● INSECTICIDE SYNERGIST FACTSHEET

PIPERONYL BUTOXIDE

Piperonyl butoxide (PBO) is a synergist used to increase the potency of insecticides like pyrethrins and pyrethroids. According to the U.S. Environmental Protection Agency (EPA), PBO is one of the most commonly used ingredients in household pesticide products.

PBO acts as a synergist by inhibiting the activity of a family of enzymes called P450s. These enzymes have many functions, including breakdown of toxic chemicals and transformation of hormones.

Symptoms of PBO exposure include nausea, diarrhea, and labored breathing.

EPA classifies PBO as a "possible human carcinogen" because it caused liver tumors and cancers in laboratory tests.

In a study conducted by PBO manufacturers, PBO caused atrophy of the testes in male rats. Other researchers found behavioral changes (a decrease in home recognition behavior) in the offspring of exposed mothers.

PBO affects a variety of hormone-related organs, including thyroid glands, adrenal glands and the pituitary gland.

PBO reduces the immune response of human lymphocytes, cells in our blood that help fight infections.

Concentrations of less than one part per million of PBO reduce fish egg hatch and growth of juvenile fish. PBO also inhibits hormone-related enzymes in fish and slows the breakdown of toxic chemicals in their tissues. PBO is very toxic to earthworms and highly toxic to aquatic animals.

BY CAROLINE COX

Piperonyl butoxide (PBO) is an insecticide synergist, a chemical that is used to make insecticides more potent. (See Figure 1 for PBO's molecular structure.) The discovery of PBO's properties as a synergist occurred in the 1940s, following the development of aerosol cans to apply insecticides.[1] PBO is now frequently used, particularly in aerosol products. About 1700 insecticide products contain PBO,[2] 8 percent of the over 20,000 pesticide products[3] registered in the U.S.

Major U.S. manufacturers of PBO pesticides include MGK (McLaughlin Gormley King Company), Prentiss, Inc. and S.C. Johnson & Son, Inc.[1]

PBO is often used as a synergist with pyrethrins (JPR 22(1):14-20) and the chemically related synthetic pyrethroids.[4] However, it also can synergize a variety of other pesticides, including the insecticides fipronil,[5] parathion,[6] dichlorvos,[7] linalool, and D-limonene,[8] the insect growth regulators methoprene, hydroprene, and fenoxycarb,[8] as well as alpha-naphthylthiourea (formerly used as a rodenticide).[9]

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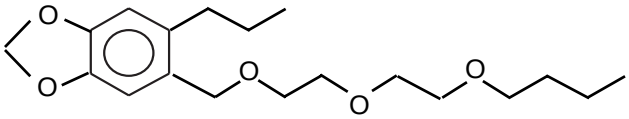
Caroline Cox is NCAP's staff scientist.

Figure 1
Piperonyl Butoxide



2-(2-butoxyethoxy)ethyl 6-propylpiperonyl ether

Use

In a household pesticide use survey done for the U.S. Environmental Protection Agency (EPA), products contained PBO more frequently than any other ingredient. Over 12 percent of the products used by households in the survey contained PBO.[10] A recent Minnesota survey had similar results.[11] The EPA survey estimates that almost 300 million applications of PBO-containing products are made indoors every year in the U.S, and almost 60 million applications outdoors.[10]

In addition to these household uses, other significant uses include use in public health pest control, commercial indoor pest control, buildings that house animals, commercial landscape maintenance, and lettuce production.[12]

Mode of Action

Piperonyl butoxide acts as a synergist by slowing the breakdown in insects of certain insecticides. The first step in the breakdown of many drugs, pesticides, and other compounds is oxidation by a family of enzymes called the P450 mono-oxygenases. PBO inhibits the activity of these enzymes. If the breakdown product is less toxic than the insecticide itself, the insecticide remains toxic longer when PBO

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inhibits the P450 enzymes.[13]

P450 enzymes have important biological functions. In addition to detoxification of synthetic compounds, they also transform sex hormones, vitamins, and other naturally occurring molecules.[13]

Inert Ingredients

Like most pesticides, commercial PBO-containing insecticides contain ingredients other than PBO many of which, according to U.S. pesticide law, are called "inert."[14] Except for acute toxicity testing, all toxicology tests required for registration of PBO products were conducted with PBO, not with the combination of ingredients found in commercial products.[15]

Most inert ingredients are not identified on product labels, and little information about them is publicly available.

For more information about the hazards of some of the inert ingredients in PBO products see "Examples of Hazardous 'Inerts'," below.

Exposure Symptoms

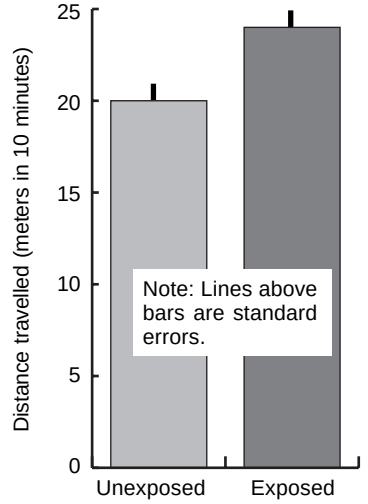
Symptoms caused by ingestion of PBO include nausea, cramps, vomiting, and diarrhea.[16] Symptoms caused by breathing PBO include tearing, salivation, and labored breathing.[17] Accumulation of fluids in the lungs can occur.[18] PBO can also cause temporary eye and skin irritation.[16]

Effects on the Nervous System

PBO causes behavioral changes in young laboratory animals. A researcher at the Tokyo Metropolitan Research Laboratory of Public Health observed behavior of mice after they had been fed PBO for six weeks. He found that exposed rats traveled longer distances and turned more frequently than unexposed animals. (See Figure 2.) The effects on travel distance occurred at all doses tested in this experiment.[19]

PBO also reduces the activity of the

Figure 2
PBO Changes Behavior



Source: Tanaka, T. 1993. Behavioral effects of piperonyl butoxide in male mice. *Toxicol. Lett.* 69: 155-161.

In a laboratory study, PBO affected motor activity at every dose level tested.

EXAMPLES OF HAZARDOUS "INERTS" IN PRODUCTS CONTAINING PIPERONYL BUTOXIDE

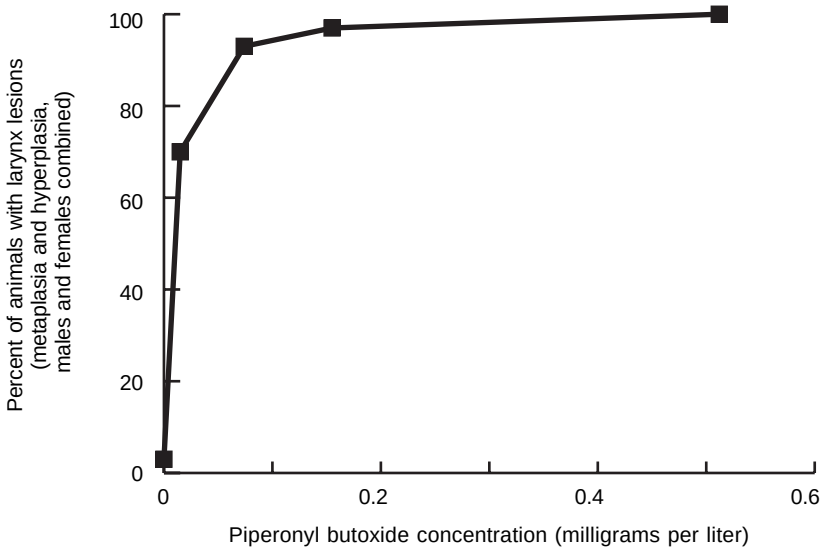
Raid Flea Killer Plus (EPA Reg. No. 4822-273) contains **butane, propane,** and **isobutane** as "inert" propellants.[1] Butane, isobutane, and propane can cause headache, dizziness, numbness, sleepiness, mental confusion, poor coordination, and memory loss. They are "extremely flammable" and "will be easily ignited by heat, sparks, or flame."[2,3,4]

Pyrenone® Crop Spray (EPA Reg. No. 432-1033) and Prentox® Pyronyl™ Fogging & Contact Spray (EPA Reg. No. 655-675) contain a **petroleum solvent**.[5,6] This solvent is called **hydrotreated kerosene** and its Chemical Abstract Service number is 64742-47-8.[7] This solvent has caused skin tumors when applied to the skin of laboratory mice.[8] Exposure to this solvent also causes dizziness, nausea, and headache. Breathing droplets of this solvent can cause aspiration pneumonia.[6]

Scourge® Insecticide with SBP-1382®/PBO 1.5%+4.5% Forla II (EPA Reg. No. 432-719) contains an **aromatic petroleum solvent** with Chemical Abstract Services number 64742-94-5[9] also called **solvent naphtha**. This solvent contains two aromatic hydrocarbons, naphthalene and 1,2,4-trimethylbenzene.[10] Naphthalene is classified by EPA as a possible human carcinogen because it causes lung tumors in mice following inhalation. Naphthalene exposure also causes headache, restlessness, lethargy, nausea, diarrhea, and anemia. Anemia in newborns can be caused by exposure during pregnancy.[11] 1,2,4-Trimethyl benzene is irritating to eyes and skin. It can depress the central nervous system and cause headache, fatigue, nausea, and anxiety. It has also caused asthmatic bronchitis.[12]

1. S.C. Johnson & Son, Inc. 2000. Material safety data sheet: Raid® Flea Killer Plus. Racine, Wisconsin. 800-725-6737.
2. Hazardous Substance Data Bank. 2002. Butane. http://toxnet.nlm.nih.gov.
3. Hazardous Substance Data Bank. 2002. Isobutane. http://toxnet.nlm.nih.gov.
4. Hazardous Substance Data Bank. 2001. Propane. http://toxnet.nlm.nih.gov.
5. Aventis. 2001. Material safety data sheet: Pyrenone® Crop Spray. www.cdms.net/ldat/mp0GC000.pdf.
6. Prentiss, Inc. 1998. Material safety data sheet: Prentox® Pyronyl Fogging & Contact Spray. www.prentiss.com/msds/pdf/655_675.pdf.
7. National Institute for Occupational Safety and Health. Undated. The registry of toxic effects of chemical substances: Kerosene (petroleum), hydrotreated. www.cdc.gov/niosh/rtecs/oa53fc00.html.
8. International Agency for Research on Cancer. 1989. Occupational exposures in petroleum refining. *IARC Monographs* 45:39. http://193.51.164.11/htdocs/monographs/Vol45/45-01.htm.
9. Aventis. 2000. Material safety data sheet: Scourge® Insecticide with SBP-1382®/PBO 1.5%+4.5% Forla II. www.cdms.net/ldat/mp57D000.pdf.
10. Shell Chemical Company. 2002. Material safety data sheet: Shellsol® A150. www.euapps.shell.com/MSDS/GotoMsds.
11. Hazardous Substance Data Bank. 2002. Naphthalene. http://toxnet.nlm.nih.gov.
12. Hazardous Substance Data Bank. 2002. 1,2,4-trimethylbenzene. http://toxnet.nlm.nih.gov.

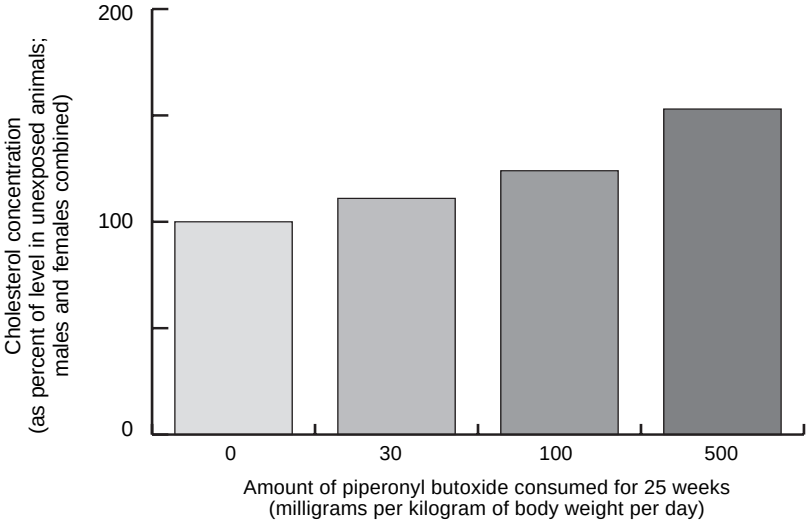
Figure 3
Breathing Piperonyl Butoxide Damages the Larynx



Source:
U.S. EPA. Office of Prevention, Pesticides and Toxic Substances. 1994. EPA Id# 067501. Piperonyl butoxide. Review of a series 82-4 subchronic inhalation toxicity study in rats. Memo from J. Doherty, Health Effects Division, to A. Dixon and B. Sidwell, Special Review and Reregistration Division. Washington, D.C., June 22.

Piperonyl butoxide damaged the larynx at all dose levels tested.

Figure 4
Exposure to Piperonyl Butoxide Increases Cholesterol Levels



Source: U.S. EPA. Office of Prevention, Pesticides and Toxic Substances. 1988. EPA Reg. No.: 4816-72. Piperonyl butoxide. Review of a chronic feeding/oncogenicity study submitted by the Piperonyl Butoxide Task Force. Memo from J. Doherty, Hazard Evaluation Division, to P. Hutton and G. Werdig, Registration Division. Washington, D.C., Apr. 28.

Piperonyl butoxide exposure increases cholesterol levels.

enzyme cholinesterase. This enzyme plays a role in transmitting nerve impulses from one nerve cell to another or to muscle cells.[20] In a long-term feeding study with rats, researchers from the laboratory mentioned in the previous paragraph found that female rats fed PBO had 30 percent less blood cholinesterase activity than unexposed rats.[21]

In addition, PBO can increase the neurotoxicity of other compounds. For example, pharmacologists at Virginia Commonwealth University fed rats PBO and methylmercury, a neurotoxic metal. They found that rats fed the combination developed neurological symptoms more frequently than rats fed methylmercury alone.[22]

Effects on the Digestive System

Researchers at the National Institute of Hygienic Sciences (Japan) found that long-term exposure to PBO caused intestinal ulcers in rats. Intestinal bleeding was also more common in exposed rats than in unexposed ones.[23]

Effects on the Larynx

The larynx is susceptible to damage from breathing PBO-contaminated air. A study conducted by a manufacturers' task force found damage at all dose levels tested.[24] (See Figure 3.) The damage consisted of metaplasia, transformation of cells to an atypical form, and hyperplasia, an abnormal increase in the number of cells in an organ.[25]

Effects on the Liver and Kidney

In laboratory toxicology studies, PBO often affects the liver. For example, in the study of the digestive system mentioned above, liver weights in all exposed rats were greater than in unexposed rats. The researchers also observed liver damage.[23] Other researchers in the same laboratory found that liver damage occurred following as little as one week of exposure.[26] In a study with dogs conducted by a manufacturers' task force, liver damage occurred at all dose levels tested.[27] A similar study with a lower dose level found liver damage at all but the

lowest level.[27] Liver damage also occurred in studies with mice.[28]

Researchers from the Tokyo Metropolitan Research Laboratory studied effects of PBO on kidneys. In a three-month feeding study with rats, they found kidney damage at all dose levels tested. Damage included atrophy and dilation of kidney structures.[29]

Effects on the Circulatory System

Long-term feeding of PBO caused anemia in rats in a study conducted by researchers from the Tokyo Metropolitan Research Laboratory of Public Health. The amount of hemoglobin (an oxygen-carrying molecule in blood) was lower in exposed rats than unexposed ones at all dose levels tested.[30]

In another study by the same group of researchers, a three-month exposure to PBO increased the blood levels of cholesterol in rats. Cholesterol levels at the highest dose were about double the level in unexposed rats.[31]

A study by a manufacturers' task force also found that PBO increased cholesterol. Rats exposed in a long-term study had higher cholesterol levels than unexposed rats at all but the lowest dose tested.[32] (See Figure 4.)

Carcinogenicity

Since 1995, EPA has classified piperonyl butoxide as carcinogen (a chemical that causes cancer). EPA's classification of piperonyl butoxide is "Group C," a possible human carcinogen. EPA based its evaluation on a study of mice conducted by a manufacturers' task force. The study found that piperonyl butoxide caused liver tumors and cancer.[33,34] (See Figure 5.)

PBO also caused liver cancer in mice in a study conducted by researchers from the Tokyo Metropolitan Research Laboratory. At the highest dose level, almost half of the mice tested developed liver cancer.[35]

PBO has also caused cancer in rats. A study conducted by PBO manufacturers found the incidence of lymph and thyroid tumors increased with increasing exposure to PBO.[36] A second study, by the Japanese researchers mentioned above, found that PBO caused liver cancer.[37]

A contaminant of PBO causes cancer. The contaminant is safrole, which the National Toxicology Program classifies as "reasonably anticipated to be a human carcinogen."[38] Researchers at the UFR de Pharmacie (France) found safrole in all of the PBO samples they tested. The samples were provided by European manufacturers.[39]

In addition, PBO can increase the carcinogenicity of other cancer-causing chemicals. Researchers at Harvard Medical School and the National Cancer Institute found that the combination of Freon (a refrigerant that was also used as a propellant in aerosol pesticides) and PBO was more carcinogenic than either chemical alone.[40] The liver carcinogen N-hydroxy-2-acetylaminofluorene also is more carcinogenic when combined with PBO than it is alone.[41]

However, the carcinogenicity of PBO is still controversial to some reviewers. The World Health Organization, in a 1995 review, identified five other studies that found no evidence that PBO exposure caused cancer.[42]

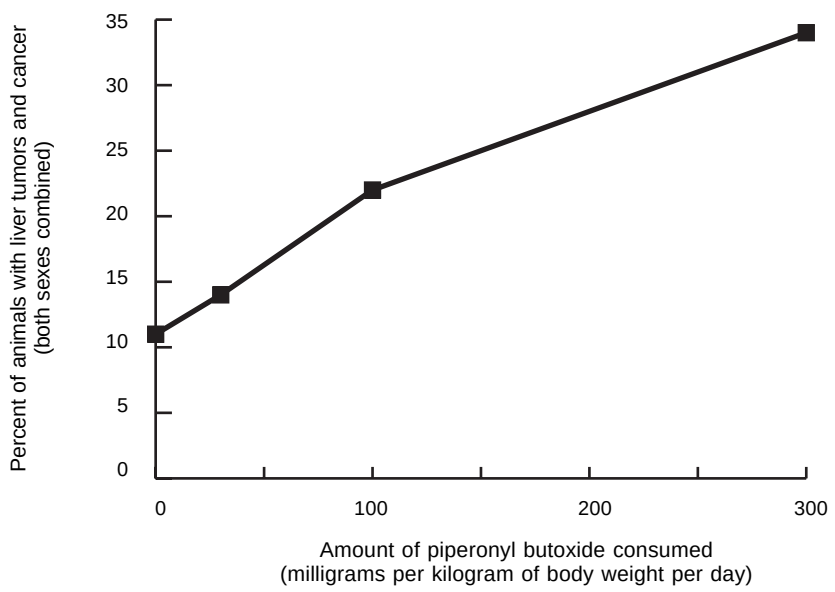
Mutagenicity (Genetic Damage)

While some tests for genetic damage have shown that PBO "does not demonstrate any significant potential for mutagenicity,"[43] this synergist does cause genetic damage in other tests.

In 1995, researchers from the Tokyo Metropolitan Research Laboratory of Public Health studied PBO's effects on a cell culture derived from human embryo cells. They found that PBO caused mutations in a gene called *OuaR*. Also, PBO caused mutations in *K-ras*, a gene "believed to be involved in neoplastic [tumorous] changes."[44]

Another study from the same laboratory found that PBO caused sister chromatid exchanges in cultures of cells from hamster ovaries.[45] (Sister chromatid exchanges are exchanges of genetic material within a chromosome.[46]) PBO's contaminant safrole also

Figure 5
Exposure to Piperonyl Butoxide Causes Cancer



Source: U.S. EPA. Office of Prevention, Pesticides and Toxic Substances. 1995. Carcinogenicity peer review of piperonyl butoxide. Memo from J. Doherty and E. Rinde , Health Effects Division, to R. Keigwin, Registration Division, and A. Dixon and B. Sidwell, Special Review and Reregistration Division. Washington, D.C., June 7.

EPA classifies PBO as a carcinogen because it causes liver tumors and cancer.

caused sister chromatid exchanges in this study.[45]

In addition, a study conducted by a PBO manufacturer found that the frequency of a mutation called HGPRT was 2 to 4 times higher in hamster ovary cells exposed to PBO than in unexposed cells. However, EPA agreed with the study authors that this was "not of biological significance."[47]

Effects on Reproduction

In laboratory tests, PBO has adversely affected a variety of reproductive functions.

Atrophy of the testes was observed in a two-year feeding study with rats conducted by a manufacturers' task force,[48] along with some decreases in weight of the seminal vesicles (sperm-producing structures).[49] (See Figure 6.) Increased incidence of testicular atrophy occurred at all dose levels tested. However, because the average weight of the testes did not decrease, EPA concluded that "the data did not provide conclusive evidence."[48]

A series of studies at the Tokyo Metropolitan Research Laboratory found other effects on reproduction. The offspring of mice that were fed PBO before, during, and after pregnancy weighed less than the offspring of unexposed mice. This decrease occurred at all the dose levels tested in this experiment. In addition, PBO caused changes in the home recognition olfactory behavior of the offspring of exposed mothers. In a test where the mice had a choice of entering a compartment with wood chips from their home cage or entering a compartment with fresh (unused) chips, the offspring of exposed mothers were less likely to enter the compartment that smelled like home than the offspring of unexposed mothers. This behavioral change occurred at all but the lowest dose level tested.[50]

A three-generation study by researchers from the same laboratory found that litter size and weight were less for exposed mothers than for unexposed ones. (Animals were fed PBO continuously from an age of 5 days in the first generation through the weaning of the third generation.) Also, nursing pups of exposed mothers weighed less than pups with unexposed mothers. In the third generation, several behaviors, including the olfactory home-recognition behavior mentioned above, were also affected by PBO exposure. The effects on the weight of nursing pups occurred at all dose levels tested, the behavioral effects occurred at all but the lowest dose level.[51]

A third study from the same laboratory used a different kind of exposure. In this study, pregnant mice were given a single dose of PBO on the ninth day of their pregnancy. The weight of fetuses from exposed mothers was less than the weight of fetuses from unexposed mothers. This effect occurred at all dose levels tested for female fetuses and all but the lowest dose level for males. The number of fetal deaths was also higher for exposed mothers. These increased fetal deaths occurred at all but the lowest dose level tested. These researchers also found that the frequency of fetuses with defective or missing fingers was higher for mothers exposed at all but the lowest dose level.[52]

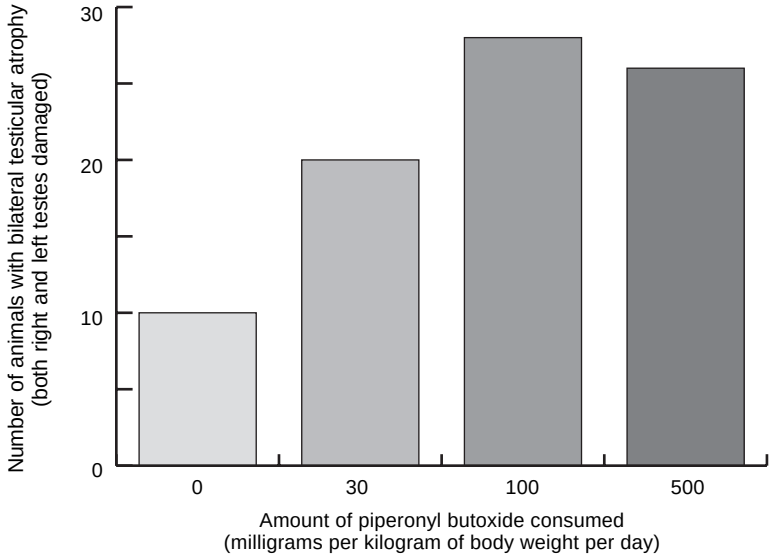
A study conducted by a manufacturers' task force found that the incidence of a bone defect was higher in the offspring of rats exposed during pregnancy than in the offspring of unexposed rats. The incidence was dose-related and was 2 to 4 times higher for exposed rats than for unexposed ones. However, EPA concurred "with the study author's conclusions that these effects were not related to treatment."[53]

Effects on the Immune System

Medical researchers first documented PBO's ability to inhibit normal functions of the immune system in 1979. Physicians from the State University of New York (Buffalo) showed that PBO inhibited the immune response of human blood cells called lymphocytes. PBO caused stronger inhibition (25 percent) than the seven other pesticide chemicals tested.[54]

In a recent (1999) study, researchers from the University of Applied Sciences

Figure 6
Exposure to Piperonyl Butoxide Causes Atrophied Testes

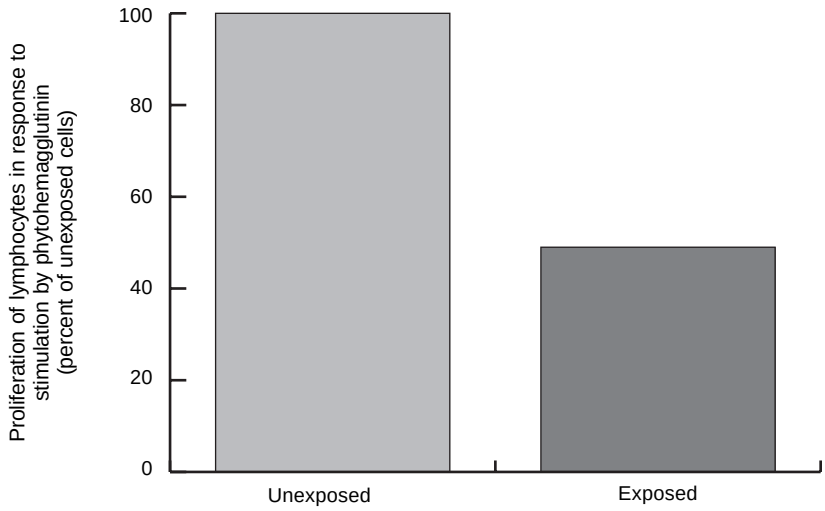


Source: U.S. EPA. Office of Prevention, Pesticides and Toxic Substances. 1988. EPA Reg. No.: 4816-72. Piperonyl butoxide. Review of a chronic feeding/oncogenicity study submitted by the Piperonyl Butoxide Task Force. Memo from J. Doherty, Hazard Evaluation Division, to P. Hutton and G. Werdig, Registration Division. Washington, D.C., Apr. 28. p. R12-R13.

Piperonyl butoxide caused atrophied testes in a long-term feeding study of rats.

(Germany) found similar results: PBO caused about a 50 percent reduction in the immune response of human lymphocytes.[55] (See Figure 7.)

Figure 7
Piperonyl Inhibits Immune System Function



Source: Diel, F. et al. 1999. Pyrethroids and piperonyl-butoxide affect human T-lymphocytes in vitro. *Toxicol. Lett.* 107: 65-74.

Piperonyl butoxide reduces the activity of immune system cells in human blood.

Effects on Hormones

The impact of environmental contaminants on the normal function of human and animal hormone systems has been a significant concern in the last decade.[56] Hormones are biologically active molecules that control all responses and functions of the body. Dramatic changes in the activity of cells in humans and other animals "are caused by extremely small amounts" of hormones or other chemicals that disrupt this system.[57]

Since the P450 enzymes inhibited by PBO break down steroids (a class of chemicals that includes many sex hormones),[13] it is not surprising that PBO can have this kind of hormonal effect. A study conducted by a task force of PBO manufacturers showed that long-term exposure of rats to PBO damages hormone-related organs. In exposed animals, thyroid glands were larger than in unexposed animals. Also, adrenal glands in exposed females were larger than in unexposed females, and pituitary glands were smaller in exposed males.[58]

Increasing Exposure to Potentially Toxic Chemicals

PBO can increase exposure of people and other species to toxic chemicals in several different ways.

First, this synergist affects the amount of certain toxic chemicals that are absorbed through skin. Veterinarians at North Carolina State University found that PBO exposure increased the absorption of the insecticide carbaryl through skin. When exposure to PBO occurred, skin absorption was about double the rate without PBO exposure. The veterinarians believe that the increased absorption was caused by PBO's ability to irritate the skin.[59]

Second, PBO can inhibit the activity of P450 enzymes in the nose that would otherwise detoxify chemicals that are inhaled. Researchers from the Lovelace Respiratory Institute showed that high levels of detoxifying enzymes occur in the noses of many species, and some of these enzymes are inhibited by PBO.[60]

Finally, PBO can inhibit the breakdown of toxic chemicals in the soil by inhibiting the enzymes in microorganisms that usually do the detoxification. For example, researchers at the Institute for Environmental Studies (Illinois) found that about 1 1/2 times as much benzidine, a carcinogen, persisted for a month when the soil was treated with both benzidine and PBO as persisted when the treatment used only benzidine.[61]

Exposure

Because PBO is frequently used for household pesticide treatments, people are frequently exposed. A recent (2002) study of pregnant women conducted by researchers from Columbia University documented how often this exposure occurs. In this study, women from northern Manhattan and the South Bronx (New York) wore personal air monitors for two days and left the monitor near their beds at night. The monitoring found PBO in air samples from over 80 percent of women in the study. PBO was the fourth most commonly detected pesticide. PBO concentrations were highest in homes that had been treated with insecticide aerosol spray cans or "bombs."[62]

> "The monitoring found PBO in air samples from over 80 percent of the women in the study."

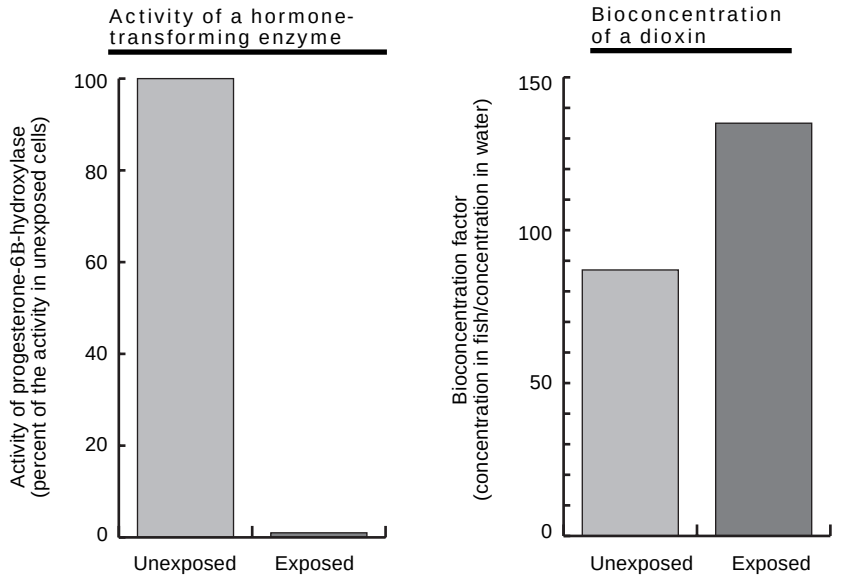
Contamination of Food

PBO is regularly found on food. The U.S. Department of Agriculture has found PBO on spinach,[63] peas, sweet potatoes,[64] tomatoes, peaches, squash,[65] strawberries,[66] bell peppers,[67] grapes, and pineapples.[68]

Effects on Birds

According to a study conducted by a manufacturers' task force, PBO adversely affected reproduction in mallard ducks. PBO affected the number of eggs laid, the number of eggs that cracked while being hatched, and the

Figure 8
Effects of Piperonyl Butoxide on Fish



Sources:
Sijm, D.T.H.M., G. Schaap, and A. Opperhuizen. 1993. The effect of the biotransformation inhibitor piperonyl butoxide on the bioconcentration of 2,8-dichlorodibenzo-p-dioxin and pentachlorobenzene in goldfish. *Aquat. Toxicol.* 27: 345-360.
Miranda, C.L., M.C. Henderson, and D.R. Buhler. 1998. Evaluation of chemicals as inhibitors of trout cytochrome P450s. *Toxicol. Appl. Pharmacol.* 148: 237-244.

Piperonyl butoxide can disrupt fish hormone systems and increase the concentration of toxic chemicals in fish tissues.

thickness of the eggshells.[69]

Researchers from the Indian Institute of Chemical Technology found that PBO inhibited "important detoxification enzymes" in the kidney, lung, brain, and heart of pigeons. These enzymes "protect the cell against chemically induced damages" so that inhibition of their activity could make the birds more susceptible to a variety of toxic chemicals.[70]

Effects on Fish

In terms of its acute toxicity (ability to cause mortality in a time period up to 96 hours long), PBO is classified as "moderately toxic" to fish. Concentrations between 3 and 7 parts per million (ppm) are sufficient to kill fish.[69]

PBO affects the ability of fish to successfully reproduce at much lower concentrations than are required for mortality. In a study conducted by a manufacturers' task force, concentrations of less than 1 ppm reduced egg hatch and larval growth in the fathead minnow, a standard test fish.[69]

PBO also increases the toxicity to fish of a variety of pesticides. For example, studies done by the New York Department of Environmental Conservation found that, compared to fish exposed to the insecticide resmethrin alone, mortality was higher and swimming stamina less in fish exposed to both PBO and resmethrin.[71,72] PBO also increased bioconcentration of the insecticide phenothrin in a study of carp conducted by the Sumitomo Chemical Co., Ltd.[73] Other studies (by researchers at the University of Wisconsin-Milwaukee and the Illinois Natural History Survey) found that PBO decreased the ability of fish to detoxify the pesticides rotenone, aldrin, methoxychlor, and trifluralin.[74,75]

PBO increases the toxicity of other chemicals to fish. A study from the University of Utrecht (The Netherlands) found that PBO slowed the transformation of the dioxin 2,8-DCDD (a chemical relative of the notorious 2,3,7,8-TCDD) into a form that goldfish can eliminate.[76] This resulted in higher bioaccumulation of the dioxin. (See Figure 8.) Researchers at the Medical College of Wisconsin found that PBO inhibited the breakdown of di-2-ethylhexyl phthalate[77] (DEHP, a chemical that causes cancer and genetic damage)[78] and nonyl phenol (an estrogen mimic that disrupts normal hormone function) in rainbow trout.[79]

In addition, PBO can disrupt fish hormone systems. Researchers from Oregon State University showed that PBO strongly inhibits the activity of an enzyme called progesterone-6ß-hydroxylase in rainbow trout livers.[80] (See Figure 8.) Progesterone regulates egg maturation in fish.[81]

Effects on Other Aquatic Animals

PBO is "highly" acutely toxic to water fleas, shrimp and oysters. Studies conducted by a manufacturers' task force found that concentrations of less than one ppm killed all three of these species.[69]

Another of the task force's studies found adverse effects on water flea reproduction at concentrations as low as 12 parts per billion.[69] Supporting these results, a study from North Carolina State University found that exposure of water fleas to PBO altered the transformation of the sex hormone testosterone. Less than one ppm inhibited most enzymes that transform testosterone over 60 percent.[82]

Effects on Insects

In addition to making other insecticides more toxic to pest insects, PBO can increase the toxicity of insecticides to beneficial insects, such as honey bees and water beetles.[83,84]

PBO also has more unexpected effects. Researchers from the University of South Australia showed that PBO exposure of fruit flies increased the genetic damage caused by X-rays and the mutagenic chemical heliotrine.[85] U.S. Dept. of Agriculture researchers showed that PBO inhibits the activity of enzymes involved in the breakdown or synthesis of insect sex pheromones,[86] chemicals insects use for communication between males and

females.[87]

Toxicity to Earthworms

University of Kentucky researchers tested the acute toxicity of a variety of chemicals to a common earthworm, *Eisenia foetida*. They found that piperonyl butoxide was "very toxic" to this earthworm.[88]

Effects on Plants

Although it is perhaps unexpected for a chemical usually used as an insecticide synergist, PBO can affect plant physiology. For example, researchers at Ibaraki University (Japan) found that PBO inhibited P450 enzymes in rice leaves that produce phytoalexins, compounds that inhibit the germination of disease-causing fungus spores.[89] PBO causes flowering in asparagus, also by inhibiting P450 enzymes.[90]

PBO also increases herbicide damage to plants. It increases the damage to corn caused by the sulfonylurea herbicides primisulfuron[91] and tribenuron,[92] the thiocarbamate herbicide EPTC,[93] and the triazine herbicides atrazine, terbutryn, and prometryn.[94] Similar increases in the toxicity of sulfonylurea herbicides have been documented in soybeans, lambsquarter, and a variety of weedy grasses.[95,96]

Persistence

Outdoors: PBO's half-life (the time required for half of applied PBO to break down or move away from the application site) is about 4 days in field tests of agricultural soils conducted by a manufacturers' task force. In the same tests, conducted in California, Georgia, and Michigan, PBO persisted (measured as the time required for all applied PBO to dissipate) up to 30 days.[97]

The manufacturers' task force also measured PBO's half-life and persistence in water and aquatic sediments. In water tested in California, Arkansas, and Mississippi, the half-life was about a day. In sediments, the half-life was up to 24 days and PBO persisted up to 120 days.[97]

Indoors: There is less information available about PBO's persistence indoors, but a study from Justus Liebig University (Germany) found that PBO persisted for at least two weeks after a cockroach treatment on toys and in dust in a kindergarten.[98]

References

1. Tozzi, A. 1998. A brief history of the development of piperonyl butoxide as an insecticide synergist. In D.G. Jones, ed. *Piperonyl butoxide: The insecticide synergist.* San Diego: Academic Press. Pp. 1-5.
2. U.S. EPA. Office of Pesticide Programs. 2002. USEPA/OPP chemical ingredients database. www.cdpr.ca.gov.
3. Aspelin, A.L. and A.H. Grube. 1999. Pesticides industry sales and usage: 1996 and 1997 market estimates. U.S. EPA. Office of Pesticide Programs. p. 20. www.epa.gov/pesticides.
4. Farnham, A.W. 1998. The mode of action of piperonyl butoxide with reference to studying pesticide resistance. In D.G. Jones, ed. *Piperonyl butoxide: The insecticide synergist.* San Diego: Academic Press. Pp. 199-213.
5. Hainzal, D., L.M. Cole, and J.E. Casida. 1998. Mechanisms for selective toxicity of fipronil insecticide and its sulfone metabolite and desulfinyl photoproduct. *Chem. Res. Toxicol.* 11: 1529-1535.
6. Levine, B.S. and S.D. Murphy. 1977. Esterase inhibition and reactivation in relation to piperonyl butoxide-phosphorothionate interactions. *Toxicol. Appl. Pharmacol.* 40: 379-391.
7. Tripathi, A.M. and R.A. Agarwal. 1998. Molluscicidal and anti-AChE activity of tertiary mixtures of pesticides. *Arch. Environ. Contam. Toxicol.* 34: 271-274.
8. Keane, P. 1998. The use of piperonyl butoxide in formulations for the control of pests of humans, domestic pets and food animals. In D.G. Jones, ed. *Piperonyl butoxide: The insecticide synergist.* San Diego: Academic Press. Pp. 290-300.
9. Carpenter, L.J. and R.A. Roth. 1988. Potentiation by piperonyl butoxide of alpha-naphthylthiourea toxicity in the isolated, perfused rat lung. *Biochem. Pharmacol.* 37: 771-772.
10. Whitmore, R.W., J.E. Kelly, and P.L. Reading. 1992. National home and garden pesticide use survey. Final report, vol. 1: Executive summary, results, and recommendations. Research Triangle Park NC: Research Triangle Institute. Table G-1.
11. Adgate, J.L. et al. 2000. Pesticide storage and use patterns in Minnesota households with children. *J. Exp. Anal. Environ. Epidemiol.* 10: 159-167.
12. Calif. Dept. of Pesticide Regulation. 2001. Summary of pesticide use report data 2000. Indexed by chemical. Preliminary data. www.cdpr.ca.gov.
13. Hodgson, E. and P.E. Levi. 1998. Interactions of piperonyl butoxide with cytochrome P450. In D.G. Jones, ed. *Piperonyl butoxide: The insecticide synergist.* San Diego: Academic Press. Pp. 41-54.
14. Federal Insecticide, Fungicide, and Rodenticide Act Sec. 2(m).
15. 40 *Code of Federal Regulations* § 158.340.
16. Prentiss, Inc. 1998. Material safety data sheet: 655-113 Prentox® piperonyl butoxide technical. www.prentiss.com/msds/pdf/655_113.pdf.
17. World Health Organization and Food and Agricultural Organization. 1996. Pesticide residues in food — Evaluations 1995. [Part II] Toxicological and environmental. Geneva, Switzerland: World Health Organization. Pp. 282.
18. Bateman, D.N. 2000. Management of pyrethroid exposure. *Clin. Toxicol.* 38: 107-109.
19. Tanaka, T. 1993. Behavioral effects of piperonyl butoxide in male mice. *Toxicol. Lett.* 69: 155-161.
20. Ware, G.W. 2000. *The pesticide book.* Fresno CA: Thomson Publications. p. 379.
21. Takahashi, O. et al. 1994. Chronic toxicity studies of piperonyl butoxide in F344 rats. Induction of hepatocellular carcinoma. *Fund. Appl. Pharmacol.* 22: 291-303.
22. Friedman, M.A. and L. R. Eaton. 1978. Potentiation of methyl mercury toxicity by piperonyl butoxide. *Bull. Environ. Contam. Toxicol.* 20: 9-10.
23. Maekawa, A. et al. 1985. Lack of evidence of carcinogenicity of technical-grade piperonyl butoxide in F344 rats: Selective induction of ileocaecal ulcers. *Fd. Chem. Toxic.* 23: 675-682.
24. U.S. EPA. Office of Prevention, Pesticides and Toxic Substances. 1994. EPA Id# 067501. Piperonyl butoxide. Review of a series 82-4 subchronic inhalation toxicity study in rats. Memo from J. Doherty, Health Effects Division, to A. Dixon and B. Sidwell, Special Review and Reregistration Division. Washington, D.C., June 22.
25. National Cancer Institute. Undated. Cancer.gov dictionary. www.nci.nih.gov/dictionary.
26. Fujitani, T., Y. Tada, and M. Yoneyama. 1993. Hepatotoxicity of piperonyl butoxide in male F344 rats. *Toxicol.* 84: 171-183.
27. Ref. #17, p. 287-288.
28. Fujitani, T., T. Tanaka, Y. Hashimoto, and M. Yoneyama. 1993. Subacute toxicity of piperonyl butoxide in ICR mice. *Toxicol.* 83: 93-100.
29. Fujitani, T., Y. Tada, and M. Yoneyama. 1993. Hepatotoxicity of piperonyl butoxide in male F344 rats. *Toxicol.* 84: 171-183.
30. Takahashi, O. et al. 1994. Chronic toxicity studies of piperonyl butoxide in F344 rats: Induction of hepatocellular carcinoma. *Fund. Appl. Pharmacol.* 22: 291-303.
31. Fujitani, T. et al. 1992. Sub-acute toxicity of piperonyl butoxide in F344 rats. *Toxicol.* 72: 291-298.
32. U.S. EPA. Office of Prevention, Pesticides and Toxic Substances. 1988. EPA Reg. No.: 4816-72. Piperonyl butoxide. Review of a chronic feeding/oncogenicity study submitted by the Piperonyl Butoxide Task Force. Memo from J. Doherty, Hazard Evaluation Division, to P. Hutton and G. Werdig, Registration Division. Washington, D.C., Apr. 28.
33. U.S. EPA. Office of Prevention, Pesticides and Toxic Substances. 1995. Carcinogenicity peer review of piperonyl butoxide. Memo from J. Doherty and E. Rinde , Health Effects Division, to R. Keigwin, Registration Division, and A. Dixon and B. Sidwell, Special Review and Reregistration Division. Washington, D.C., June 7.
34. National Cancer Institute. Undated. Cancer.gov dictionary. www.nci.nih.gov/dictionary.
35. Takahashi, O. et al. 1997. Chronic toxicity studies of piperonyl butoxide in CD-1 mice: Induction of hepatocellular carcinoma. *Toxicol.* 124: 95-103.
36. Ref. #17, p. 291
37. Takahashi, O. et al. 1994. Chronic toxicity studies of piperonyl butoxide in F344 rats. Induction of hepatocellular carcinoma. *Fund. Appl. Pharmacol.* 22: 291-303.
38. U.S. Department of Health and Human Services. Public Health Service. National Toxicology Program. 2001. *9th report on carcinogens.* Safrole.

http://ehp.niehs.nih.gov/roc/ninth/rahc/safrole.pdf.
39. Schreiber-Deturmeny, E.M., A.M. Pauli, and J.L. Pastor. 1993. Determination of safrole, dihydro-safrole, and chloromethyldihydrosafrole in piperonyl butoxide by high-performance liquid chromatography. *J. Pharmaceut. Sci.* 82: 813-816.
40. Epstein, S. et al. 1967. Synergistic toxicity and carcinogenicity of 'Freons' and piperonyl butoxide. *Nature* 214: 526-528.
41. Fujii, K. and S. Epstein. 1979. Effects of piperonyl butoxide on the toxicity and hepatocarcinogenicity of 2-acetylaminofluorene and 4-acetylaminobiphenyl, and their N-hydroxylated derivatives, following administration to newborn mice. *Oncol.* 36: 105-112.
42. Ref. #17, pp. 288-293.
43. Breathnach, R. 1998. The safety of piperonyl butoxide. In D.G. Jones, ed. *Piperonyl butoxide: The insecticide synergist.* San Diego: Academic Press. p. 23.
44. Suzuki, H. and N. Suzuki. 1995. Piperonyl butoxide mutagenicity in human cells. *Mut. Res.* 344: 27-30.
45. Tayama, S. 1996. Cytogenetic effects of piperonyl butoxide and safrole in CHO-K1 cells. *Mut. Res.* 368: 249-260.
46. U.S. EPA. Prevention, Pesticides and Toxic Substances. 1998. Health effects test guidelines: OPPTS 870.5915. In vivo sister chromatid exchange assay. www.epa.gov/pesticides.
47. U.S. EPA. Office of Pesticides and Toxic Substances. 1985. Piperonyl butoxide-mutagenicity study submitted in response to Special Review DCI. Accession No. 257430. Registration No. 1021-974. Caswell: 670. Memo from I. Mauer, Hazard Evaluation Div. to T. Gardner and P. Shroeder, Registration Div. Washington, D.C., June 27.
48. Ref. # 32, p. R12-13.
49. Ref. # 43, p. 20.
50. Tanaka, T. 1992. Effects of piperonyl butoxide on F1 generation mice. *Toxicol. Lett.* 60: 83-90.
51. Tanaka, T., O. Takahashi, and S. Oishi. 1992. Reproductive and neurobehavioral effects in the three-generation toxicity study of piperonyl butoxide administered to mice. *Fd. Chem. Toxic.* 30: 1015-1019.
52. Tanaka, T. et al. 1994. Developmental toxicity evaluation of piperonyl butoxide in CD-1 mice. *Toxicol Lett.* 71: 123-129.
53. U.S. EPA. Office of Prevention, Pesticides and Toxic Substances. 1993. EPA ID # 067501. Memo from G.B. Reddy, Health Effects Division, to B. Sidwell and T. Chin, Reregistration Division.. Washington, D.C., Mar. 23. See attached data evaluation record, pp. 8-9.
54. Lee, T.-P., R. Moscati, and B.H. Park. 1979. Effects of pesticides on human leukocyte function. *Res. Comm. Chem. Pathol. Pharmacol.* 23: 597-609.
55. Diel, F. et al. 1999. Pyrethroids and piperonyl-butoxide affect human T-lymphocytes in vitro. *Toxicol. Lett.* 107: 65-74.
56. National Research Council. Commission on Life Sciences. Board on Environmental Studies and Toxicology. Committee on Hormonally Active Agents in the Environment. 1999. *Hormonally active agents in the environment.* Washington, D.C.: National Academy Press, p. 10.
57. Eubanks, M.W. 1997. Hormones and health. *Environ. Health Persp.* 105: 482-487.
58. Ref. #17, 290-291.
59. Baynes, R.E. and J.E. Riviere. 1998. Influence of inert ingredients in pesticide formulations on dermal absorption of carbaryl. *AJVR* 59: 168-175.
60. J.R. Thornton-Manning, and A.R. Dahl. 1997. Metabolic capacity of nasal tissue interspecies comparisons of xenobiotic-metabolizing enzymes. *Mut. Res.* 380: 43-59.
61. Lu, P.-Y. 1977. The environmental fate of three carcinogens: benzo-(a)-pyrene, benzidine, and vinyl chloride evaluated in laboratory model ecosystems. *Arch. Environ. Contam. Toxicol.* 6: 129-142.
62. Whyatt, R.M. 2002. Residential pesticide use during pregnancy among a cohort of urban minority women. *Environ. Health Persp.* 110: 507-514.
63. U.S. Dept. of Agriculture. Agricultural Marketing Service. Science and Technology Division. 1997. Pesticide data program: Annual summary calendar year 1995. Appendix E. p. 9.
64. U.S. Dept. of Agriculture. Agricultural Marketing Service. Science and Technology Division. 1998. Pesticide data program: Annual summary calendar year 1996. Appendix D. p. 23.
65. U.S. Dept. of Agriculture. Agricultural Marketing Service. Science and Technology Division. 1998. Pesticide data program: Annual summary calendar year 1997. Appendix E. p. 23.
66. U.S. Dept. of Agriculture. Agricultural Marketing Service. Science and Technology Division. 2000. Pesticide data program: Annual summary calendar year 1998. Appendix E. p. 29.
67. U.S. Dept. of Agriculture. Agricultural Marketing Service. Science and Technology Division. 2001. Pesticide data program: Annual summary calendar year 1999. Appendix E. p. 30.
68. U.S. Dept. of Agriculture. Agricultural Marketing Service. Science and Technology Division. 2001. Pesticide data program: Annual summary calendar year 2000. Appendix E. p. 39.
69. Osimitz, T.G. and J.F. Hobson. 1998. An ecological risk assessment of piperonyl butoxide. In D.G. Jones, ed. *Piperonyl butoxide: The insecticide synergist.* San Diego: Academic Press. Pp. 122-135.
70. Siddiqui, M.K.J. et al. 1993. Alterations in extra hepatic glutathione S-transferase activity in pigeon exposed to dimethoate, piperonyl butoxide and DDT. *Indian J. Exper. Biol.* 31: 278-279.
71. Paul, E.A., and H.A. Simonin. 1995. Comparison of the toxicity of a synergized and non-synergized insecticide to young trout. *Bull. Environ. Contam. Toxicol.* 55: 453-460.
72. Paul, E.A. and H.A. Simonin. 1996. Effects of naled, synergized and non-synergized resmethrin on the swimming performance of young trout. *Bull. Environ. Contam. Toxicol.* 57: 495-502.
73. Miyamoto, M. et al. 1992. Effect of metabolism on bioconcentration of geometric isomers of d-phenothrin in fish. *Chemosphere* 24: 2001-2007.
74. Erickson, D.A., F.E. Laib, and J.J. Lech. 1992. Biotransformation of rotenone by hepatic microsomes following pretreatment of rainbow trout with inducers of cytochrome P450. *Pest. Biochem. Physiol.* 42: 140-150.
75. Reinbold, K.A. and R.L. Metcalf. 1976. Effects of the pesticide synergist piperonyl butoxide on metabolism of pesticides in green sunfish. *Pest. Biochem. Physiol.* 6: 401-412.
76. Sijm, D.T.H.M., G. Schaap, and A. Opperhuizen. 1993. The effect of the biotransformation inhibitor piperonyl butoxide on the bioconcentration of 2,8-dichlorodibenzo-p-dioxin and pentachlorobenzene in goldfish. *Aquat. Toxicol.* 27: 345-360.
77. Melancon, M.J., J. Saybolt, and J.L. Lech. 1977. Effect of piperonyl butoxide on disposition of di-2-ethylhexyl phthalate by rainbow trout. *Xenobiotica* 7: 633-640.
78. U.S. EPA. 1998. Integrated Risk Information System. Di(2-ethylhexyl)phthalate (DEHP). www.epa.gov/iris/subst/0014.htm
79. Meldahl, A.C., K. Nithipatikom, and J.J. Lech. 1996. Metabolism of several 14C-nonylphneol isomers by rainbow trout (*Oncorhynchus mykiss*): *In vivo* and *in vitro* microsomal metabolites. *Xenobiotica* 26: 1167-1180.
80. Miranda, C.L., M.C. Henderson, and D.R. Buhler. 1998. Evaluation of chemicals as inhibitors of trout cytochrome P450s. *Toxicol. Appl. Pharmacol.* 148: 237-244.
81. Paolucci, M., N. Custodia, and I.P. Callard. 1998. Progesterone effects and receptors, subavian species. In Knobil, E. and J.D. Neill. *Encyclopedia of reproduction.* Vol. 4. San Diego: Academic Press. Pp. 16-23.
82. Baldwin, W.S. and G. A. LeBlanc. 1994. Identification of multiple steroid hydroxylases in *Daphnia magna* and their modulation by xenobiotics. *Environ. Toxicol. Chem.* 13: 1013-1021.
83. Hagler, J.R., G.D. Waller, and B.E. Lewis. 1989. Mortality of honeybees (Hymenoptera: Apidae) exposed to permethrin and combinations of permethrin with piperonyl butoxide. *J. Apicult. Res.* 28: 208-211.
84. Federle, P.F. and W.J. Collins. 1976. Insecticide toxicity to three insects from Ohio ponds. *Ohio J. Sci.* 76: 19-24.
85. Lindsay, R.J. and A.M. Clark. 1978. The effect of microsomal enzyme inhibitor, piperonyl butoxide, on mutagenesis in *Drosophila melanogaster.* (Abstract.) *Mut. Res.* 53: 221.
86. Fang, N., P.E.A. Teal, and J.H. Tumlinson. 1995. Characterization of oxidase(s) associated with the sex pheromone gland in *Manduca sexta* (L.) females. *Arch. Insect Biochem. Physiol.* 29: 243-257.
87. Ref. # 20, p. 387.
88. Roberts, B.L. and H.W. Dorough. 1984. Relative toxicities of chemicals to the earthworm Eisenia foetida. *Environ. Toxicol. Chem.* 3: 67-78.
89. Kato, H., O. Kodama, and T. Akatsuka. 1995. Characterization of an inducible P450 hydroxylase involved in the rice diterpene phytoalexin biosynthetic pathway. *Arch. Biochem. Biophys.* 316: 707-712.
90. Tanigaki, F. 1993. Interaction of microsomal cytochrome P-450s and n-phenylcarbamates that induce flowering in *Asparagus* seedlings. *Z. Naturforsch.* 48 c: 879-885.
91. Simarmata, M. and D. Penner. 1993. Protection from primisulfuron injury to corn (*Zea mays*) and sorghum (*Sorghum bicolor*) with herbicide safeners. *Weed Technol.* 7: 174-179.
92. Kotoula-Syka, E. and K.K. Hatzios. 1996. Interactions of tribenuron with four safeners and piperonyl butoxide on corn (*Zea mays*). *Weed Sci.* 44: 215-218.
93. Cottingham, C.K., K.K. Hatzios, and S.A. Meredith. 1993. Comparative responses of selected corn (*Zea mays* L.) hybrids to EPTC and metolachlor. *Weed Res.* 33: 161-170.
94. Varsano, R. and B. Rubin. 1991. Increased herbicidal activity of triazine herbicides by piperonyl butoxide. *Phytoparasitica* 19: 225-236.
95. Kwon, C.S., J.J. Kells, and D. Penner. 1995. Combined effects of acetolactate synthase-inhibiting herbicides with terbufos and piperonyl butoxide on corn (*Zea mays*) and soybean (*Glycine max*). *Weed Technol.* 9: 696-702.
96. Kwon, C. and D. Penner. 1996. The effect of piperonyl butoxide and adjuvants on sulfonylurea herbicide activity. *Weed Technol.* 10: 127-133.
97. Arnold, D.J. 1998. The fate and behavior of piperonyl butoxide in the environment. In D.G. Jones, ed. *Piperonyl butoxide: The insecticide synergist.* San Diego: Academic Press. Pp. 105-117.
98. Fischer, A, and T. Eikmann. 1996. Improper use of an insecticide at a kindergarten. *Toxicol. Lett.* 88: 359-364.