#### ●INSECTICIDE FACTSHEET

# **DIAZINON: TOXICOLOGY**

Diazinon is an organophosphate insecticide with agricultural, commercial, and household uses. Household uses predominate, with 75 million applications in the U.S. annually totalling over 5 million pounds.

Diazinon is toxic to the nervous system. Symptoms of acute diazinon poisoning include headache, nausea, dizziness, tearing, and sweating. Some symptoms, including blurred vision, headaches, and memory problems, can last for months or years.

In laboratory tests, feeding diazinon to pregnant animals has caused a decrease in the endurance, coordination, and growth of their offspring. In addition, the sexual development of offspring of both sexes was delayed.

Diazinon exposure has been associated with an increased risk of brain cancer in children and the cancer non-Hodgkin's lymphoma in farmers.

Infants are especially susceptible to diazinon. In addition, 9 - 16 percent of people have a slow form of an important detoxification enzyme and thus are particularly susceptible.

The U.S. Environmental Protection Agency estimated exposure to household residents following use of diazinon insecticide products and found that exposure following lawn care applications of liquid products and following indoor applications exceed the agency's "levels of concern."

#### By CAROLINE COX

Diazinon (see Figure 1) is an organophosphate insecticide, chemically related to other common insecticides like malathion and chlorpyrifos. It was first registered in the U.S. in 1956<sup>2</sup> and is sold under a variety of brand names, including DZN<sup>3</sup> and Knox Out 2FM.<sup>4</sup>

Use

Diazinon has agricultural, commercial, and household uses, but household uses predominate. Estimated agricultural use is 1.5 million pounds annually. Crops using the most diazinon are almonds, berries, pecans, and nectarines.<sup>5</sup> About 75 million household applications are made annually, 18 million indoors and 57 million outdoors.<sup>6</sup> Home, lawn, and garden use totals 5.5 million pounds per year.<sup>5</sup>

Mode of Action

Inside living things, diazinon is

Caroline Cox is JPR's editor.

Figure 1
Diazinon and Diazoxon

H
$$(CH_3)_2$$
 $C$ 
 $N$ 
 $O$ -P
 $OC_2H_5$ 
 $CH_3$ 
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transformed into a molecule called diazoxon. (See Figure 1.) Diazinon, and the more potent diazoxon, kill insects by interfering with nervous system function, as do all members of the organophosphate chemical family. Normally, impulses are transmitted chemically from the end of one nerve cell to the beginning of another; one of the chemical transmitters used in animal nervous

systems is called acetylcholine. After transmitting the nerve impulse, acetylcholine is destroyed by an enzyme called acetylcholinesterase (AChE) in order to clear the way for another transmission. Organophosphates attach to AChE and prevent it from destroying acetylcholine, causing overstimulation of the nerves.<sup>8</sup>

Mammal and insect nervous systems are similar enough that effects of organophosphates are similar.<sup>1</sup>

It is worth noting that not all of diazinon's toxicological effects stem from its inhibition of AChE. Diazinon and other organophosphates inhibit numerous enzymes with molecular structures that are similar to AChE. For example, an enzyme involved in the metabolism of the amino acid tryptophan is strongly inhibited by diazinon and diazoxon.<sup>9</sup>

## Inert Ingredients

Like most pesticide products, most commercial diazinon products contain ingredients other than diazinon that are misleadingly labelled as "inert." Public information about the identity of these inerts is scanty. See "Toxicology of Inert Ingredients," below, for information about inert ingredients that have been publicly identified.

Effects of Acute Exposure

Symptoms of acute (short-term) diazinon poisoning in people are similar

to the symptoms of any organophosphate insecticide poisoning: headache, nausea, dizziness, tearing, sweating, salivation, drowsiness, agitation, anxiety, 10 and influenza-like symptoms. 11 Symptoms of higher exposure include an abnormal heart rate (either too slow or too rapid), 12

muscle weakness, muscle twitching, pinpoint pupils, <sup>1</sup> lung congestion, <sup>13</sup> cardiac arrest, <sup>14</sup> and seizures. <sup>15</sup>

Other symptoms observed in laboratory animals after acute exposure include abnormal walking, reduced activity, <sup>16</sup> increased blood sugar levels, <sup>17</sup> low blood

# TOXICOLOGY OF INERT INGREDIENTS

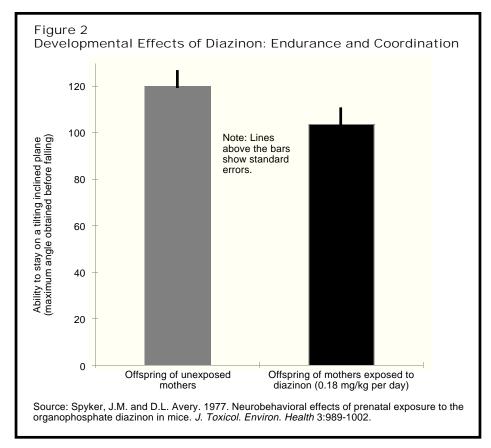
Ingredients in commercial diazinon products that have been publicly identified<sup>1</sup> include the following:

- 1,2-Benzisothiazolin-3-one is a preservative that has caused allergic skin reactions.<sup>2</sup>
- Calcium Silicate caused an increase in the frequency of abnormal chromosomes in a laboratory study using human blood cells. The incidence of a second kind of genetic damage, called sister chromatid exchanges, was also increased.<sup>3</sup>
- Cumene is a primary eye and skin irritant. It depresses the central nervous system. Symptoms of exposure include burning sensations, headache, dizziness, confusion, and drowsiness.<sup>4</sup>
- Diethylenetriamine is a potent skin irritant and also causes allergic skin sensitization. Other symptoms of exposure include irritation of the cornea and conjunctiva of the eye, asthmatic breathing, and nausea. In laboratory tests, it caused an increase in the incidence of a kind of genetic damage, sister chromatid exchanges.<sup>5</sup>
- Ethylbenzene can cause severe lung injury if inhaled. The offspring of rats who breathed ethylbenzene during their pregnancy had an increased incidence of birth defects. In laboratory animals who breathed ethylbenzene, the incidence of several cancers increased. 6
- **Isobutane** depresses the central nervous system. It is also extremely flammable and can be an explosion

hazard.<sup>7</sup>

- Polyvinyl Alcohol causes anemia in laboratory tests.<sup>8</sup>
- **Propane** is flammable and a severe explosion hazard.<sup>9</sup>
- Silica (crystalline) has been classified as "carcinogenic to humans" by the International Agency for Research on Cancer<sup>10</sup> and as "known to be a human carcinogen" by the National Toxicology Program.<sup>11</sup> It causes emphysema and obstructive airway disease and has also caused genetic damage in exposed people and laboratory tests.<sup>11</sup>
- Sodium sulfite. It may cause eye and skin irritation with vomiting and diarrhea<sup>12</sup> as well as skin allergies.<sup>13</sup> Exposure to small amounts can cause severe allergic reactions.<sup>14</sup>
- •1,2,4-Trimethylbenzene damages the central nervous system and is irritating to eyes, skin, and the upper respiratory tract. 15
- **Xylenes** are central nervous system depressants, <sup>16</sup> cause eye and skin irritation, headaches, nausea, and confusion. In laboratory tests they have caused kidney damage, <sup>17</sup> a reduction in fetal growth, an increase in fetal death, and an increase in the incidence of birth defects. <sup>16</sup>
- Inert ingredients identified under the Freedom of Information Act (U.S. EPA. Office of Prevention, Pesticides, and Toxic Substances. 1999. Letter from Calvin Furlow to Marcy Trice, Aug. 5), and on material safety data sheets from www.cdms.net, www.ortho.com, and www.bonideproducts.con. For detailed information see NCAP's web page, www.pesticide.org.
- Damstra, R.J., W.A. van Vloten, and C.J.W. van Ginkel. 1992. Allergic contact dermatitis from the preservative 1,2-benzisothiazolin-3-one (1,2-BIT; Proxel<sup>®</sup>): a case report, its prevalence in those occupationally at risk and in the general dermatological population, and its relationship to allergy

- to its analogue Kathon®CG. Cont. Dermat. 27:105-109.
- Aslam, M., Fatima, N. and Rahman, Q. 1993. Cytotoxic and genotoxic effects of calcium silicates on human lymphocytes in vitro. *Mut. Res.* 300(1):45-48.
- National Library of Medicine. Toxicology and Environmental Health Information Program. Hazardous Substance Database. 2000. Cumene. www.toxnet.nlm.nih.gov, Mar. 28.
- National Library of Medicine. Toxicology and Environmental Health Information Program. Hazardous Substance Database. 2000. Diethylenetriamine. www.toxnet.nlm.nih.gov, Mar. 28.
- National Library of Medicine. Toxicology and Environmental Health Information Program. Hazardous Substance Database. 2000. Ethylbenzene. www.toxnet.nlm.nih.gov, Feb. 8.
- National Library of Medicine. Toxicology and Environmental Health Information Program. Hazardous Substance Database. 2000. Isobutane. www.toxnet.nlm.nih.gov, Feb. 8.
- National Library of Medicine. Toxicology and Environmental Health Information Program. Hazardous Substance Database. 2000. Polyvinyl alcohol. www.toxnet.nlm.nih.gov, Feb. 8.
- National Library of Medicine. Toxicology and Environmental Health Information Program. Hazardous Substance Database. 2000. Propane. www.toxnet.nlm.nih.gov. Feb. 8.
- International Agency for Research on Cancer. 1997. Silica. http://193.51.164.11/htdocs/ Monographs/Vol68/SILICA.HTM.
- U.S. Dept. of Health and Human Services. Public Health Service. National Toxicology Program. 2000. Ninth Report on Carcinogens. http://ehis.niehs.nih.gov/roc/toc9.html.
- Acros Organics. 1997. Material safety data sheet: sodium sulfite. www.fisher1.com/fb/ itv?16..f97.1.msa0013.666..1.9., Sept. 2.
- Lodi, A. et al. 1993. Contact allergy to sodium sulfite contained in an antifungal preparation. Cont. Dermatit. 29:97.
- Anonymous. 1986. MSDS for sodium sulfite, anhydrous. www.chem.utah.edu/MSDS/S/ SODIUM\_SULFITE,\_ANHYDROUS, Aug. 18.
- Aldrich Chemical Co, Inc. 1998. Material safety data sheet: 1,2,4-Trimethylbenzene. Milwaukee WI. www.sigma-aldrich.com.
- National Library of Medicine. Toxicology and Environmental Health Information Program. Hazardous Substance Database. 2000. Xylenes. www.toxnet.nlm.nih.gov, Apr. 20.
- U.S. Dept. of Health and Human Services. Agency for Toxic Substances and Disease Registry. 1995. Toxicological profile for total xylenes. Atlanta GA.



Offspring of mice exposed to diazinon during pregnancy had less endurance and coordination than offspring of unexposed mothers.

pressure,  $^{18}$  and inflammation of the pancreas.  $^{19}$ 

Physicians have reported that symptoms of acute diazinon exposure in children are different than those in adults. Tearing, sweating, 1 slow heart rate and muscle twitches, 20 common in adults, are infrequent in children. Seizures are much more common in children than in adults. 20 Inflammation of the pancreas is another symptom that is "not rare" in children with diazinon poisoning. 21

Whether acute exposure to diazinon and other organophosphate insecticides can cause long-term health problems has been a controversial issue. Recently (1998), however, a U.S. Environmental Protection Agency (EPA) review found that "symptoms may persist for months or years after the initial exposure." Persistent symptoms include blurred vision, headaches, muscle weakness, lethargy, short term memory impairment, inabil-

ity to concentrate, confusion, lowered intelligence test scores, depression, and irritability.  $^{23}$ 

## Skin Allergies

Both diazinon and the diazinon-containing insecticide Diazinon 4E caused allergic skin reactions in people. Although pesticides in general are tested on laboratory animals, diazinon and Diazinon 4E were tested on a group of 56 people. About 10 percent of them showed "positive dermal sensitization." In this test, diazinon is applied to the skin of the subjects twice. If the reaction to the second exposure is greater than the reaction to the first exposure, the chemical causes sensitization. <sup>25</sup>

# Effects of Subchronic and Chronic Exposure

Laboratory studies of subchronic (medium-term) and chronic (long-term) ex-

posure typically expose animals by feeding them the test chemical over a period of several months, for subchronic tests, or over a one to two year period, for chronic tests. In subchronic and chronic tests with diazinon, the primary effect studied is inhibition of acetylcholinesterase (AChE), diazinon's target enzyme. In five studies ( a six-week study of people; a one-year, a three-month, and a one-month study of dogs; and a onemonth study of female rats) AChE inhibition occurred at strikingly low doses: the animals were fed less than 50 micrograms per kilogram of animal body weight per day. 26,27,28

A study of rats who were exposed by breathing diazinon-contaminated air measured AChE inhibition at a similar level of exposure (26 micrograms per kilogram (µg/kg) of body weight per day).<sup>29</sup>

At somewhat higher feeding levels (500 µg/kg per day), other effects occur. Two studies from Simon Fraser University found that medium-term exposure caused reduced weight gain, liver injury, and reduced levels of four chemicals (other than acetylcholine) that are used to transmit nervous system impulses in the brain. 30,31

For pets, one form of chronic exposure is from wearing flea collars. Several studies have shown that the activity of acetylcholinesterase was inhibited in dogs and cats wearing flea collars. Inhibition continued for the entire time the collar was worn, up to 315 days.<sup>32</sup>

Only four of the above studies, the tests of flea collars on pests and the one-month study of people, used commercial diazinon-containing products.

#### Effects on Reproduction

Diazinon exposure of pregnant animals in laboratory tests has demonstrated that this insecticide can cause a variety of reproductive problems, including damage to the developing nervous system, delays in sexual development, stillbirths, death of newborn offspring, and birth defects.

The effects on the developing nervous system are most significant. An EPA-funded study using mice exposed to low levels of diazinon in their food (0.18 mil-

ligrams per kilogram, mg/kg, per day) found that the endurance and coordination of the offspring was impaired. They were unable to remain on as steep of an inclined plane as mice born to unexposed mothers. (See Figure 2.) In addition, their ability to climb developed later than mice born to unexposed mothers.<sup>33</sup>

This study also showed that diazinon exposure of pregnant mice delayed the sexual development of their offspring. Sexual maturity (measured by the age when vaginal opening occurred in females and descent of the testes in males) was delayed about 6 percent in offspring of exposed mothers. <sup>33</sup>

A study of dogs that were fed diazinon (1 mg/kg per day) during pregnancy showed that their exposure increased the number of stillbirths. Less than 6 percent of the offspring of unexposed mothers were stillborn, while 15 percent of offspring of mothers fed diazinon were stillborn. The researchers who conducted this study, from the Food and Drug Administration, noted that diazinon made the mothers "extremely high strung" resulting in stillbirths as the mothers "would not lay still while giving birth."

These researchers also found that feeding diazinon (5 mg/kg per day) to pregnant pigs increased the incidence of skull deformities in the offspring.<sup>34</sup>

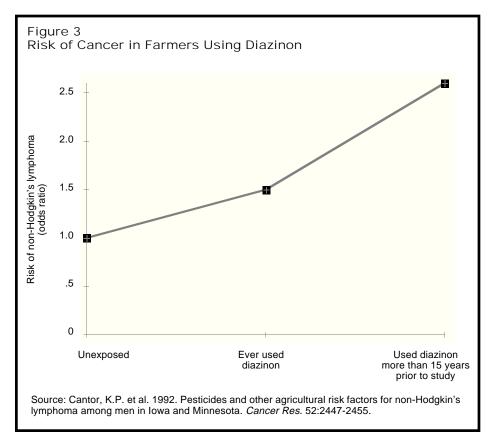
Finally, a study of pregnant rats fed diazinon (7 mg/kg per day) found that the number of offspring that died was greater in litters from exposed mothers than for litters from unexposed mothers. The offspring of exposed mothers also grew more slowly while they were nursing. This study was conducted by a diazinon manufacturer.<sup>35</sup>

Diazinon also has caused reproductive problems in male animals. Dogs fed diazinon (20 mg/kg per day) developed atrophied testicles.<sup>36</sup>

There is no publicly available information about the reproductive effects of commercial diazinon-containing products.

#### **Endocrine Disruption**

Problems caused by synthetic chemicals that disrupt the normal functioning



Use of diazinon has been associated with an increased risk of cancer in several studies, including this National Cancer Institute study of farmers in Iowa and Minnesota.

of our hormone systems have been well publicized in the last decade. Of particular concern are chemicals that interfere with the activity of estrogen, often called the female sex hormone. Estrogen has recently been shown to affect the development and growth of cells in the lining of the colon. The result of abnormal growth of these cells is colon cancer. In tests with cultures of cells from a human colon, low concentrations of diazinon had growth-promoting effects, suggesting diazinon had interfered with the normal activity of estrogen.<sup>37</sup>

There is no publicly available information about the endocrine-disrupting effects of commercial diazinon-containing products.

# Carcinogenicity

Diazinon's carcinogenicity (its ability to cause cancer) has been studied in laboratory animals with negative results; as a result it has been classified as "not likely" to be a carcinogen by EPA.<sup>38</sup>

Studies of people who have used diazinon, however, show just the opposite: there is an association between diazinon use and the risk of certain types of cancer. In a study of children in Missouri, garden diazinon use by the parents was associated with an increased risk of brain cancer in their children.<sup>39</sup> In a study of Iowa and Minnesota farmers conducted by the National Cancer Institute (NCI), use of diazinon was associated with an increased risk of non-Hodgkin's lymphoma.<sup>40</sup> (See Figure 3.) Similar results were found in an NCI study of Nebraska farmers.<sup>41</sup>

#### Mutagenicity

Diazinon's mutagenicity, its ability to cause genetic damage, is controversial. The World Health Organization, in its review of the effects of diazinon on human health and the environment, wrote that diazinon "gave no evidence of a mutagenic potential."<sup>42</sup>

However, a series of other studies show that diazinon in fact can damage genes in human blood cells, in cells from laboratory animals, and in bacteria.

A study conducted at by Italian cancer researchers used concentrations of diazinon equivalent to those found in an Italian food monitoring study. They found that this low level of exposure increased the occurrence in human blood cell cultures of a type of genetic damage called micronuclei. And Micronuclei are broken or separated chromosomes produced when a cell divides. Micronuclei were about 50 percent more frequent in exposed cells than in unexposed cells.

Two older studies of human blood cell cultures found that diazinon was mutagenic. Abnormal chromosomes were more frequent in human blood cell cultures exposed to diazinon than they were in unexposed cells, <sup>45</sup> as was a type of genetic damage called sister chromatid exchanges. <sup>46</sup> (Sister chromatid exchanges are exchanges of genetic material between parts of a duplicating chromosome. <sup>47</sup>)

A fourth study, conducted by the National Institute of Hygenic Sciences in Japan, found that diazinon exposure increased the frequency of abnormal chromosomes in hamster lung cell cultures.<sup>48</sup>

Finally, a study of *Salmonella* bacteria found that diazinon was mutagenic to one of the four strains tested.<sup>49</sup>

There is no publicly available information about the mutagenicity of commercial diazinon-containing products.

#### Sensitive Populations

Physicians have long noted that infants appear to be particularly susceptible to diazinon poisoning. For example, in 1970, 3-week old twins were poisoned by a diazinon cockroach treatment in the other half of the duplex in which they lived. Both twins required five days of hospitalization, although none of the adults or older children living in either half of the duplex were ill.<sup>50</sup> In another example, a two-month old infant devel-

oped symptoms of cerebral palsy after a diazinon treatment of her home. Symptoms persisted for seven months, until her family moved out of the treated home. <sup>51,52</sup> One reason for infants' increased susceptibility is that newborns have low levels of the enzyme that usually breaks down diazoxon, the active form of diazinon. <sup>53</sup>

Individuals whose body chemistry is less efficient at breaking down diazoxon are also more sensitive to this insecticide. The enzyme that breaks down diazoxon is produced by a gene called PON1. Each person has two PON1 genes. One form of this gene, called the R form, produces an enzyme that is less efficient at breaking down diazoxon, so people with two R genes are most susceptible to diazinon. About 9 percent of people of northern European descent have two R genes, while about 16 percent of people of Hispanic origin have two R genes. This means that a substantial fraction of the population will be particularly sensitive to diazinon and suggests an additional hazard for farmworkers, since in the U.S. many farmworkers are of Hispanic origin.<sup>54</sup>

Another sensitive population may be those who are malnourished. Studies with laboratory animals have found that rats fed a protein deficient diet were almost twice as susceptible to diazinon as rats fed an adequate diet. 55,56

# Synergistic Effects

A wide variety of chemicals interact synergistically with diazinon, meaning that their toxicity together is greater than the sum of their individual toxicities. This synergism has been observed with compounds from strikingly different chemical families, including other pesticides, drugs, and nutrients. (See Table 1) The length of the list in Table 1 is sobering, since real-life exposures are often to multiple chemicals while most toxicological testing and most regulation of hazardous chemicals is based on single exposures.

#### Toxic Breakdown Products

If a diazinon-containing product is contaminated with a trace of water, some

Table 1
Synergistic Interactions with Diazinon

Other Pesticides	
clotrimazole (fungicide)	(1)
captan (fungicide)	(2)
dieldrin (insecticide)	(3)
carbaryl (insecticide)	(4)
atrazine (herbicide)	(5)
Drugs	
cimetidine (ulcer medication)	(6)
succinylcholine (anesthetic)	(7)
cocaine (narcotic)	(8)

#### Nutrients

ascorbic acid (Vitamin C) (9 tryptophan (amino acid) (10

- Ronis, M.J.J. and T.M. Badger. 1995. Toxic interactions between fungicides that inhibit ergosterol biosynthesis and phosphorodithioate insecticides in the male rat and bobwhite quail. *Toxicol. Appl. Pharmacol.* 130:221-228.
- Stromberg, K.L. 1977. Seed treatment pesticide effects on pheasant reproduction at sublethal doses. *J. Wildl. Manage*. 41:632-642.
- Abdelsam, E.B. and E.J.H. Ford. 1986. Effect of pretreatment with hepatic microsomal enzyme inducers on the toxicity of diazinon in calves. Res. Vet. Sci. 41:336-330
- Keplinger, M.L. and W.B. Deichmann. 1967. Acute toxicity of combinations of pesticides. *Toxicol. Appl. Pharmacol.* 10:586-595.
- Lichtenstein, E.P., T.T. Laing, and B.N. Anderegg. 1973. Synergism of insecticides by herbicides. *Science* 181: 847-849.
- 6. Kurt, T.L. 1988. Letter to the editor. *Vet. Hum. Toxicol.* 30:268.
- Ware, M.R. et al. 1990. Electroconvulsive therapy complicated by insecticide ingestion. J. Clin. Psychopharmacol. 10:72-73.
- Roth, L. et al. 1992. Cocaine hepatoxicity: Influence of hepatic enzyme inducing and inhibiting agents on the site of necrosis. Hepatol. 15:934-940.
- Enan, E.E. et al. 1982. In-vivo interactions of some organophosphorous insecticides with different biochemical targets in white rats. J. Environ. Sci. Health B17:549-570.
- Abdelsam, E.B. and E.J.H. Ford. 1987. The effect of induced liver, kidney, and lung lesions on the toxicity of levamisole and diazinon in calves. J. Comp. Path. 97:619-627.

of the diazinon in the product breaks down into two chemicals that are extremely potent acetylcholinesterase inhibitors, monothiotepp and sulfotepp. Monothiotepp has been reported to be 14,000 times more toxic than diazinon itself. In the early 1990s, when several Australian dogs died after being washed with a diazinon product and some of their handlers became ill, regulatory authorities suspected monothiotepp or sulfotepp contamination and screened diazinon-containing products from pesticide retailers. They tested 169 products and found that about 5 percent were contaminated with the two breakdown products and traces of water.<sup>57</sup> The contamination of these products greatly increases their toxicity. NCAP located no similar studies of U.S. products.

# Exposure

Exposure to diazinon is a complicated subject. "Organophosphates are efficiently absorbed by inhalation, ingestion, and skin penetration," according to EPA, and exposure by "multiple routes can lead to serious additive toxicity." For exposures following residential applications, a single application can lead to exposure via all three routes. 58

For example, researchers from British Columbia and from North Carolina State University studied broadcast applications to carpets and "crack and crevice" applications, thin streams of pesticide applied just to the kind of site usually inhabited by cockroaches. (Crack and crevice applications are an alternative to broadcast sprays and in general use a smaller amount of insecticide.) After application, diazinon was found both in the air of the treated rooms and on horizontal surfaces in the rooms.<sup>59-61</sup> Diazinon in air leads to inhalation exposure. Diazinon settling on horizontal surfaces leads to both exposure through the skin (if people contact the horizontal surfaces) and ingestion (if, for example, a hand contacts a contaminated surface and then is put in the mouth).

These exposures can be persistent. Air was contaminated for 21 days following crack and crevice application, 59 horizontal surfaces were contaminated for six weeks following application. 60 The British Columbia researchers recommended not entering any unventilated rooms for at least two days

after treatment.61

EPA surveys of air inside houses, not specifically ones recently treated with diazinon, found that diazinon is surprisingly common in indoor air. In surveys in Florida, 62 Texas, 63 and Arizona, 64 between 53 and 100 percent of homes were contaminated with diazinon.

Outdoor applications also lead to exposure through inhalation (breathing of contaminated air), dermal (skin contact with treated turf), and ingestion (inadvertent hand-to-mouth transfers).<sup>65</sup>

EPA recently (April, 2000) estimated exposure via multiple routes following both lawn care and crack-and-crevice indoor applications. They found that exposures following lawn care applications of liquid diazinon products and following indoor crack and crevice treatments exceeded EPA's "level of concern" for both adults and children. 66

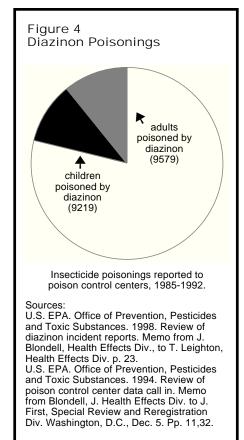
Contaminated house dust has recently been studied and can also result in multiple routes of exposure. Skin can contact dust particles, the particles can be inhaled, and they can be ingested.<sup>67</sup> Dust is believed to be a particularly important source of exposure to children.<sup>68</sup>

Diazinon-contaminated dust can be common. Surveys in Florida, New Jersey, California, Texas, and Arizona found diazinon in the dust from 53 to 80 percent of the houses tested. 63,64,68-70

Farmworker children may be particularly at risk of exposure to diazinon via dust. The California study found higher and more frequent diazinon contamination in farmworker homes than in non-farmworker homes in the same town.<sup>70</sup>

#### **Human Poisonings**

Diazinon's frequent use and significant toxicity means that poisonings of people are frequent. EPA characterizes diazinon as "one of the leading causes of acute reactions to insecticide use reported as poisoning incidents in the United States." A review of data collected by poison control centers nationwide between 1985 and 1992 showed that diazinon was the second most frequent cause of nonoccupational insecticide poi-



Diazinon is a leading cause of insecticiderelated poisonings, responsible for 23 percent of reported incidents.

sonings. Almost one-quarter of the insecticide poisonings reported to the centers were caused by diazinon. Nearly half of these poisonings involved children under six years of age. 72

## References

- Reigart, J.R. and J.R. Roberts. 1999. Recognition and management of pesticide poisoning. Fifth edition. Washington, D.C.: U.S. EPA. Pp. 34-38.
   U.S. EPA. Office of Pesticides and Toxic Sub-
- U.S. EPA. Office of Pesticides and Toxic Substances. 1988. Guidance for the reregistration of pesticide products containing diazinon as the active ingredient. Washington, DC, Dec. p. 6.
- Novartis Crop Protection, Inc. 1999. DZN Diazinon 50W material safety data sheet. Greensboro NC, Feb. 24. www.cdms.net.
- Elf Atochem North America, Inc. 2000. Knox out 2FM material safety data sheet. Philadelphia PA, Mar. 22. www.cdms.net.
- U.S. EPA. 1999. Quantitative usage analysis for diazinon. Washington, DC, Jan. 29. www.epa.gov/pesticides/op.
- 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.
- 7. U.S. Dept. of Health and Human Services. Public Health Service. Agency for Toxic Substances

- and Disease Registry. 1996. Toxicological profile for diazinon. Atlanta, GA, Aug. p.82.
- Ware, G.W. 2000. The pesticide book. Fresno, CA: Thomson Publications. p. 181.
- Seifert, J. and T. Pewnim. 1992. Alteration of mice L-tryptophan metabolism by the organophosphorous acid triester diazinon. Biochem. Pharmacol. 44:2243-2250.
- U.S. EPA. Office of Prevention, Pesticides and Toxic Substances. 1997. Review of chlorpyrifos poisoning data. Memo from J. Blondell, Health Effects Div., to L. Propst, Special Review and Reregistration Div. Washington, D.C. p.9
- 11. Murray, V.S. et al. 1992. Health effects of organophosphate sheep dips. Brit. Med. 305(6861):1090.
- Forbat, I.N. and J.D. Skehan. 1992. Health effects of organophosphate sheep dips. Brit. Med. J. 305:1502-1503.
- Rude, C, P. Markers, and M. Døssing. 1984. Pulmonary oedema following absorption of an insecticide, brought over the counter, through the skin. Ugeskr Laeger 146:2400-2401.
- Wecker, L., R. Mrak, and W.-D. Dettbarn. 1985. Evidence of necrosis in human intercostal muscle following inhalation of an organophosphate insecticide. J. Environ. Pathol. Toxicol. Oncol. 6:171-175.
- 15. Halle, A. and D.D. Sloas. 1987. Percutaneous organophosphate poisoning. South. Med. J. 80.1179-1181
- U.S. EPA. Office of Pesticide Programs. Health Effects Div. 1998. Tox Oneliner: Diazinon. Washington, DC, Aug. 10. Pp. 23. Matin, M.A., K. Husain, and S.N. Khan. 1990.
- Modification of diazinon-induced changes in carbohydrate metabolism by adrenalectomy in rats. Biochem. Pharmacol. 30:1781-1786.
- Kojimo, T., S. Tsuda, Y. Shirasu. 1992. Noncholinergic mechanisms underlying the acute lethal effects of P=S type organophosphorus insecticides in rats. J. Vet. Med. Sci. 54(3):529-533.
- Frick, T.W. et al. 1987. Effects of insecticide, diazinon, on pancreas of dog, cat and guinea pig. J. Environ. Pathol. Toxicol. 7:1-11.
- Zwiener, R.J. and C.M. Ginsburg. 1988. Organophosphate and carbamate poisoning in infants and children. Pediat. 81: 121-126.
- Weizman, Z. and S. Sofer. 1992. Acute pancreatitis in children with anticholinesterase insecticide intoxication. Pediat. 90:204-206.
- U.S. EPA. Office of Prevention, Pesticides and Toxic Substances. 1998. Review of diazinon incident reports. Memo from J. Blondell, Health Effects Div., to T. Leighton, Health Effects Div. p. 49.
- Ref #10, p. 37. U.S. EPA. 2000. Diazinon: Toxicology chapter for the RED as revised 3/30/00 in response to the Novartis Crop Protection, Inc. responses submitted February 9, 2000 to the RED. Memo from Doherty, J., Health Effects Div., to Chambliss, B., Special Review and Reregistration Div. and Eiden, C., Health Effects Chambliss, Div. Washington, DC. www.epa.gov/pesticides/
- 25. U.S. EPA. Prevention. Pesticides and Toxic Substances. 1998. Health effects test guidelines. OPPTS 870.2600. Skin sensitization. Washington, DC: Aug.
- 26. Ref. # 16, Pp. 32,38.
- Davies, D.B. and B.J. Holub. 1980. Toxicological evaluation of dietary diazinon in the rat. Arch. Environ. Contam. Toxicol. 9:637-650.
- Ref.#24. Pp.7-8.
- U.S. EPA. Office of Prevention, Pesticides and Toxic Substances. 2000. Diazinon: Revised HED preliminary human health risk assessment for

- the reregistration eligibility decision (RED) D262343. PC Code: 057801. List A Case No. 0238. Memo from Eiden, C. Reregistration Branch III, Health Effects Div. to Chambliss, B., Special Review and Reregistration Div. Washington, DC. www.epa.gov/pesticides/op. p. 26.
- Anthony, J., E. Banister, and P.C. Oloffs. 1986. Effect of sublethal levels of diazinon: histopathology of the liver. Bull. Environ. Contam. Toxicol. 37:501-507.
- Rajendra, W., P.C. Oloffs, and E.W. Banister. 1986. Effects of chronic intake of diazinon on blood and brain monoamines and amino acids. Drug Chem. Toxicol. 9:117-131.
- Ref. # 16, Pp. 29,38,39.
- Spyker, J.M. and D.L. Avery. 1977. Neurobehavioral effects of prenatal exposure to the organophosphate diazinon in mice. J. Toxicol. Environ. Health 3:989-1002.
- Earl, F.L. et al. 1973. Reproductive, teratogenic, and neonatal effects of some pesticides and related compounds in beagle dogs and miniature swine. In Pesticides and the environment: Continuing controversy. 8th Inter-Am. Conf. Occup. Med., ed. Deichmann, W.B. New York NY: intercontinental Medical Book Corp.
- Ref. #24, Pp.13,23.
- Earl, F.L. et al. 1971. Diazinon toxicity comparative studies in dogs and swine. *Toxicol. Appl. Pharmacol.* 18:285-295.
- Greenman, S.B. et al. 1997. Herbicide/pesticide effects on intestinal epithelial growth. Environ. Res. 75:85-93.
- U.S. EPA. 1999. Office of Pesticide Programs listing of chemicals evaluated for carcinogenic potential. Washington, DC, Aug. 25. p.11.
- Davis, J.R. et al. 1993. Family pesticide use and childhood brain cancer. Arch. Environ. Contam. Toxicol. 24:87-92.
- Cantor, K.P. 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.
- Zahm, S.H. et al. 1988. A case-control study of non-Hodgkin's lymphoma and agricultural factors in eastern Nebraska. (Abstract.) Am. J. Epidemiol. 128:901.
- World Health Organization. 1998. Diazinon. Environmental Health Criteria 198. Geneva, Switzerland. p.4.
- Bianchi-Santamaria, A. et al. 1997. Human lymphocyte micronucleus genotoxicity test with mixtures of phytochemicals in environmental concentrations. Mut. Res. 388:27-32.
- U.S. EPA, Prevention, Pesticides and Toxic Substances. 1998. Health effects tests guidelines. OPPTS 870.5395. Mammalian erythrocyte micronucleus test. Washington, DC, Aug. p.1.
- Lopez, D.E. and E. Carrascal. 1987. Sensitivity of human lymphocyte chromosome to diazinon at different times during cell culture. Bull. Environ. Contam. Toxicol. 38:125-130.
- Sobti, R.C., A. Krishan, and C.D. Pfaffenberger. 1982. Cytokinetic and cytogenetic effects of some agricultural chemicals on human lymphoid cells in vitro: organophosphates. Mut. Res. 102:89-102
- U.S. EPA. Office of Prevention, Pesticides and Toxic Substances. 1998. Health effects test guidelines: OPPTS 870.5915 In vivo sister chromatid exchange assay. www.epa.gov/docs/ OPPTS\_Harmonized/870\_Health\_Effects\_ Test\_Guidelines.
- Matsuoka. A., M. Hayashi, and M. Ishidate. 1979. Chromosomal aberration tests on 29 chemicals combined with S9 mix in vitro. Mut. Res. 66:277-290.
- Wong, P.K., C.C. Wai, and E. Liong. 1989. Comparative study on mutagenicities of

- organophosphorous insecticides in Salmonella. Chemosphere 18:2413-2422.
- English, T. et al. 1970. Organic phosphate poisoning - Cleveland, Ohio. Morb. Mort. Weekly Rep. 19(40):403-404.
  Wagner, S.L. 1995. Pitfalls in the laboratory di-
- agnosis of pesticide intoxication. J. AOAC Intern. 78(1):1-3.
- Wagner, S.L. and D.L. Orwick. 1994. Chronic organophosphate exposure associated with transient hypertonia in an infant. Pediat. 94:94-97.
- Mueller, R.F. et al. Plasma paraoxonase polymorphism: A new enzyme assay, population, family, biochemical, and linkage studies. Am. J. Hum. Genet. 35:393-408.
- Davies, H.G. et al. 1996. The effect of the human serum polymorphism is reversed with diazoxon, soman, and sarin. Nat. Gen. 14:334-336.
- Charbonneau, S.M. and I.C. Munro. 1983. Dietary factors affecting pesticide toxicity. In Miyamoto, J. and P.C. Kearney, ed. *Pesticide* chemistry: Human welfare and the environment. Oxford: Pergamon Press. Pp. 521-525. Boyd, E.M. and E. Carsky.
- Kwashiorkorigenic diet and diazinon toxicity. *Acta* Pharmacol, Toxicol, 27:284-294.
- Allender, W.J. and A.G. Britt. 1994. Analyses of liquid diazinon formulations and breakdown products: An Australia-wide survey. Bull. Environ. Contam. Toxicol. 53:902-906.
- Ref. #29, p. 6.
- Leidy, R.B., C.G. Wright, and H.E. Dupree, Jr. 1982. Concentration and movement of diazinon in air. J. Environ. Sci. Health B17:311-319.
- Wright, C.G., R.B. Leidy, and H.E. Dupree, Jr. 1984. Chlorpyrifos and diazinon detection on surfaces in dormitory rooms. Bull. Environ. Contam. Toxicol. 32:259-264.
- Currie, K.L. et al. 1990. Concentrations of diazinon, chlorpyrifos, and bendiocarb after application in offices. Am. Ind. Hyg. Assoc. J.
- 62. U.S. EPA. Office of Research and Development. Atmospheric Research and Exposure Assessment Laboratory. 1990. Nonoccupational pesticide exposure study (NOPES). Final Report. Research Triangle Park, NC. Pp.24-25.
- Mukerjee, S. et al. 1997. An environmental scoping study in the Lower Rio Grande Valley of Texas - III. Residential microenvironmental monitoring for air, house dust, and soil. Environ. Intern. 23:657-673.
- Gordon, S.M. et al. 1999. Residential environmental measurements in the National Human Exposure Assessment Survey (NHEXAS) pilot study in Arizona: preliminary results for pesticides and VOCs. J. Exp. Anal. Environ. Epidemiol. 9:456-470.
- Ref.#29, Pp. 104-108. Ref. #29, p. 13-14.
- 66
- Ref #62, p.42
- Roinestad, K.S., J.B. Louis, and J.D. Rosen. 1993. Determination of pesticides in indoor air and dust. *J. AOAC Intern.* 76:1121-1126. Roberts, J.W. et al. 1989. Development and field
- testing of a high volume sampler for pesticides and toxics in dust. Total Exposure Assessment Methodology Symp., Las Vegas, NV, Nov. 29.
- Bradman, M.A. et al. 1997. Pesticides exposures to children from California's Central Valley: Results of a pilot study. J. Exp. Anal. Environ. Epidemiol. 7:217-233.
- Ref. #29, p. 7.
- U.S. EPA. Office of Prevention, Pesticides and Toxic Substances. 1994. Review of poison control center data call in. Memo from Blondell, J. Health Effects Div. to J. First, Special Review and Reregistration Div. Washington, D.C., Dec. 5. Pp. 11,32.

#### ●INSECTICIDE FACTSHEET

# DIAZINON: ECOLOGICAL EFFECTS AND ENVIRONMENTAL CONTAMINATION

Diazinon is an organophosphate insecticide that is toxic to birds and has caused what the U.S. Environmental Protection Agency calls "widespread and repeated mortality." Exposure to diazinon also reduces the number of eggs birds lay and the survival of eggs and nestlings.

Diazinon is toxic to many species of fish. In addition to killing fish, exposure to low concentrations (one part per billion or less) also causes genetic damage, disrupts behaviors that are crucial for reproduction and protection from predators, and kills aquatic animals on which fish depend for food.

Exposure to diazinon also causes abnormalities in developing tadpoles at concentrations of several parts per billion. The abnormalities include stunting of their tails and underdevelopment of their gills.

Diazinon reduces the ability of soil organisms to transform (fix) atmospheric nitrogen into a form that is usable to plants.

Rivers and streams are frequently contaminated with diazinon. In a national monitoring study, diazinon was the most commonly detected insecticide in both urban and agricultural watersheds. Research has shown that residential diazinon applications made according to label directions contaminate surface water. Diazinon is a frequent air contaminant; a compilation of U.S. air monitoring studies found diazinon in 90 percent of the samples tested.

#### BY CAROLINE COX

Diazinon (see Figure 1) is a commonly used insecticide in the organophosphate chemical family. The hazards of diazinon to human health were summarized in the first part of this factsheet (JPR 20(2):15-21). This article discusses diazinon's hazards to birds, fish, beneficial insects, and plants, as well as diazinon contamination of water and air.

#### Effects on Birds

Acute toxicity: Diazinon is notorious because of its acute (short-term) toxicity to birds. For many species, its acute toxicity is less than 10 milligrams per kilogram (mg/kg), placing it in the U.S. Environmental Protection Agency's (EPA's) highest acute toxicity category. Sensitive species include Canada goose, house sparrow, mallard duck, bobwhite quail, red-

Caroline Cox is JPR's editor.

winged blackbird,<sup>1</sup> and American wigeon.<sup>2</sup>

Symptoms of acute poisoning in birds are lack of coordination, wing spasms, diarrhea, salivation, and seizures.<sup>3</sup>

Diazinon-related bird kills are common. According to EPA, "Diazinon has caused widespread and repeated mortality of birds." Only the carbamate insecticide carbofuran has caused more pesticide-related bird kills than diazinon. These hazards led to cancellation of diazinon's uses on golf courses and sod farms, where diazinon-caused bird kills were frequent, in 1988. Even though

these uses have ended, EPA's incident reporting system indicates that the number of diazinon-caused bird kills has increased steadily since the 1980s.<sup>5</sup>

Both liquid and granular diazinon products are hazardous to birds. Liquid products leave residues on vegetation, which can then be eaten by birds. Diazinon also washes into puddles during rainfall or irrigation, and birds drink the contaminated water. Birds eat granular products when they stick to food or pick them up directly as grit. A single diazinon granule has killed house sparrows in laboratory tests, and five granules have killed blackbirds.

EPA's assessment of diazinon's acute risks to birds concluded that the agency's "levels of concern" were exceeded for all uses evaluated. EPA stated that its past efforts to mitigate these risks by lowering application rates and adding label warnings "are not adequate to prevent mortality." <sup>10</sup>

**Problems with Reproduction:** In addition to its acute toxicity, diazinon re-

duces the reproductive success of birds. Examples include the following problems:

- A decrease in the successful hatching of eggs. Robin eggs in Christmas tree plantations sprayed with diazinon hatched at a lower rate than did eggs in unsprayed nests. <sup>11</sup> In a laboratory study, the hatching rate of eggs from chickens fed 0.1 parts per million (ppm) in their food was 87 percent, compared to 94 percent from birds fed uncontaminated food. <sup>12</sup>
- A decrease in the survival of nestlings. Mortality of song sparrow nestlings in Christmas tree plantations sprayed with diazinon was greater than mortality in unsprayed nests. 11 (See Figure 2.) In a laboratory study, survival of mallard ducklings whose mothers were fed 16 ppm of diazinon in their food was significantly less than the survival of ducklings whose mothers were fed uncontaminated food. 13
- A decrease in the number of eggs laid. The number of eggs produced by bobwhite quail fed 35 ppm of diazinon in their food was reduced compared to quail fed uncontaminated food. <sup>14</sup>
- An increase in the number of deformities in developing chicks. Injection of small amounts of diazinon (6.25 micrograms (µg)) into developing eggs caused the chicks to develop twisted necks. Higher amounts of diazinon caused additional defects in quail and chicken including folding of the spinal chord, shortening of the neck, shortening and twisting of vertebrae, shortening development of ribs and breastbone, clause, and reduced calcification of bones, curled claws, and reduced growth of leg and wing bones.

Endangered species: EPA's assessment of diazinon's hazards concluded that the agency's "levels of concern" for endangered bird species were exceeded by all the uses of diazinon evaluated, including liquid and granular diazinon products used in both agricultural and urban settings. <sup>19</sup> These concerns are supported by a study of loggerhead shrikes in Virginia. Diazinon contaminated 29 percent of the kidney, liver, and brain samples tested. <sup>20</sup>

**Exposure:** Studies of diazinon exposure to birds are rare. However, the few studies obtained by NCAP indicate that

diazinon exposure could be widespread. In California's Central Valley, diazinon was found on the feathers of 45 percent of the hawks studied.<sup>21</sup> Diazinon also was found in a goose that died after a condominium lawn was treated with diazinon.<sup>22</sup>

Lack of ability to detoxify organophosphates: Birds have lower levels of a group of enzymes used to break down diazinon than do mammals.<sup>23</sup> According to researchers, "this appears to be the main reason why birds are much more susceptible than mammals"<sup>23</sup> to diazinon and related insecticides.

Special susceptibility of juveniles: Young birds appear to be more susceptible to diazinon poisoning than mature birds. In a study of starlings, newly hatched nestlings were 20 times more sensitive than birds that had fledged.<sup>24</sup>

## Effects on Fish

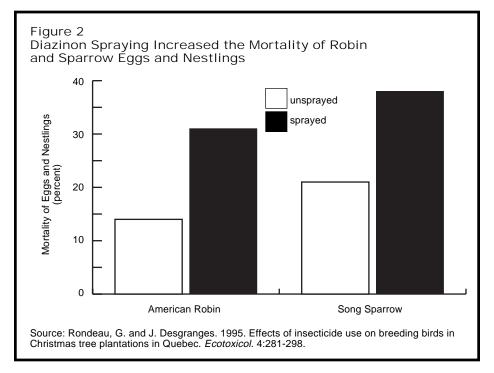
Acute toxicity: According to EPA, diazinon is highly toxic (median lethal concentration,  $LC_{50}$ , less than 1 ppm) or very highly toxic ( $LC_{50}$  less than 0.1 ppm) to about 60 percent of the fish species for which the agency has data. These in-

clude bluegill sunfish; brook, cutthroat, lake, and rainbow trout; and striped mullet.<sup>25</sup> Fish species that are sensitive to diazinon have enzymes that activate diazinon more quickly, enzymes that break it down more slowly, or nerves that are more affected by diazinon.<sup>26</sup>

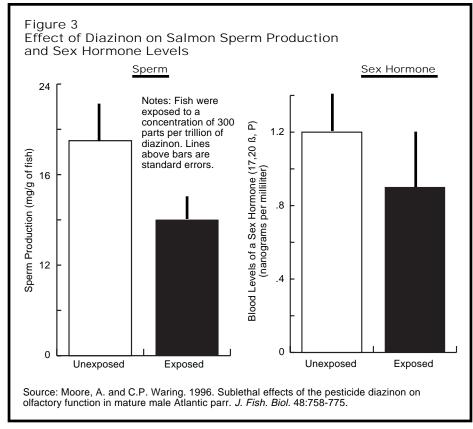
EPA has calculated that acute risks to fish from diazinon's use on urban lawns and many of its agricultural uses exceed the agency's "levels of concern." 27

Genetic damage: Diazinon has caused genetic damage in the central mudminnow, a fish used as a model for testing of genetic effects. In aquarium studies, diazinon at the low concentration of 160 parts per trillion caused an increase in genetic damage called sister chromatid exchanges (SCEs).<sup>28</sup> SCEs are exchanges of genetic material between parts of a chromosome as it duplicates.<sup>29</sup>

Effects on reproduction: Diazinon can disrupt the physiology of reproducing fish. Male Atlantic salmon returning to spawning streams normally react to the smell of urine from female salmon who have recently ovulated. In response to this smell, the levels of sex hormones in males'



In nests from Christmas tree plantations sprayed with diazinon, mortality of eggs and nestlings was nearly twice the mortality in nests from plantations not sprayed during nesting season.



Male Atlantic salmon exposed to diazinon produced less sperm than unexposed salmon. Levels of one of their sex hormones were also reduced.

blood rise and their production of milt (sperm) increases. Concentrations of diazinon above 300 ppt reduced these responses. <sup>30</sup> (See Figure 3.)

Diazinon also impaired reproduction at only slightly higher concentrations (560 ppt) in a study of sheepshead minnows. <sup>31</sup> Reproduction was impaired during diazinon exposure and for up to a month after exposure. In a similar study of brook trout, diazinon reduced the growth of offspring at concentrations of 550 ppt. <sup>32</sup>

Effects on antipredator behavior: A study by the National Marine Fisheries Service found that diazinon affects the behavior of young chinook salmon. Concentrations (1 and 10 ppb) which "emulate diazinon pulses in the natural environment" affect the olfactory system (their sense of smell) and disrupt antipredator behaviors that are normally initiated when the fish smell alarm chemicals given off by other fish in the water. <sup>33</sup>

Effects on food: As an insecticide,

diazinon kills insects and other aquatic animals on which many fish feed. For details about effects on these food resources, see "Effects on Aquatic Insects and Crustaceans," below.

**Bioconcentration:** Diazinon bioconcentrates in fish, meaning that the concentration in fish is greater than that in the water in which the fish lives. Bioconcentration factors (the ratio between the concentration in the fish and that in the water) vary from 18 to 300.<sup>34-38</sup>

Damage to gills: Concentrations of diazinon as low as 15 ppb have damaged gills of the bluegill sunfish. At higher concentrations, diazinon "may result in severe physiological problems, ultimately leading to the death of fish." Gills may be particularly susceptible to diazinon because diazinon bioconcentrates more strongly in gills (bioconcentration factor 2300) than in some other fish tissues.

**Liver damage:** Another organ in which diazinon bioconcentrates is the liver.

Bioconcentration factors as high as 1850 have been measured.<sup>42</sup> In the livers of exposed fishes, concentrations of 150-200 ppb cause cell membranes to rupture<sup>43</sup> and cavities to form.<sup>43,44</sup>

**Effects on Vision:** Exposure of eggs to diazinon causes areas of dead cells to form in the retina of developing medaka.<sup>45</sup>

Hazards to Endangered Species: EPA's risk assessment considered 19 agricultural uses and 1 household use (broadcast treatment of lawns). The agency's "levels of concern" for endangered freshwater and marine fish species were exceeded by all 20 uses evaluated.<sup>27</sup>

# Effects on Frogs

Low concentrations of diazinon affect the survival and development of frog eggs and tadpoles. About 3 parts per billion (ppb) of the diazinon-containing insecticide Basudin 500 EC caused half of tested green frog eggs to fail to hatch. Slightly higher concentrations (5 ppb) of diazinon alone had a similar effect. Even lower concentrations (0.5 ppb Basudin 500 EC and 1 ppb diazinon) produced swelling and blistering of the head or abdomen, stunting of the tail, and underdevelopment of gills. Since the researchers measured diazinon contamination up to .78 ppb in wetlands in four orchards, they believe that it impacts tadpoles at "environmentally relevant concentrations."46

## Effects on Earthworms

Application of the diazinon-containing insecticide Diazinon 14G to turf reduces earthworm populations about 60 percent. (See Figure 4.) Supporting evidence comes from the  $LC_{50}$  (the median lethal concentration, the concentration needed to kill 50 percent of test animals) for a common earthworm, 18 ppb. (48)

# Effects on Aquatic Insects and Crustaceans

Diazinon is "very highly toxic" to most many aquatic insects and crustaceans, according to EPA. For example,  $LC_{50}$ s for waterfleas, scuds, and larval caddisflies are less than 1 ppb.<sup>49-51</sup> Other effects occur at lower exposures: 0.5 ppb reduced

waterflea feeding<sup>52</sup>; 1 ppt reduced growth and reproduction of waterfleas; 0.1 ppb disrupted shrimp feeding<sup>53</sup>; and 0.6 ppb induced stress proteins in a crustacean.<sup>54</sup>

In a field study, cadddisflies and waterfleas were the two groups of animals most sensitive to diazion.<sup>55</sup>

Diazinon can be surprisingly persistent in aquatic animals. For example, diazinon was found in fiddler crabs 6 weeks after a mosquito treatment.<sup>56</sup>

# Effects on Beneficial Insects and Mites

Given that diazinon is an insecticide, it is not surprising that it harms beneficial insects and mites, those that are useful in agriculture.

For example, diazinon is classified by EPA as "highly toxic" to honeybees.<sup>57</sup> It also shortens the life span of worker honey bees.<sup>58,59</sup> (See Figure 5.) Newly emerged bees are most sensitive.<sup>59</sup> Alfalfa leafcutting bees and alkali bees, also used as pollinators, are as sensitive to diazinon as honey bees are.<sup>60</sup>

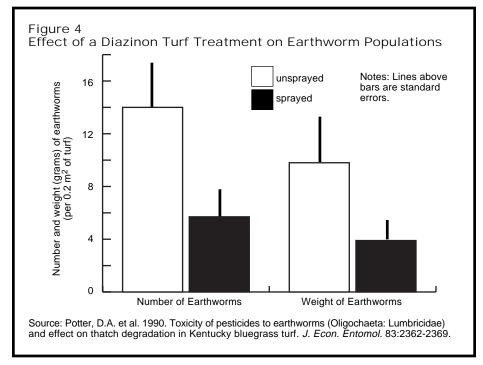
In an international screening program of beneficial insects and mites, diazinon was in the highest toxicity category for most of the species tested: parasitic wasps and predatory mites, as well as lacewings, ladybugs, and other predatory insects. <sup>61</sup> Diazinon has similar effects on predators and parasites of the pecan aphid; diazinon caused 100 percent mortality of the four species studied. <sup>62</sup>

# Effects on Pest Insects

Diazinon increases the economic damage caused by the brown plant hopper, an important pest of rice in tropical areas. Treatment with diazinon increased planthopper populations and rice damage. The planthoppers' feeding rate and egg production also increased on treated rice. <sup>63</sup>

#### Effects on Plant Growth

Although perhaps unexpected for an insecticide, diazinon can reduce plant growth. The most sensitive species studies in tests submitted to EPA for diazinon's registration was cucumber. A



Treatment of turf with diazinon caused significant earthworm mortality.

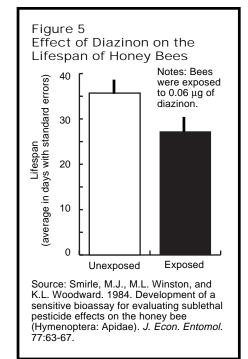
reduction in shoot height<sup>64</sup> occurred at applications of less than the recommended 4 pounds per acre.<sup>65</sup> EPA assessed diazinon's risks to endangered plant species using this data and concluded that the agency's "levels of concern" were exceeded for many agricultural uses.<sup>66</sup>

## Genetic Damage in Plants

Diazinon has also caused genetic damage in plants. In onions<sup>67</sup> and barley<sup>67,68</sup> diazinon treatment resulted in abnormal chromosomes. In addition, seeds from diazinon-treated plants grew into seedlings with mutations that reduced the chlorophyll in their cells.<sup>67</sup> Abnormal chromosomes in pollen cells were also caused by diazinon treatment of barley seeds or seedlings.<sup>69</sup>

# Effects on Nitrogen Fixation

Diazinon can reduce the ability of soil organisms to fix nitrogen, an important plant nutrient. Nitrogen fixation is the process that transforms atmospheric nitrogen into a form that is usable by plants. In a study of *Rhizobium*, a nitrogen-fixing bacteria that lives on the root of the soybean, diazinon treatment at the rec-



Exposure of bees to diazinon (1/5 of the amount that will kill a bee) shortens their lifespan.

ommended rate reduced the activity of an important nitrogen-fixing enzyme. The nitrogen content of the soybean was reduced by about half. <sup>70</sup> A study of nitro-

gen-fixing bacteria on water hyacinth had similar results: diazinon reduced the growth of the bacteria by almost half. The activity of an enzyme found in soil microorganisms that "plays an important role" in nitrogen cycling was inhibited by diazinon. Diazinon also reduced the amount of nitrogen compounds in soil. The similar to the same than the similar to the same than the similar to the same than the similar to the simi

# Contamination of Rivers and Streams

Diazinon was the most commonly detected insecticide in the U.S. Geological Survey's National Water-Quality Assessment Project (NAWQA). NAWQA is a nationwide monitoring program that eventually will study 60 river basins across the country. Currently (2000), data from 20 basins are available. Diazinon contaminated rivers or streams in all of the basins studied so far. (See Figure 6.)

NAWQA also found associations between pesticide pollution and land use. Diazinon was the most commonly detected insecticide in both agricultural and urban areas. About 75 percent of samples from urban watersheds were contaminated with diazinon, as were almost 20 percent of samples from agricultural watersheds, 74 but concentrations were highest in agricultural areas with heavy diazinon use. 76

This widespread contamination has led to studies on local or regional scales which have documented several important patterns. These include the following:

- Diazinon applications made at recommended rates and following label directions contaminate streams. In Castro Valley, California, researchers treated two single family homes with diazinon in accordance with the product label, then measured diazinon contamination in runoff from patios, driveways, and roof drains. Runoff was contaminated for seven weeks after application.<sup>77</sup> (See Figure 7.)
- Diazinon pollution is not limited seasonally or to certain weather conditions. A study in the Hudson River Basin, New York, found that diazinon contamination was more frequent in the spring and summer, when applications are typically made, but 60 percent of the fall and win-

ter samples analyzed were also contaminated. River Basin (Colorado and Nebraska) found diazinon in samples taken after rain storms, when runoff might be expected, but also found "substantial" contamination during dry weather.

- Pollution of rivers and streams by diazinon is related to the amount of use. This is true both in urban and agricultural areas. For example, in King County, Washington, diazinon was the insecticide with the most retail sales, and was found in 100 percent of the streams sampled. 80 In the Tuolumne River Basin, California, diazinon was the insecticide with the highest reported use, and was also found in the highest concentration. 81
- Diazinon use does not have to be widespread in order to contaminate streams. In Castro Valley, California, researchers calculated that use of diazinon by four percent of the homes would account for the contamination they observed.<sup>77</sup>
- Pollution of urban streams is related primarily to residential use of diazinon, not use on commercial properties. In Stockton and Sacramento, California, researchers

compared stormwater from a residential and a commercial drainage. Concentrations were two times higher in the residential area than in the commercial area. 82

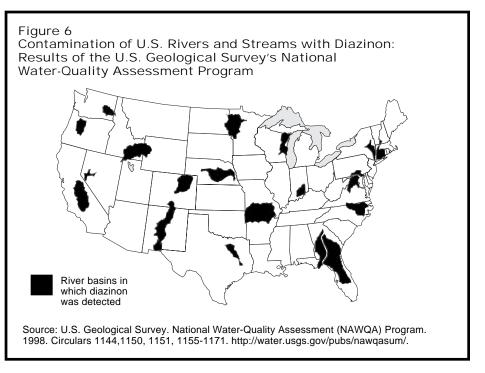
# Contamination of Wells

Diazinon contaminates wells, but not as frequently as it contaminates surface water. NAWQA found diazinon in about 2 percent of tested wells.<sup>83</sup> Contaminated wells were located in 10 of the 20 basins studied so far.<sup>75</sup>

It is striking that deep wells are as vulnerable as shallow wells to diazinon. In the NAWQA study, contamination was as common in deep wells in major aquifers as it was in shallow wells.<sup>84</sup> Diazinon was found in Missouri drinking water wells that were 80 feet deep; a similar study in Virginia found diazinon in wells that were 200 feet deep.<sup>85</sup>

# Toxic Effluent from Wastewater Treatment Plants

Diazinon is a major cause of toxicity in the effluent from wastewater treatment plants. A nationwide survey found that 65 percent of effluent samples were con-



The U.S. Geological Study found diazinon in all twenty river basins they have studied as part of the National Water-Quality Assessment Program.

taminated. Across the country, 47 treatment plants have failed Clean Water Act toxicity tests because of diazinon contamination. Ref. Although researchers believe that this contamination comes from diazinon's varied commercial and residential uses, Ref. one study identified pet kennels as a source of wastewater with high concentrations of diazinon.

Diazinon is not removed from wastewater by the standard techniques used at wastewater treatment plants.<sup>89</sup>

#### Contamination of Air

Diazinon frequently pollutes air. In 1998, the USGS compiled local, state, multi-state, and national air monitoring studies from the U.S and found that nearly 90 percent of the samples were contaminated with diazinon. Diazinon was the fifth most frequently detected pesticide. 90

As with diazinon's contamination of water, both urban and agricultural uses contribute significantly to contamination of air, with urban uses being particularly important. A 1998 study along the Mississippi River found diazinon in every sample tested, regardless of land use. However, concentrations were highest in "reaches of the river that included a major metropolitan area." 91

Airborne diazinon can travel large distances. In California, diazinon has been found in the air in the Sierra Nevada Mountains, 15 miles (25 kilometers, km) from the application site.<sup>92</sup>

# Contamination of Rain, Snow, and Fog

Diazinon contaminates rain and snow along the Mississippi and in California's Sierra Nevada mountains. 92-94 One California study found diazinon in rain 25 miles (40 km) from the application site. 92

During the last two decades, diazinon has been repeatedly found in fog in California's Central Valley and in coastal areas. 95-98 Diazinon in fog water that drips from trees is transformed into diazoxon, a more biologically active and toxic molecule than diazinon, resulting in "enhanced toxicity" 98 to orchard workers.

Figure 7
Levels of Diazinon in Runoff after Household Treatments

300
Concentration of diazinon in runoff from roof drains, patios, and driveways

100
EPA's one-day health advisory level for a child
Image: Sources:
Schueler, T.R. 1999. Diazinon sources in runoff from the San Francisco Bay region. Watershed Protection Techniques 3:613-616.
U.S. EPA. Office of Water. 2000. Drinking water standards and health advisories. Washington D.C.

Diazinon concentrations in runoff following a household application made according to label directions were above EPA's acceptable level for a child to drink. Concentrations remained above this level for seven weeks after application.

## References

- U.S. EPA. 2000. Environmental risk assessment for diazinon. (Preliminary.) Washington, DC. www.epa.gov/pesticides/op/diazinon.htm. Released May 19. Pp. 75-78.
- Kendall, R.J. et al. 1992. American wigeon mortality associated with turf application of diazinon AG500. J. Widl. Dis. 28:263-267.
- 3. Ref. #1, pp. 78-79.
- 4. Ref. #1, p. 148. 5. Ref. #1, p. 144.
- 6. Ref. #1, p. 144
- 7. Ref. #1, p. 145.
- Balcomb, R., R. Stevens, and C. Bowen II. 1984. Toxicity of 16 granular insecticides to wild-caught songbirds. *Bull. Environ. Contam. Toxicol*. 33:302-307.
- 9. Ref. #1, p.16.
- 10. Ref. #1, p.16-17.
- Rondeau, G. and J. Desgranges. 1995. Effects of insecticide use on breeding birds in Christmas tree plantations in Quebec. *Ecotoxicol.* 4:281-298.
- Sauter, E.A. and E.E. Steele. 1972. The effect of low level pesticide feeding on the fertility and hatchability of chicken eggs. *Poult. Sci.* 51:71-76.
- 13. Ref. #1, p.81.
- Stromberg, K.L. 1981. Reproductive tests of diazinon on bobwhite quail. In Avian and mammalian wildlife toxicology. Second conference, ed. D.W. Lamb and E.E. Kenaga. ASTM Special Technical Publication 757. Phildelphia PA.
- Wyttenbach, C.R. and J.D. Hwang. 1984. Relationship between insecticide-induced short and wry neck and cervical defects visible histologically shortly after treatment of chick embryos. J. Exp. Zool. 229:437-446.
- Meneely, G.A. and C.R. Wyttenbach. 1989. Effects of the organophosphate insecticides diazinon and parathion on bobwhite quail

- embryos: Skeletal defects and acetylcholinesterase activity. *J. Exp. Zool.* 252:60-70.
- Cho, J. and Lee, C. 1991. Studies on diazinoninduced inhibition of skeletal mineralization in chick embryo. Res. Rept. RDA(V) 33:41-60.
- Cho, J. and Lee, C. 1990. Effects of diazinon on the anatomical and embryological changes in the developing chick embryo. Res. Rept. RDA(V) 32:35-47.
- 19. Ref. #1, pp.107-113.
- Blumton, A.K. et al. 1990. Pesticide and PCB residues for loggerhead shrikes in the Shenandoah Valley, Virginia, 1985-1988. Bull. Environ. Contam. Toxicol. 45:697-702.
- Wilson, B.W. et al. 1991. Orchard dormant sprays and exposure of red-tailed hawks to organophosphates. *Bull. Environ. Toxicol.* 47:717-724.
- Frank, R. et al. 1991. Deaths of Canada geese following spraying of turf with diazinon. Bull. Environ. Contam. Toxicol. 46:852-858.
- C.H. Walker and M.I. Mackness. 1987. "A" esterases and their role in regulating the toxicity of organophosphates. Arch. Toxicol. 60:30-33.
- Hooper, M.J. et al. 1990. An integrated laboratory and field approach for assessing hazards of pesticide exposure to wildlife. In *Pesticide effects on* terrestrial wildlife. ed. L. Somerville and C.H. Walker, 278-279. London UK: Taylor & Francis.
- 25. Ref. #1, pp. 91-92, 98.
- Keizer, J. et al. 1995. Enzymological differences of AChE and diazinon hepatic metabolism: correlation of in vitro data with the selective toxicity of diazinon to fish species. Sci. Tot. Environ. 171:213-220
- 27. Ref. #1, pp.125-128.
- Vigfusson, N.V. et al. 1983. In vivo induction of sister chromatid exchange in *Umbra limi* by the insecticides endrin, chlordane, diazinon and guthion. *Mut. Res.* 118:61-68.
- U.S. EPA. Prevention, Pesticides and Toxic Substances. 1998. Health effects test guidelines:

- OPPTS 870.5915 In vivo sister chromatid exchange assay. Washington, DC, Aug. www.epa.gov/pesticides.
- 30. Moore, A. and C.P. Waring. 1996. Sublethal effects of the pesticide diazinon on olfactory function in mature male Atlantic parr. J. Fish. Biol. 48:758-775.
- 31. Ref. #1, p.99.
- 32. Ref. #1, p.93.
- 33. Scholz, N.L. et al. 2000. Diazinon disrupts antipredator and homing behaviors in chinook salmon (Oncorhynchus tshawytscha). Can. J. Fish. Aquat. Sci. 57: 1911-1918
- 34. Kanazawa, J. 1978. Bioconcentration ratio of diazinon by freshwater fish and snail. Bull. Environ. Contam. Toxicol. 20:613-617.
- 35. Tsuda, T. et al. 1995. Accumulation and excretion of diazinon, fenthion, and fenitrothion by killifish: Comparison of individual and mixed pesticides. Wat. Res. 29:455-458.
- 36. Seguchi, K. and S. Asaka. 1981. Intake and excretion of diazinon in freshwater fishes. Bull. Environ. Contam. Toxicol. 27:244-249.
- Tsuda, T. et al. 1992. Pesticides in water and fish from rivers flowing into Lake Biwa (II). Chemosphere 24:1523-1531.
- Keizer, J., G. D'Agostino, and L. Vittozzi. 1991. The importance of biotransformation in the toxicity of xenobiotics to fish. I. Toxicity and bioaccumulation of diazinon in guppy (Poecilia reticulata) and zebra fish (Brachydanio rerio). Aquat. Toxicol. 21:239-254.
- 39. Dutta, H.M., C.R. Richmonds, and T. Zeno. 1993. Effects of diazinon on the gills of the bluegill sunfish Lepomis macrochirus. JEPTO 12:219-227.
- Sancho, E. et al. 1992. Organophosphorus diazinon induced toxicity in the fish Anguilla anguilla L. Comp. Biochem. Physiol. 103: 351-356.
- Sancho, E. et al. 1993. Bioconcentration and excretion of diazinon by eel. Bull. Environ. Contam. Toxicol. 50:578-585.
- 42. Sancho, E. et al. 1992. Acute toxicity, uptake, and clearance of diazinon by the European eel. 1992. J. Environ. Sci. Health B27:209-221.
- Sastry, K.A. and K. Sharma. 1981. Diazinoninduced histopathological and hematological alterations in a freshwater teleost. Ophiocephalus punctatus. Ecotoxicol. Environ. Safety 5:329-340.
- 44. Anees, M.A. 1978. Hepatic pathology in a freshwater teleost Channa punctatus (Bloch) exposed to sub-lethal and chronic levels of three organophosphorus insecticides. Bull. Environ. Contam. Toxicol. 19:524-527.
- 45. Hamm, J.T., B.W. Wilson, and D.E. Hinton. 1998. Organophosphate-induced acetylcholinesterase inhibition and embryonic retinal cell necrosis in vivo in the teleost (Oryzias latipes). Neurotoxicol. 19:853-870.
- 46. Harris, M.L. et al. 1998. The functional integrity of northern leopard frog (Rana pipiens) and green frog (Rana clamitans) populations in orchard wetlands. II. Effects of pesticides and eutrophic conditions on early life stage development. Environ. Toxicol. Chem. 17:1351-1363.
- 47. Potter, D.A. et al. 1990. Toxicity of pesticides to earthworms (Oligochaeta: Lumbricidae) and effect on thatch degradation in Kentucky bluegrass turf. J. Econ. Entomol. 83:2362-2369.
- Ebere, A.G. and A. Akintonwa. 1995. Acute toxicity studies with earthworms, Lumbricus terrestris. Bull. Environ. Contam. Toxicol. 55:766-770.
- Ref. #1, pp. 94-95.
  Bailey, H.C. et al. 1997. Joint acute toxicity of diazinon and chlorpyrifos to Ceriodaphnia dubia. Environ. Toxicol. Chem. 16: 2304-2308.
- van der Geest, H.G. et al. 1999. Survival and behavioral responses of larvae of the caddisfly Hydropsyche angustipennis to copper and diazinon. Environ. Toxicol. Chem. 18:1965-1971.
- 52. Fernandez-Casalderrey, A., M.D. Ferrando, and

- E. Andreu-Moliner. 1994. Effect of sublethal concentrations of pesticides on the feeding behavior of Daphnia magna. Ecotoxicol. Environ. Safety 27:82-89.
- Chu, K. H. and P.Y. Lau. 1994. Effects of diazinon, malathion, and paraquat on the behavioral response of the shrimp Metapenaeus ensis to chemoattractants. Bull. Environ. Contam. Toxicol. 53: 127-133.
- Werner, I. and R. Nagel. 1997. Stress proteins HSP60 and HSP70 in three species of amphipods exposed to cadmium, diazinon, dieldrin, and fluoranthene. Environ. Toxicol. Chem. 16:2393-2403.
- Ref. #1, pp.95-98.
- Marganian, V.M. and W.J. Wall. 1972. Dursban® and diazinon residues in biota following treatment of intertidal plots on Cape Cod-1967-69. Pest. Monitor. J. 6:160-165.
- Ref. #1, p. 85.
- Smirle, M.J. M.L. Winston, and K.L. Woodward. 1984. Development of a sensitive bioassay for evaluating sublethal pesticide effects on the honey bee (Hymenoptera: Apidae). J. Econ. Entomol. 77:63067.
- MacKenzie, K. and M.L. Winston. 1989. Effects of sublethal exposure to diazinon on longevity and temporal division of labor in the honey bee (Hymenoptera: Apidae). J. Econ. Entomol. 82:75-82.
- Johansen, C.A. et al. 1983. Pesticides and bees. Environ. Entomol. 12:1513-1518.
- Hassan, S.A. et al. 1988. Results of the fourth joint pesticide testing programme carried out by the IOBC/WPRS-Working Group "Pesticides and Beneficial Organisms." J. Appl. Ent. 105:321-
- Mizell, R.F. and D.E. Schiffhauer. 1990. Effects of pesticides on pecan aphid predators Chrysoperla rufilibris (Neuroptera: Chrysopidae), Hippodamia convergens, Cycloneda sanguinea (L.), Olla v-nigrum (Coleoptera: Coccinellidae), and Aphilinus perpallidus (Hymenoptera: Encyrtidae). J. Econ. Entomol. 83:1906-1812.
- Riley, T.J. 1988. Plant stress from arthropods: Insecticide and acaricide effects on insect, mite, and host plant biology. In Plant stress-insect interactions, ed. E.A. Heinricks, 187-204. New York NY: John Wiley & Sons
- Ref. #1, p.102.
- Novartis Crop Protection, Inc. 1997. D-z-n diazinon AG500 insecticide. Specimen label. Greensboro NC. www.cdms.net.
- Ref. #1, p.129-143.
- Grover, I.S. et al. 1987. Genotoxic effects of pesticides. Nucleus [India] 30:160.
- Kour, P. and I.S. Grover. 1985. Cytological effects of some organophosphorus pesticides. I. Mitotic effects. Cytol. 50:187-197.
- Kour, P. and I.S. Grover. 1985. Cytological effects of some organophosphorus pesticides. II. Meiotic effects. Cytol. 50:199-211.
- Mallik, M.A.B. and K. Tesfai. 1985. Pesticidal effect on soybean-rhizobia symbiosis. Plant and Soil 85:33-41.
- Patil, V.D. and V. Isawaran. 1981. Effect of pesticides on the growth of epiphytic organisms of water hyacinth Eichlornia crassipes Mort. Solms. Sci. Cult. 47:257-258.
- Frankenberger, W.T. and M.A. Tabatabai. 1991. Factors affecting L-asparaginase activity in soils. Biol. Fertil. Soils 11:1-5.
- Ingham, E.R. and D.C. Coleman. 1984. Effects of streptomycin, cycloheximide, Fungizone, captan, carbofuran, Cygon, and PCNB on soil microorganisms. Microb. Ecol. 10:345-358.
- U.S. Geological Survey. 1999. The quality of our nation's waters: Nutrients and pesticides. USGS Circular 1225. p.60
- 75. U.S. Geological Survey. National Water-Quality Assessment (NAWQA) Program. 1998. Circulars

- 1144,1150, 1151, 1155-1171. http:// water.usgs.gov/pubs/nawqasum/.
- Ref. #74, p.68.
- Schueler, T.R. 1999. Diazinon sources in runoff from the San Francisco Bay region. Watershed Protection Techniques 3:613-616.
- U.S. Geological Survey. 1997. Pesticides in surface waters of the Hudson River Basin -Mohawk River subbasin. Fact Sheet FS-237-96. http://wwwdnyalb.er.usgs.gov.
- U.S. Geological Survey. 1998. Pesticides in surface water in agricultural and urban areas of the South Platte River Basin, from Denver, Colorado, to North Platte, Nebraska, 1993-1994. Water-Resources Investigations Rep. 97-4230. Denver CO
- 80. U.S. Geological Survey. 1999. Pesticides detected in urban streams during rainstorms and relations to retail sales of pesticides in King County, Washington. USGS Fact Sheet 097-99. Tacoma WA, Apr.
- 81. U.S. Geological Survey. 1998. Pesticides in storm runoff from agricultural and urban areas in the Tuolumne River Basin in the vicinity of Modesto, California. Water-Resources Investigations Rep. 98-4017.
- Bailey, H.C. et al. 2000. Diazinon and chlorpyrifos in urban waterways in northern California, USA. Environ. Toxicol. Chem. 19:82-87.
- Ref. #74, p.74.
- U.S. EPA. Office of Prevention, Pesticides and Toxic Substances. 1999. Water resources for diazinon. Memo from R. Matzner and E. Waldman, Environmental Fate and Effects Div. to C. Eiden, Health Effects Div. and B. Chambliss, Special Review and Reregistration Div. Washington, D.C., May 10. p.17.
- 85. Ref. #84, pp. 27-28.
- Ref. #84, p.7.
- Amato, et al. 1992. An example of the identification of diazinon as a primary toxicant in an effluent. Environ. Toxicol. Chem. 11:209-216.
- Brandenburg, B. 1995-1996. Central San's experience with diazinon and chlorpyrifos. RMP Reg. Monitor. News 2(1):1,10,11.
- Monteith, H.D. et al. 1995. Modeling the fate of pesticides in municipal wastewater treatment. Water Environ. Res. 87:964.
- 90. Majewski, M.S. and P.D. Capel. 1995. Pesticides in the atmosphere: Distribution, trends, and governing factors, Chelsea, MI: Ann Arbor Press, Inc. Pp.78-80.
- 91. Majewski, M.S. et al. 1998. Airborne pesticide residues along the Mississippi River. Environ. Sci. Technol. 32:3689-3698.
- Zabik, J.M and J.N Seiber. 1993. Atmospheric transport of organophosphate pesticides from California's Central Valley to the Sierra Nevada mountains. J. Environ. Qual. 22:80-90.
- Ref. #84, p.37.
- McConnell, L.L. et al. 1995. Wet deposition of current-use pesticides in the Sierra Nevada mountain range, California, USA. Environ. Toxicol. Chem. 17:1908-1916.
- Glotfelty, D.E., J.N Seiber, and L.A. Liljedahl. 1987. Pesticides in fog. Nature 325:602-605.
- Glotfelty, D.E., M.S. Majewski, and J.N. Seiber. 1990. Distribution of several organophosphorous insecticides and their oxygen analogues in a foggy atmosphere. Environ. Sci. Technol.
- Schomberg, C.J., D.E. Glotfelty, and J.N. Seiber. 1991. Pesticide occurrence and distribution in fog collected near Monterey, California. Environ. Sci. Technol. 25:155-160.
- Seiber, J.N., B.W. Wilson, and M.M. McChesney. 1993. Air and fog deposition residues of four organophosphate insecticides used on dormant orchards in the San Joaquin Valley, California. Environ. Sci. Technol. 27:2236-2243.