* Food Chem. Toxicol. 30, 1015-1019. As cited in WHO, 1995.

Tanaka, T, T Fujitana, O Takahashi, and S Oishi. 1994. *Developmental Toxicity Evaluation of Piperonyl Butoxide in CD-1 Mice.* Toxicol. Lett., 71, 123-129. As cited in WHO, 1995.

United States Environmental Protection Agency (USEPA). 2000. *Tox Oneliners for Piperonyl Butoxide.* Office of Pesticide Programs. Last Updated May 26, 2000. As cited in Westchester County, 2002.

United States Environmental Protection Agency (USEPA). 2002. *Chemicals Evaluated for Carcinogenic Potential.* Health Effects Division Office of Pesticide Programs, May 8, 2002.

Wester, RC, DAW Bucks, and HI Maibach. 1994. *Human In Vivo Percutaneous Absorption of Pyrethrin and Piperonyl Butoxide.* Food Chem. Toxicol, 32, 51-53. As cited in WHO, 1995.

Westchester County. 2002. *Comprehensive Mosquito-Borne Disease Surveillance and Control Plan*, Generic Environmental Impact Statement. February, 2002.

Whyatt, R, DE Camann, PL Kinney, A Reyes, J Ramirez, J Dietrich, D Diaz, D Holmes, and FP Perera. 2002. *Residential Pesticide Use During Pregnancy Among a Cohort of Urban Minority Women.* Environmental Health Perspectives, Vol. 110/Number 5/May 2002.

World Health Organization (WHO). 1992. *Pesticide Residues in Food: 1992 Evaluations Part II Toxicology; Piperonyl Butoxide.* Joint Meeting on Pesticide Residues (JMPR), Geneva.

World Health Organization (WHO). 1995. *Pesticide Residues in Food 1995.* Toxicological evaluations Piperonyl Butoxide. Joint Meeting on Pesticide Residues (JMPR), Geneva.

5. Domestic Animal Toxicity Literature Reviews

5.1 Larvicides

5.1.1 *Bacillus thuringiensis israelensis (Bti)*

There is no data available on the reproductive or developmental effects of *Bti* on non-target organisms due to a lack of significant disease and toxicity in studies. Extensive studies of *Bti* have not been conducted because the USEPA only requires that further investigation be conducted for microbial pesticides that have shown significant adverse health effects in prior studies, which *Bti* has not (NPTN, 2000a). According to Beasley (2000) when administered to domestic animals orally, *Bti* is practically nontoxic. An acute oral study showed that dosages of up to 10,000 mg/kg did not produce any signs of toxicity (EXTOXNET 1996e).

5.1.2 *Bacillus sphaericus (Bs)*

There was no information found regarding the effects of *Bs* on domestic animals.

5.1.3 *Methoprene*

The acute oral LD_{50} for dogs has been reported as greater than 5,000 mg/kg (EXTOXNET, 1996a; Garg and Donahue, 1989; WHO, 1984a; USEPA, 2001; Beasley, 2000; Siddall, 1976). In a study in which methoprene was fed to dogs at doses of 0, 250, 500 and 5000 ppm in the diet (0, 6.25, 12.5, or 125 mg/kg/day), the No Observed Effect Level (NOEL) was 500 ppm and the Lowest Observed Adverse Effects Level (LOAEL) was 5,000 ppm based on increased liver weights. In addition, there were no irreversible adverse effects at the 5,000 mg/kg level (USEPA, 2001; USEPA, 2002; WHO, 1984a).

Short-term effects of methoprene, were examined when four beagles were administered methoprene via a stomach tube at a dose of 10 g/kg bw. The two females died within 40 minutes of treatment, and one male died after 109 minutes. The final male was euthanized after 3 hours.

Observed clinical signs of exposure included:

- aggressive behavior
- piloerection
- pupil dilation

- salivation
- increased respiratory rate
- shallow breathing
- loss of gait
- convulsions
- vomiting
- opisthotonos (a spasm in which the head and feet bend back, while the spine arches forward)
- nystagmus (rapid, involuntary oscillation of the eyeball)

Gross examination of the bodies revealed congestion of the kidneys, liver, lungs, and scleral vessels, and telangiectasia (dilation of capillaries causing red blotches) was observed in the liver. Evidence of cardiac effects were also observed, as well as signs of congestion in the central nervous system, haematopoietic tissues, the reproductive tract, and the digestive tract (Hill, 1972a [as cited in WHO, 2001]).

During a 21-day study dogs were fed methoprene at doses of 1, 2, or 5 g/kg bw. There were no clinical signs of toxicity observed and, after sacrifice, no pathological changes were found (Hill, 1972b [as cited in WHO, 2001]).

In an inhalation study, dogs were exposed to methoprene mist. The mist had a particle size of 2-5 µm technical grade, and was delivered as a 2 percent aqueous solution for six hours with an estimated dose of 30 mg/kg bw (29 mg/kg bw when corrected for purity). During this study, no animals died. The concentration in air was not reported. The clinical signs observed included increased heart rate and respiration, vomiting, salivation and exhaustion, but it was unclear whether this was the result of methoprene exposure or due to the experimental exposure procedure itself (Saito, 1975 [as cited in WHO 2001]).

The doses in the aforementioned studies experienced by the exposed beagles are extreme and are not relevant to the type of expected exposure from vector control activities, except for accidental ingestion of a container of pesticide. Therefore, as studies have focused on extreme dosages, no

acute (single exposure) or short-term (one to seven days) endpoint for methoprene has been identified (USEPA, 2002). In addition to ingestion studies, methoprene has been found to be non-irritating to the skin or eyes of dogs. Studies have not revealed any clinical signs of toxicosis from methoprene in dogs under realistic conditions (Garg and Donahue, 1989).

5.2 Pyrethrins/Pyrethroids

Pyrethrins and pyrethroids are reported by the National Pesticides Telecommunication Network (1998) as some of the least poisonous pesticides to mammals because they are quickly broken down in the body and eliminated in the urine and feces.

According to Dorman and Beasley (1991) and Murphy (1994), clinical signs associated with toxicosis in dogs and cats include:

- tremors
- increased salivation
- ataxia
- vomiting
- depression
- hyperexcitability
- diarrhea
- seizures
- depression
- dyspnea
- hyper-, then hypothermia
- death

In general, toxicosis develops within hours, but it can be delayed if it results from dermal absorption or grooming. In sublethally exposed animals, the syndrome is considered reversible and most animals recover within 72 hours (Dorman and Beasley, 1991).

5.2.1 Permethrin

According to Grant (1984), when used as recommended , permethrin powder and sprays are characterized by low mammalian toxicity. Martin and Campbell (2000) presented information from the Veterinary Poisons Information Service (VPIS) regarding feline adverse reactions to permethrin-based products. Feline toxicosis from permethrin typically resulted when cats were inadvertently treated with topical flea-control products intended for use on dogs, or when the cats were exposed to companion animals that had been treated with the permethrin products. The clinical signs of toxicosis from permethrin include the following:

- convulsions
- muscle fasciculation/tremor
- salivation/frothing/foaming
- mydriasis (extended dilation of pupil of eye)
- hyperaesthesia (increased sensitivity to stimuli)
- incoordination
- shaking/shivering
- pyrexia/hyperthermia

These symptoms typically have an onset within one to three hours of exposure, but it can be delayed up to 12 hours. Additionally, the symptoms can last three days or more. According to VPIS, permethrin toxicosis in dogs is far less common than permethrin toxicity in cats. In addition to the clinical signs of permethrin toxicosis in cats, Gray (2000) also includes the following:

- Collapse
- Coma
- Blindness
- dyspnea (difficulty breathing)
- loss of smell

- loss of taste
- emesis (vomiting)

Multiple studies have been conducted to examine the effects of permethrin on dogs. In a short-term study, two beagles were administered permethrin at 500 mg/kg bw/day for 14 days. There were no clinical signs of toxicity or significant effects on body weight (WHO, 1990a).

During a three-month study, beagle dogs were fed permethrin in capsules daily at dose levels of 0, 5, 50 or 500 mg/kg bw/day. This study resulted in an NOAEL of 5 mg/kg/day. There was no mortality observed, but at the highest dosage level the dogs exhibited signs of poisoning. Liver to body weight ratios increased at dosages of 50 mg/kg or more (WHO, 1990a; EXTOXNET, 1996c). In a study using doses of 10, 100, or 5,000 mg/kg bw/day for three months, at 5,000 mg/kg bw/day transient clinical signs of toxicity appeared and liver to body weight ratios increased (WHO, 1984b). Beagles were also administered permethrin in capsules for 13 weeks at dose levels of 0, 10, 100 and 2,000 mg/kg bw. There was no mortality, but evidence of poisoning was observed at 2,000 mg/kg, with slight liver weight gain for animals in the 2,000 mg/kg/day group. In a six-month study, Beagles were administered encapsulated permethrin at 0, 10, 50, 250 mg/kg. There were no signs of toxicity and no effects on body weight. Necropsy results also indicated that there were no pathological findings. Therefore, it was concluded that under the exposure conditions of this study, oral doses up to 250 mg/kg for beagles do not cause adverse effects (WHO, 1990a).

5.2.2 Resmethrin

The symptoms of resmethrin exposure via any route may include:

- incoordination
- twitching
- loss of bladder control
- seizures

Dermal exposure may result in numbness, itching, burning, and tingling near the site of exposure (EXTOXNET, 1996d).

Short-term studies have not shown any adverse effects to domestic animals from exposure to resmethrin. Beagles injected intravenously with 25 mg/kg bw/day of resmethrin for 15 days showed no toxic effects or compound related enzyme changes (WHO, 1996). Bioresmethrin, also referred to as trans-resmethrin, was administered to dogs daily at dose levels of 0 and 500 mg/kg for seven days, followed by an increased dose of 1000 mg/kg for 14 days. No effects were noted on mortality, behavior, body weight, haematology, blood chemistry, urinalysis, or electrocardiograms. Dogs were also administered bioresmethrin at doses of 0, 25, 80, or 250 mg/kg bw, with the highest dose increased to 500 mg/kg bw in the seventh week, for 90 days. There were no observed effects on mortality, body weight, food consumption, ophthalmology or urinalysis. Red blood cell count, haemoglobin content and packed cell volume values were slightly reduced at the highest dose level (WHO, 1989). Blood-urea nitrogen increased after 12 weeks at the highest dose level. No adverse effects were observed on gross examination. The dogs had a NOAEL of 80 mg/kg bw/day (WHO 1989; WHO, 1996).

In a sub-chronic toxicity study, dogs were fed 0, 10, 30, or 300 mg/kg bw/day of resmethrin for 180 days. The NOAEL was 10 mg/kg bw/day, while dogs fed 30 mg/kg for the 180 days showed increased liver weights (WHO 1989; WHO 1996; EXTOXNET, 1996d).

5.2.3 Sumithrin (d-phenothrin)

During a 26-week feeding study, beagles were fed sumithrin at doses of 0, 100, 300 or 1,000 mg/kg. There were no abnormal findings in mortality, clinical signs, body weight, food consumption, ophthalmology, gross or microscopic pathology, haematology or urinalysis studies. The only effects were an increase in alkaline phosphatase activity in males fed 300 mg/kg and females fed 1000 mg/kg, and there was slight liver weight gain in males fed 1000 mg/kg. The resulting NOEL was 300 ppm (Pence et al., 1981 [as cited in WHO 1990b and WHO 2002]).

In a one-year study, beagles were fed sumithrin at doses of 0, 100, 300, 1000, or 3,000 mg/kg. There were no significant effects on clinical signs, body weight, food consumption, ophthalmology, or urinalysis. There were decreases in erythrocyte count, haemoglobin concentration, haematocrit, and total blood protein in males and females fed 3,000 mg/kg. Additionally, there was an increase in mean liver weight. In one male dog fed 1,000 mg/kg, and four dogs fed 3,000 mg/kg, there was focal degeneration of the adrenal cortex. Also, hepatocytes

appeared enlarged in one dog fed 1,000 mg/kg and seven fed 3,000mg/kg. The NOEL was determined to be 300 mg/kg (8.24 mg/kg bw) for males and 1,000 mg/kg (26.77 mg/kg bw) for females (Cox et al., 1987 [as cited in WHO 1990b and WHO 2002]).

5.3 Organophosphates

A panel of veterinarians presented information regarding organophosphate poisoning in dogs and cats, including exposure routes, symptoms, and recovery rates. Common exposure routes for organophosphate poisoning are insecticidal dips that have been improperly used and, for cats and kittens, ingestion due to licking a household insecticide from their feet or fur. It should be noted that though exposure to organophosphates results from proper use, it is at a low enough level that poisoning is not likely to occur. Organophosphate poisoning can be classified as mild or severe. Mild cases exhibit symptoms of excessive salivation and diarrhea. Severe symptoms include excessive salivation, diarrhea, muscle tremors, incoordination, lacrimation (secretion of tears), convulsions, mitosis, dyspnea (difficulty breathing), paralysis, cardiac irregularities, and coma. Animal recovery rates are good if the animals are not comatose or cyanotic, have not been convulsing for hours, or were not paralyzed when presented for treatment. Results are best when treatment is administered within hours of exposure (Carbone et al., 1976). Due to a relatively stable formation of an enzyme-phosphate complex, resulting in AChE inhibition, recovery from organophosphate exposure may be somewhat prolonged when compared with recovery from other insecticide exposures (Grant, 1984). Death from exposure to organophosphates is often due to hypoxia resulting from bronchoconstriction, excessive secretions in the bronchial tree, and erratic slowed heartbeat (Buck, 1979).

5.3.1 Malathion

Bell et al. (1955) performed a series of tests to determine if malathion (referred to as malathon in this study) could be applied to dogs in sufficient concentrations for effective external parasite control, while being safe for the animals. A reference to the manufacturer's technical bulletin reports that 100 mg/kg intravenously produced no effects in dogs, but 200 mg/kg produced death (American Cyanamid Company, 1953 [as cited in Bell et al., 1955]). Bell et al. (1955) fed seven dogs malathion (95 percent technical material) in doses ranging from 500-3,500 mg/kg. All dogs vomited within 35 minutes, but did not show signs of toxicosis. Two dogs were given 3,500

mg/kg via a stomach tube while under anesthesia, to prevent vomiting. These dogs also did not exhibit signs of toxicosis. Additionally, four dogs were dipped four times a day, at four-day intervals, in a 2 percent malathion solution. Under this course of treatment, there were no apparent signs of toxicity. All animals that survived the course of the study were euthanized and necropsied. There were no findings of toxicity lesions. The results of these tests indicate that whether administered orally or applied as a dip, malathion has low toxicity for dogs (Bell et al., 1955).

An experiment by Cross and Folger (1956) investigated the toxicity of malathion on cats. There was no evidence of toxicity to cats under the following experimental conditions: exposed to dip (8 or 16 cc. of 50 percent emulsion/gallon), powdered daily with 25 percent powder for 14 days, or fed 2 g of 25 percent powder. To produce poisoning, a four-month-old kitten was dipped in a 50 percent malathion solution. The kitten died within 48 hours. There were no gross lesions revealed upon necropsy.

A study in which researchers fed dogs malathion for one year did not reveal any signs of overt toxicity, despite decreased cholinesterase activity at all dosage levels (NPTN, 2001). The LD_{50} for malathion in young dogs has been reported as greater than 1,000 mg/kg i.p. (i.p. is defined as injection into the abdominal area) (Cooperative Extension, 1981).

In a one-year oral toxicity study, 95 percent malathion in gelatin capsules was administered in to groups of six male and six female beagles at dose levels of 0, 62.5, 125, or 250 mg/kg/day. No mortality was observed. The LOAEL was 62.5 mg/kg/day based on inhibition of plasma and erythrocyte cholinesterase activity in both males and females and greater than 250 mg/kg day for brain cholinesterase inhibition. The NOAEL for cholinesterase inhibition for both sexes was less than 62.5 mg/kg/day for plasma and erythrocyte cholinesterase and 250 mg/kg/day for brain cholinesterase. According to the USEPA, this study was unacceptable for a chronic toxicity study in dogs because NOAELs were not established for inhibition of cholinesterase activity for plasma and erythrocytes in either males or females (USEPA, 2000).

5.3.2 Isomalathion

No specific information on the effects of isomalathion on domestic animals was found other than a general guidance from the Cooperative Extension University of California Toxicology

Newsletter (1981) noting that isomalathion is reportedly about six times more toxic to mammals than malathion.

5.4 Synergists

5.4.1 Piperonyl Butoxide (PBO)

PBO is a synergist, a chemical that does not have any pesticidal effects, but enhances the pesticidal properties of other chemicals (NPTN, 2000b).

During an eight-week study, beagles were fed diets containing 90.78 percent pure PBO at concentrations of 500, 1000, 2000, or 3000 ppm active ingredient. All animals survived to study termination. All males and females receiving greater than 1000 ppm had reduced body weight gains. At 3000 ppm decreased food consumption was observed. There were no haematological changes. Dogs fed greater than 2000 ppm had alkaline phosphatase activities 1.5 times greater than control values. In addition, they had liver and gall-bladder weight increases. There were no macroscopic changes resulting from treatment. Microscopic changes included mild hypertrophy of hepatocytes in all males and females treated with the highest doses. An NOAEL was not established because of effects on the liver (Goldenthal, 1993a [as cited in WHO, 1995]).

PBO is classified by the USEPA as "low to very low" in toxicity when eaten by animals (NPTN, 2000b). During a year-long study, dogs were given PBO in capsules orally six days of the week. The dosages were 0, 3, 32, 106 and 320 mg/kg. All dogs at the highest dose level died. Additionally, prior to death, they lost weight. However, there was a large variation in weight loss; and the small sample number prevented meaningful comparisons and conclusions. The NOAEL for this study was 3 mg/kg/day. There were observed dose-related weight increases in the liver, kidney and adrenals (Sarles & Vandergrift, 1952 [as cited in WHO, 1995 and NPTN, 2000b, & NPTN 2000c]).

Goldenthal (1993b, [as cited in WHO 1995]) performed a year-long study in which beagles were fed diets with concentrations of PBO equal to 100, 600 and 2000 ppm. All animals survived to the end of the study with no relevant clinical or opthalmological signs. At the 2000 ppm dosage weight gain was significantly lower than in the control group. Males fed dosages greater than or equal to 600 ppm experienced decreases in food consumption. There were no statistically significant or dose related decreases of food consumption in females. There were no

haematological changes related to treatment. Alkaline phosphatase levels increased to three to five times the control values at 2000 ppm after six to 12 months. Males and females at the 2000 ppm dosage had increases in liver and gall-bladder weights and small increases in thyroid and parathyroid weights were observed in females. There were no macroscopic changes observed. Histopathological changes were limited to diffuse, mild hypertrophy of hepatocytes in males and females at 2000 ppm. The NOAEL was 100 ppm (16mg/kg bw) per day.

5.5 Additional Primary List Mosquito Control Agents

No information for effects on domestic animals was found for:

- Garlic Oil
- Malaoxon

Table 28
Domestic Animal End Points for Primary List Mosquito Control Agents

Active Ingredient	Common Name	Study Duration	End Point		Toxicological Endpoint	Units	Source	Comments
Malathion	Dog	NR	LD50	>	1,000	mg/kg ip	Cooperative Extension, 1981	young dogs
Malathion	Dog	NR	NOEL		100	mg/kg ip	Malathon Technical Bulletin, 1953	administered by IV
Malathion	Dog	1yr	LOAEL		62.5	mg/kg/day	USEPA, 2000	oral study - overall cholinesterase LOAEL based on plasma and erythrocyte inhibition in males and females
Malathion	Dog	1yr	NOAEL	<	62.5	mg/kg/day	USEPA, 2000	for plasma and erythrocyte cholinesterase
Malathion	Dog	1yr	NOAEL		250	mg/kg/day	USEPA, 2000	for brain cholinesterase
Malathion	Dog	1yr	LOAEL		62.5	mg/kg/day	USEPA, 2000	for plasma and erythrocyte cholinesterase
Malathion	Dog	1yr	LOAEL	>	250	mg/kg/day	USEPA, 2000	for brain cholinesterase
Malathion	Dog	1yr	NOAEL		250	mg/kg/day	USEPA, 2000	systemic
Malathion	Cat	NR	no evidence of toxicity		8	cc	Cross and Folger (1956)	exposed to dip - 50% emulsion/gallon
Malathion	Cat	NR	no evidence of toxicity		16	cc	Cross and Folger (1956)	exposed to dip - 50% emulsion/gallon
Malathion	Cat	14d	no evidence of toxicity		25	%	Cross and Folger (1956)	exposed to powder
Malathion	Cat	NR	no evidence of toxicity		2	g	Cross and Folger (1956)	fed 25% powder
Methoprene	Dog	NR	LD50	>	5,000	mg/kg	EXTOXNET, 1996a; Garg and Donahue, 1989; WHO No. 47; USEPA, 2001; Beasley, 2000; Sidall, 1976	
Methoprene	Dog	NR	NOEL		12.5	mg/kg/day	USEPA, 2001	
Methoprene	Dog	NR	LOAEL		5,000	ppm	USEPA, 2002	
Methoprene	Dog	90d	no evidence of toxicity		500	mg/kg	WHO, No. 47	also all effects were reversible at 5,000mg/kg
Methoprene	Dog	21d	no evidence of toxicity		5	g/kg bw	Hill, 1975 as cited in WHO, 2001	
Permethrin	Dog	90d	NOAEL		5	mg/kg/day	EXTOXNET 1996c	
Permethrin	Dog	3mo	signs of poisoning		500	mg/kg/day	WHO 1990a	
Permethrin	Dog	3mo	adverse effects observed		50	mg/kg/day	WHO, 1990a	liver weight increased
Permethrin	Dog	13wk	signs of poisoning		2,000	mg/kg/day	WHO, 1990a	
Permethrin	Dog	13wk	adverse effects observed		2,000	mg/kg/day	WHO, 1990a	liver weight increased
Resmethrin	Dog	180d	NOAEL		10	mg/kg/ bw/day	WHO 1989; WHO 1996; EXTOXNET, 1996d	
Resmethrin	Dog	180d	adverse effects observed		30	mg/kg/ bw/day	WHO 1989; WHO 1996; EXTOXNET, 1996d	liver weight increased
Resmethrin	Dog	15d	no evidence of toxicity		25	mg/kg bw/day	WHO, 1996	administered by IV
Resmethrin	Dog	90d	NOAEL		80	mg/kg bw/day	WHO 1989; WHO, 1996	
Bioresmethrin	Dog	90d	adverse effects observed		250 then 500	mg/kg bw/day	WHO 1989; WHO, 1997	RBC, haemoglobin and packed cell counts reduced; after 12 weeks blood-urea nitrogen increased

Active Ingredient	Common Name	Study Duration	End Point		Toxicological Endpoint	Units	Source	Comments
Bioresmethrin	Dog	21d	no evidence of toxicity		500 (7days) then 1000 (14 days)	mg/kg	WHO, 1989	
Sumithrin	Dog	26wk	NOEL		300	ppm	Pence et al., 1981, as cited in WHO 1990b and WHO 2002	
Sumithrin	Dog	26wk	adverse effects observed		300	ppm	Pence et al., 1981, as cited in WHO 1990b and WHO 2003	increase in alkaline phosphatase in males
Sumithrin	Dog	26wk	adverse effects observed		1000	ppm	Pence et al., 1981, as cited in WHO 1990b and WHO 2004	increase in alkaline phosphatase in females
Sumithrin	Dog	26wk	adverse effects observed		1000	ppm	Pence et al., 1981, as cited in WHO 1990b and WHO 2005	liver weight gain in males
Sumithrin	Dog	52wk	NOEL		300	mg/kg	Cox et al., 1987, as cited in WHO 1990b and WHO 2002	for males
Sumithrin	Dog	52wk	NOEL		1000	mg/kg	Cox et al., 1987, as cited in WHO 1990b and WHO 2002	for females
Sumithrin	Dog	52wk	adverse effects observed		3000	mg/kg	Cox et al., 1987, as cited in WHO 1990b and WHO 2003	decreases in erythrocyte count, haemoglobin concentration, haematocrit, and total blood protein in males and females
Sumithrin	Dog	52wk	adverse effects observed	>	1000	mg/kg	Cox et al., 1987, as cited in WHO 1990b and WHO 2004	focal degeneration of the adrenal cortex
PBO	Dog	1yr	adverse effects observed		320	mg/kg	WHO, 1995	dogs lost weight
PBO	Dog	1yr	adverse effects observed		100 & 120	mg/kg	Sarles & Vandergrift, 1952, as in WHO, 1995	increases in kidney and adrenal weights
PBO	Dog	8wk	adverse effects observed	>	1000	ppm	Goldenthal, 1993a as in WHO, 1995	reduced body weight gains
PBO	Dog	8wk	adverse effects observed		3000	ppm	Goldenthal, 1993a as in WHO, 1995	decreased food consumption
PBO	Dog	8wk	adverse effects observed	>	2000	ppm	Goldenthal, 1993a as in WHO, 1995	alkaline phosphatase activities 1.5x greater than control; liver and gall bladder weight increases;
PBO	Dog	1yr	adverse effects observed		2000	ppm	Goldenthal, 1993b as in WHO 1995	weight gains significantly lower than control; alkaline phosphatese levels incr 3X to 5X after 6 to 12 mnths; incr in liver & gall bladder weights & small incr in thyroid & parathyroid weights in females; histopathological changes - diffuse, mily hypertrophy of hepatocytes
PBO	Dog	1yr	adverse effects observed	>	600	ppm	Goldenthal, 1993b as in WHO 1995	decreased in food consumption in males
PBO	Dog	1yr	NOAEL		100	ppm	Goldenthal, 1993b as in WHO 1995	(16mg/kg bw)

No End Point Information was found for the following Primary List mosquito control agents:
***Bti, Bs* , Garlic Oil, Malaoxon, Isomalathion**

Reference List

Domestic Animal Toxicity

American Cyanamid Company. 1953. Malathon Technical Bulletin No. 3, 30 Rockefeller Plaza, New York 20, NY as cited in Bell 1955

Beasley, V. 2000. Comparative toxicology of mosquito control strategies. In, R.A. Cood (Ed.) Proceedings of the West Nile Action Workshop, hosted by the Wildlife Conservation Society, and sponsored by the New York State Assembly. Tarrytown, NY (January 19-21, 2000).

Bell, RR, MA Price, and RD Turk. 1955. Toxicity of Malathon and Chlorthion to Dogs and Cats. *Journal of the American Veterinary Medical Association.* 126(937): 302-303

Buck, W. 1979. Clinical toxicosis induced by insecticides in dogs and cats. *Veterinary Medicine/Small Animal Clinician.* 1119-1124

Carbone, MG, L Sherman, GT Barrows, J McPeak, MR Hook, and RK Pickard. 1976. Panel Report: Organophosphate Poisoning in Dogs and Cats. *Modern Veterinary Practice.* 57(8):638-644

Cooperative Extension University of California. 1981. Environmental Toxicology Newsletter. Malathion. Vol 1. No. 2 January 5, 1981

Cox et al. 1987. Acute toxicity study in dogs with Sumithrin technical grade. Hazleton Laboratories, Sumitomo report # ET-0108. Unpublished. As cited in WHO 2002.

Cross, RF and GC Folger. 1956. The Use of Malathion on Cats and Birds. *J.A.V.M.A.* 129(2):65-66

Dorman, D and V Beasley. 1991. Neurotoxicology of Pyrethrin and the Pyrethroid Insecticides. *Vet Hum Toxicol.* 33(3):238-243

Extension Toxicology Network (EXTOXNET). 1996a. Pesticide Information Profiles: Methoprene http://extoxnet.orst.edu/pips/methoprene.htm

Extension Toxicology Network (EXTOXNET). 1996b. Pesticide Information Profiles: Malathion

Extension Toxicology Network (EXTOXNET). 1996c. Pesticide Information Profiles: Permethrin http://extoxnet.orst.edu/pips/permethr.htm

Extension Toxicology Network (EXTOXNET). 1996d. Pesticide Information Profiles: Resmethrin http://extoxnet.orst.edu/pips/resmethr.htm

Extension Toxicology Network (EXTOXNET). 1996e. Pesticide Information Profiles: Bacillus thuringiensis http://extoxnet.orst.edu/pips/bacillus.htm

Garg, RC and WA Donahue. 1989. Pharmacologic profile of methoprene, an insect growth regulator, in cattle, dogs, and cats. *JAVMA.* Feb 194 (3): 410-412

Goldenthal, EI. 1993a. Evaluation of piperonyl butoxide in an eight week toxicity study in dogs. Unpublished report No. 542-004 from International Research and Development Corp., Mattawan, Michigan, USA. Submitted to WHO by Endura SA, Bologna, Italy, for the Piperonyl Butoxide Task Force, Washington DC, USA. As cited in WHO 1995.

Goldenthal, EI. 1993b. Evaluation of piperonyl butoxide in a one year chronic toxicity study in dogs. Unpublished report No. 542-005 from International Research and Development Corporation, Mattawan, Michigan, USA. Submitted to WHO by Endura SA, Bologna, Italy, for the Piperonyl Butoxide Task Force, Washington DC, USA.

Grant, D. 1984. Insecticides and their use in the dog and cat. *In Practice* 6(4):121,123,125,127

Gray, A. 2000. Permethrin Toxicity in Cats. *The Veterinary Record: Journal of the British Veterinary Association.* 147(19):556

Hill, R. 1972a. Effects of a single oral dose of 10g/kg of ZR 515 in dogs. Report No. 99-D-70-ZR 515-PO-TX. Syntex Research, California, USA. Submitted to WHO by Novartis Animal Health Australasia Ltd, Pendle Hill, NSW, Australia. As cited in WHO 2001.

Hill, R. 1972b. Acute oral toxicity of ZR for dogs. Report No. 99-D-72-ZR 515-PO-TX. Syntex Research, California, USA. Submitted to WHO by Novartis Animal Health Australasia Ltd, Pendle Hill, NSW, Australia. As cited in WHO 2001.

Martin, A and A Campbell. 2000. Permethrin toxicity in cats. *The Veterinary Record: Journal of the British Veterinary Association* 147(22):639

Murphy, M. 1994. Toxin exposures in dogs and cats: Pesticides and biotoxins. *JAVMA.* Aug 205(3):414-421

National Pesticide Telecommunications Network (NPTN). 1998. Pyrethrins & Pyrethroids General Fact Sheet

National Pesticide Telecommunications Network (NPTN). 2000a. *Bacillus thuringiensis* General Fact Sheet

National Pesticide Telecommunications Network (NPTN). 2000b. Piperonyl Butoxide General Fact Sheet

National Pesticide Telecommunications Network (NPTN). 2000c. Piperonyl Butoxide Technical Fact Sheet

National Pesticide Telecommunications Network (NPTN). 2001. Malathion General Fact Sheet

Pence et al. 1981. Subchronic toxicity study of S-2539F in dogs. Final report (26 weeks). Hazelton Laboratories, Sumitomo report # ET-0066. Unpublished. As cited in WHO 2002.

Saito, M. 1975. Investigation of Altosid toxicity. VII. Determination of toxic consequences of acute inhalation exposure of beagle dogs to Altosid. Nomura Research Laboratory, Japan. Submitted to WHO by Novartis Animal Health Australasia Ltd, Pendle Hill, NSW, Australia. As cited in WHO 2001.

Sarles, MP and WB Vandergrift. 1952. Chronic oral toxicity and related studies on animals with the insecticide and pyrethrum synergist, piperonyl butoxide. Am. J. Trop. Med. Hyg., 1: 862-883. As cited in WHO 1995.

Siddall, J. 1976. Insect Growth Regulators and Insect Control: A Critical Appraisal. *Environmental Health Perspectives* 14:119-126.

United States Environmental Protection Agency (USEPA). 2000. Memorandum from Yung G. Yang to Paula A. Deschamp, Malathion: The Toxicology Chapter for the RED http://www.epa.gov/oppsrrd1/op/malathion/toxicology.pdf.

United States Environmental Protection Agency (USEPA). 2001. Methoprene R.E.D. Fact Sheet: update of the March 1991 fact sheet.

United States Environmental Protection Agency (USEPA). 2002. Memorandum from K. Boyle and K Leifer to Robert Forrest, IIFG Decision Document on Tolerance Reassessment for Methoprene – Aug. 1, 2002.

World Health Organization (WHO). 1984a. Data Sheets on Pesticides No. 47: Methoprene http://www.inchem.org/documents/pds/pds/pest47_e.htm.

World Health Organization (WHO). 1984b. Data Sheets on Pesticides No. 51: Permethrin http://www.inchem.org/documents/pds/pds/pest51_e.htm.

World Health Organization (WHO). 1989. Environmental Health Criteria 92 – Resmethrins: Resmethrin, Bioresmethrin, Cisresmethrin.

World Health Organization (WHO). 1990a. Environmental Health Criteria 94 – Permethrin http://www.inchem.org/documents/ehc/ehc/ehc94.htm.

World Health Organization (WHO). 1990b. Environmental Health Criteria 96 – d-Phenothrin http://www.inchem.org/documents/ehc/ehc/ehc96.htm.

World Health Organization (WHO). 1995. Data Sheet on Piperonyl Butoxide – Pesticide residues in food: 1995 evaluations Part II.

World Health Organization (WHO). 1996. WHO/FAO Data Sheets on Pesticides No. Resmethrin.

World Health Organization (WHO). 2001. Pesticide residues in food 2001: Toxicological evaluations for methoprene and S-methoprene http://www.inchem.org/documents/jmpr/jmpmono/2001pr09.htm.

World Health Organization (WHO). 2002. WHO Specifications and Evaluations for Public Health Pesticides: d-phenothrin (3-phenpxybenzyl (1R)-sic, trans-chrysanthemate).