HERBICIDE FACTSHEET

2,4-D: TOXICOLOGY, PART 1

2,4-D is the most widely used herbicide in the world. Almost 60 million pounds are used annually in the U.S. An estimated 35 million lawn and garden applications are made each year.

Symptoms of 2,4-D poisoning include drowsiness, vomiting, convulsions, kidney and liver injury, and muscle twitching. 2,4-D, and its salts that are used in herbicide products, are severe eye irritants. Three of these salts cause skin lesions.

2,4-D is unusual among herbicides in that it causes an array of adverse effects to the nervous system: myotonia (the inability of muscles to relax), disruption of the activity of nervous system chemicals, and behavioral changes. Maturing nervous systems may be particularly vulnerable: in laboratory tests juvenile rats exposed to 2,4-D developed smaller brains than unexposed rats.

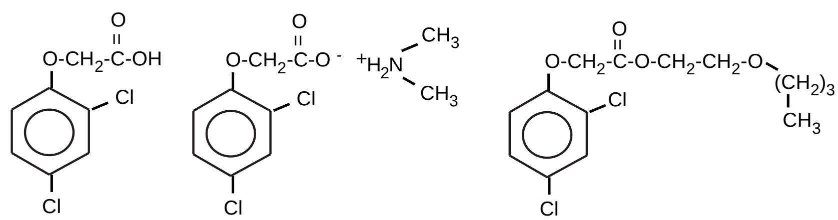
The ability of blood to carry oxygen and to form clots is reduced by 2,4-D.

2,4-D has also caused genetic damage in tests using both cell cultures and laboratory animals. It increased the frequency of a gene mutation in hamster muscle cell cultures, increased the frequency of abnormal chromosomes in bone marrow cells of rats and mice, and increased the number of breaks in human DNA (the molecule from which chromosomes are made).

BY CAROLINE COX

The herbicide 2,4-D (2,4-dichlorophenoxyacetic acid; see Figure 1) is a widely used member of the phenoxy family.[1] It is currently manufactured by AGRO-GOR, Dow AgroSciences, and Nufarm, U.S.A[2] and is sold under a immense variety of brand names, including many "weed & feed" home use products.[3] It is a selective herbicide, with highest toxicity to broadleaf plants.[1] The acid form is occasionally used in commercial herbicide products; three salts (the dimethylamine, triisopropanolamine, and isopropylamine) and two esters (the isooctyl ester and the butoxyethyl ester) are commonly used.[4] (See Figure 1 for examples.)

Figure 1
2,4-D and Some of Its Salts and Esters



2,4-D | dimethylamine salt of 2,4-D | butoxyethyl ester of 2,4-D

2,4-D was first registered for use in the U.S. in 1948,[5] and is now undergoing the reregistration process in which health and safety testing for older pesticides is brought up to current standards.[6]

Use

2,4-D is "the most widely used herbicide in the world," according to a consortium of 2,4-D manufacturers.[2] The U.S. Environmental Protection Agency (EPA) estimates that use in the U.S. is 58 million pounds per year, with lawn and garden uses accounting for 9 million pounds; industrial, commercial and government uses accounting for 13 million; and agriculture accounting for 36 million.[7] (See Figure 3.)

U.S. households make an estimated 35 million applications of 2,4-D annually.[8]

The U.S. Department of Agriculture surveyed agricultural 2,4-D use patterns in 1996 and found that major uses included control of unwanted plants in pasture, fallow land, rangeland, wheat, corn, and turf.[9]

"Inert" Ingredients

Like most pesticide products, many commercial 2,4-D products contain "inerts," ingredients added to make pesticides more potent or easier to use whose identity is often not publicly available. Where available, information about the toxicology of commercial 2,4-D products,

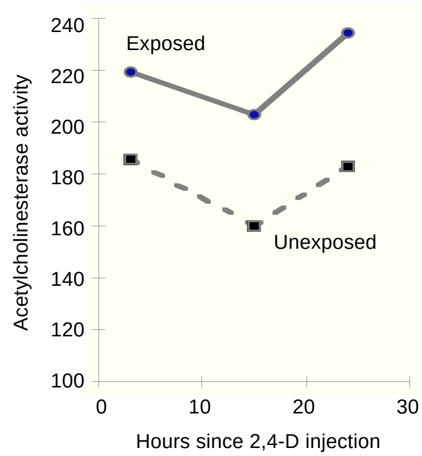
Caroline Cox is JPR's editor. The second part of NCAP's summary of 2,4-D's toxicology will be published in the Summer 1999 issue of JPR.

TOXIC MECHANISMS: HOW DOES 2,4-D KILL PLANTS AND INJURE ANIMALS?

Plants: 2,4-D mimics plant hormones called auxins which control "a multitude of plant growth and development processes."[1] Concentrations of auxins normally fluctuate in order to properly direct growth. In cells exposed to 2,4-D, however, levels of this auxin mimic remain high because 2,4-D is more stable and persistent than auxins.[1,2] As a result, 2,4-D stimulates the synthesis of nucleic acids[3] and proteins and causes abnormal growth.[1] Death occurs when the plant's transport system (xylem and phloem) is crushed and plugged by this growth.[1,2] Other physiological processes are also disrupted by 2,4-D, including the activity of certain enzymes, energy production,[4] and cell division.[3]

Animals: 2,4-D also has striking effects on biological processes in animals. Energy production in animal cells is disrupted[5]: ATP (adenosine triphosphate), the molecule that serves as the cell's energy "currency," is depleted,[6] and two enzymes in the mitochondria (where cellular energy production occurs) are inhibited.[7] 2,4-D also inhibits an enzyme involved in the metabolism of lipids (fatty organic molecules).[8,9] Protein synthesis is inhibited[10,11]: 2,4-D inhibits an enzyme (ornithine decarboxylase) that synthesizes molecules called polyamines that in turn are required for making proteins.[12,13] 2,4-D also inhibits the synthesis of DNA, the molecule that makes up genetic material.[10,11] Enzymes used by the liver to detoxify hazardous molecules are inhibited by 2,4-D,[14,15] and a peptide used in detoxification is depleted.[16] 2,4-D increases the activity of a muscle cell enzyme (p-nitrophenylphosphatase) that transports ions across membranes,[17] and also inhibits four blood enzymes.[18] 2,4-D causes overexpression of a gene involved in regulating transport of lipids in the liver.[19] Finally, 2.4-D disrupts the nervous system: it binds with acetylcholine[20] (used to transmit nerve impulses from one nerve to another), inhibits the enzyme acytylcholinesterase,[21,22] (see Figure 2) and increases levels of another neurotransmitter, serotonin.[23]

Figure 2
2,4-D Inhibits a Nervous System Enzyme



Source: Ref.

1. Hess, F.D. 1993. Herbicide effects on plant structure, physiology and biochemistry. In *Pesticide interactions in crop production: Beneficial and deleterious effects,* ed. Altman, J. Boca Raton: CRC Press.
2. Zimdahl, R.L. 1993. *Fundamentals of weed science.* San Diego CA: Academic Press.
3. Cherry, J.H. 1970. Effect of auxin-herbicides on plant and animal biochemistry. *HortScience* 5: 205-207.
4. Munro, I.C. et al. 1992. A comprehensive, integrated review and evaluation of the scientific evidence relating to the safety of the herbicide 2,4-D. *J. Amer. Coll. Toxicol.* 5:559-664.
5. Zychlinski, L. and S. Zolnierowicz. 1990. Comparison of uncoupling activities of chlorophenoxy herbicides in rat liver mitochondria. *Toxicol. Lett.* 52:25-34.
6. Palmeira, C.M., A.J. Moreno, and V.M.C. Madeira. 1994. Metabolic alterations in hepatocytes promoted by the herbicides paraquat, dinoseb, and 2,4-D. *Arch. Toxicol.* 68:24-31.
7. Palmeira, C.M., A.J. Moreno, and V.M.C. Madeira. 1994. Interactions of herbicides 2,4-D and dinoseb with liver mitochondrial bioenergetics. *Toxicol. Appl. Pharmacol.* 127:50-57.
8. El Kebbaj, M.S., M. Cherkaoui Malki, and N. Latruffe. 1995. Effect of peroxisomes proliferators and hypolipemic agents on mitochondrial inner membrane linked D-3-hydroxybutyrate dehydrogenase (BDH). *Biochem. Mol. Biol. Intern.* 35:65-77.
9. M. Cherkaoui Malki et al. 1991. Effect of different hypolemic agents on rat liver peroxisomal and mitochondrial functions and biogenesis. *Cell. Mol. Biol.* 37:723-733.
10. Rivarola, V., J.R. Bergesse, and H. Balegno. 1985. DNA and protein synthesis inhibition in Chinese hamster ovary cells by dichlorophenoxyacetic acid. *Toxicol. Lett.* 29:137-144.
11. Rivarola, V. and H. Balegno. 1991. Effects of 2,4-dichlorophenoxyacetic acid on polyamine synthesis in Chinese hamster ovary cells. *Toxicol. Lett.* 56:151-157.
12. Rivarola, V. and H. Balegno. 1991. 2,4-Dichlorophenoxyacetic acid effects on polyamine biosynthesis. *Toxicol.* 68:109-119.
13. Rivarola, V., G. Mori, and H. Balegno. 1992. 2,4-Dichlorophenoxyacetic acid action on in vitro protein synthesis and its relation to polyamines. *Drug Chem. Toxicol.* 15:245-257.
14. Singh, S.V. and Y.C. Awasthi. 1985. Inhibition of human glutathione S-transferases by 2,4-dichlorophenoxyacetate (2,4-D) and 2,4,5-trichlorophenoxyacetate (2,4,5-T). *Toxicol. Appl. Pharmacol.* 81:328-336.
15. Mustonen, R. et al. 1989. Effects of commercial chlorophenolate, 2,3,7,8-TCDD, and pure phenoxyacetic acids on hepatic peroxisome proliferation, xenobiotic metabolism, and sister chromatid exchange in the rat. *Arch. Toxicol.* 63:203-208.
16. Palmeira, C.M., A.J. Moreno, and V.M.C. Madeira. 1995. Thiol metabolism is altered by the herbicides paraquat, dinoseb and 2,4-D: A study in isolated hepatocytes. *Toxicol. Lett.* 81:115-123.
17. Brody, I.A. 1973. Myotonia induced by monocarboxylic aromatic acids. *Arch. Neurol.* 28: 243-246.
18. Sekeroglu, M.R., I Celik, and O. Arslan. 1997. Influence of some pesticides on activity of seven serum enzymes (*in vitro*). *J. Environ. Sci. Health* A 32:1975-1980.
19. Miranda, S. et al. 1997. Overexpression of mdr2 gene by peroxisome proliferators in the mouse liver. *J. Hepatol.* 26:1331-1339.
20. Sastry, B.V.R. et al. 1997. Cellular toxicity of 2,4,5-trichlorophenoxyacetic acid: formation of 2,4,5-trichlorophenoxyacetylcholine. *Cell. Mol. Biol.* 43:549-557.
21. Bernard, P.A. et al. 1985. 2,4-Dichlorophenoxyacetic acid (2,4-D) reduces acetylcholinesterase activity in rat muscle. *Exper. Neurol.* 87:544-556.
22. Kuntz, D.J. et al. 1990. Toxicity of mixtures of parathion, toxaphene and/or 2,4-D in mice. *J. Appl. Toxicol.* 10:257-266.
23. Elo, H.A. and E. MacDonald. 1989. Effects of 2,4-dichlorophenoxyacetic acid (2,4-D) on biogenic amines and their acidic metabolites in brain and cerebrospinal fluid of rats. *Arch. Toxicol.* 63:127-130.

including "inerts," is included in the following discussion. Specific information about the toxicology of "inerts" used in 2,4-D products will be summarized in the next part of this factsheet.

Acute Toxicity

According to the National Toxicology Program, symptoms of short-term exposure to 2,4-D include drowsiness, nausea, vomiting, convulsions, coma, kidney and liver injury, hepatitis, diarrhea, weakness, muscle twitching, loss of reflexes, headache, numbness or pain in the arms and legs, sweating, and incontinence.[10]

Case reports published by physicians provide more detailed accounts. One patient spilled about 1/4 cup (60 milliliters) of a 10 percent solution of a 2,4-D ester on his forearms. That evening he felt fatigued, and for ten days suffered from nausea, vomiting, and a 20 pound weight loss. A second patient accidentally sprayed a 2,4-D ester solution on his sleeves and pant legs, and inhaled the spray. The following day he had a headache, and vomited.[11]

Eye Irritation

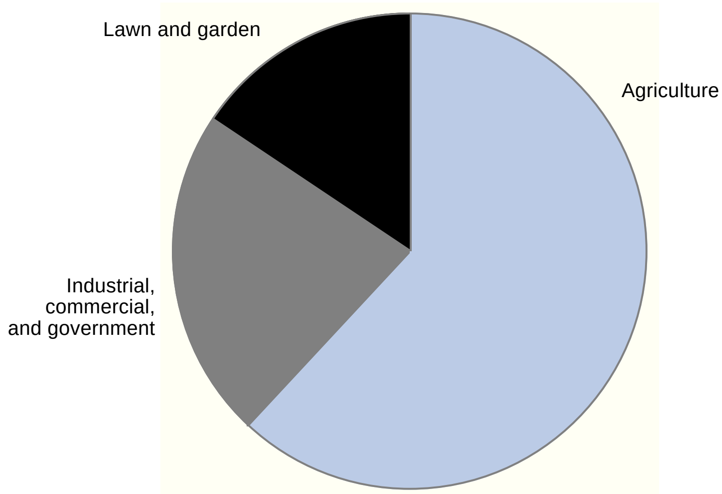
2,4-D and its dimethylamine, diethanolamine, isopropylamine, and triisopropanolamine salts are "severe eye irritants" which cause eye lesions lasting at least 3 days. In long-term feeding studies at relatively high doses, 2,4-D has also caused degeneration of the retina and cataracts.[12]

For information about the eye irritation caused by commercial 2,4-D products, NCAP surveyed material safety data sheets (MSDSs) and labels for 56 products. Of the products surveyed, 50 warned of eye irritation hazards. Over 20 of these were "corrosive" or caused "substantial" or "irreversible" eye damage. The others warned of moderate damage.[4]

Skin Irritation

In rabbits, 2,4-D is "mildly irritating" to the skin. Three 2,4-D salts (dimethylamine, diethanolamine, and isopropylamine) cause skin lesions.[12] In NCAP's survey of MSDSs and labels of commercial 2,4-D products, we found that half of them contained warnings of skin irritation, including skin sensitization or the development of allergic reactions in susceptible people.[4]

Figure 3
Estimated Use of 2,4-D in the United States



Source: Aspelin, A.L. 1997. Pesticides industry sales and usage: 1994 and 1995 market estimates. U.S. EPA. Office of Pesticide Programs. Biological and Economic Analysis Division. Washington, D.C., Aug.

Lawn and garden use of 2,4-D accounts for 16 percent of total use while agriculture accounts for 26 percent.

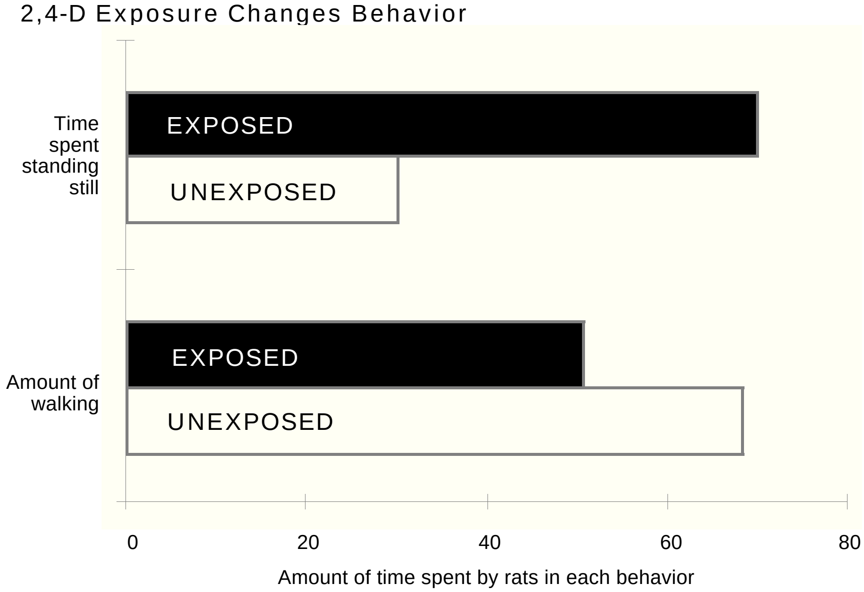
Neurotoxicity

Although many common insecticides target the nervous system, 2,4-D is unusual among herbicides because it has an array of adverse effects on the nervous system. One of the most common neurotoxic symptoms associated with 2,4-D exposure is myotonia. Myotonia occurs when muscles are unable to relax after a voluntary contraction.[13] Myotonia is routinely induced in laboratory experiments by administering 2,4-D.[13-15]

2,4-D has also caused peripheral neuropathy, a condition involving unusual sensations, numbness, and pain in the arms and legs, as well as incoordination and unsteadiness when walking.[16] This disability can be protracted, and recovery incomplete. Examples of cases reported by physicians include a farmer who lost the ability to walk six weeks after spilling 2,4-D on his clothing. A year later the patient was still using crutches to walk, and two years later had not regained movement of his toes. A homeowner developed peripheral neuropathy after kneeling on her 2,4-D-treated lawn. She lost 20 pounds and was unable to walk for a period of weeks. Three years later she was still "clumsy on her feet," according to her physician, and was only 2/3 recovered.[17] Another farmer lost hand control after spraying 2,4-D on his cornfield; a year later he still complained of intermittent numbness.[17] Individuals seem to vary considerably in how susceptible they are to neuropathy caused by 2,4-D.[17]

In experiments with laboratory animals, 2,4-D affects behavior. In rats, single oral doses of 2,4-D decreased how much they walked, decreased the number of times they reared on their hind legs, and increased the amount of time they were still. (See Figure 4.) These behavioral effects were associated with changes in the levels of serotonin and its breakdown product in the brain.[18] Serotonin is one of the chemicals used in the

Figure 4
2,4-D Exposure Changes Behavior

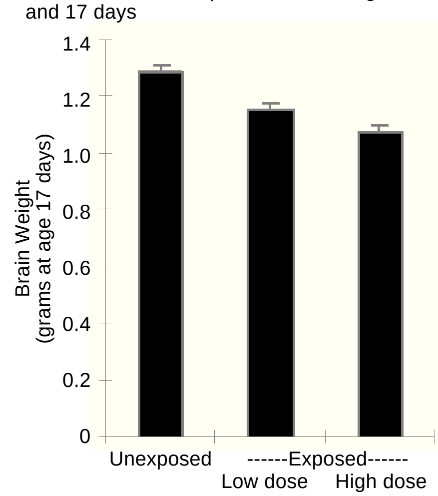


Source: Oliveira, G.H. and J. Palermo-Neto. 1993. Effects of 2,4-dichlorophenoxyacetic acid (2,4-D) on open-field behavior and neurochemical parameters of rats. *Pharmacol. Toxicol.* 73:79-85.

2,4-D causes laboratory rats to increase the time they spend standing still and decrease the amount of walking they do.

Figure 5
2,4-D Exposure of Juveniles Reduces Brain Size

Notes: Lines above bars are standard errors. Rats were exposed between ages 7 and 17 days



Source: Rosso, S.B. et al. 1997. Effects of 2,4-dichlorophenoxyacetic acid on central nervous system of developmental rats: Associated changes in ganglioside pattern. *Brain Res.* 769:163-167.

2,4-D causes juvenile rats to develop smaller brains.

brain to transmit nerve impulses from one nerve cell to another. Another experiment showed that 2,4-D reduced the frequency of learned behaviors in rats.[19] Similar behavioral effects have been caused by 2,4-D butoxyethyl ester: a reduced amount of walking and an increase in incoordination.[20,21] The incoordination is probably caused by n-butanol, a breakdown product of the butoxyethyl ester, but the effects on movement appear to be caused by 2,4-D itself.[21]

Laboratory experiments also indicate that children's nervous systems might be especially affected by 2,4-D exposure. In rats, exposure of growing juvenile rats (during the first 2-3 weeks after birth) reduced the size of the brain and altered components of the membranes in nerve cells.[22] (See Figure 5.) When juvenile rats are exposed to 2,4-D through their mother's milk, they develop less myelin (a covering on nerve cells) than normal.[23] Exposure of juvenile rats to the butoxyethyl ester of 2,4-D resulted in reduced brain weight, changes in the levels of neurotransmitters, and impaired learning ability.[24] When juvenile rats were exposed both during pregnancy and nursing, levels of serotonin in the brain permanently increased.[25]

These studies showing how 2,4-D affects the nervous system are supported by studies showing that 2,4-D travels to the brain after animals are dosed with the herbicide. At high doses, 2,4-D damages the blood-brain barrier, allowing 2,4-D to penetrate brain tissue,[26] but 2,4-D also reaches the brain at doses as low as 1/100 of the acute lethal dose.[27]

Effects on the Circulatory System

2,4-D disrupts the ability of the blood to carry oxygen, and also inhibits the blood clotting process.

Scanning electron micrographs of human red blood cells show that 2,4-D has a dramatic effect on the shape of red blood cells (cells that carry oxygen). (See Figure 6.) This effect is caused by 2,4-D's ability to perturb red blood cell membranes.[28] 2,4-D also decreases the affinity that hemoglobin has for oxygen.[29]

In human blood, platelet aggregation (the process of blood clotting) is inhibited by 2,4-D.[30,31] Inhibition of clotting also occurs in rabbit blood following injection of 2,4-D.[30]

There are no publicly available laboratory studies of the effect of commercial 2,4-D products on the circulatory system. However, NCAP's survey of label and MSDSs for 56 2,4-D products found that seven of them warn that exposure can cause a decrease in blood pressure.[4]

Subchronic Toxicity

In medium-term (subchronic) toxicity tests using rats, EPA found the most significant adverse effects were found in the blood and kidneys.[32] After 7 weeks, hemoglobin (oxygen-carrying protein) levels in the blood decreased, as did the number of red blood cells, consistent with the studies summarized in the previous section. The decrease was statistically significant at all but the lowest dose tested (1 milligram per kilogram (mg/kg) of body weight per day). EPA set 2,4-D's reference dose, the "daily exposure that is likely to be without an appreciable risk of deleterious effects during a lifetime," based on this study,[32] although 2,4-D manufacturers support a 15-fold increase.[1] In addition, at all but the two lowest doses tested in this study, kidney weights increased and pathology of the kidney was observed.

Other medium-term studies have found 2,4-D caused loss of muscle and body weight, or decreased weight gain.[33-35]

Chronic Toxicity

Chronic (long-term) feeding studies with laboratory animals show effects similar to those found in the subchronic studies. In a two-year study with rats, 2,4-D caused kidney lesions at all but the lowest dose tested (1 mg/kg of body weight). In a one-year study with dogs, 2,4-D caused a decrease in weight gain and lesions in the liver and kidney, again at all doses except the lowest. The World Health Organization used these results to set its "acceptable daily intake."[12]

Chronic effects of 2,4-D have also been reported in people. Several physicians have published reports of liver disease (hepatitis) associated with exposure to 2,4-D. In both cases the patients were golfers who habitually licked their golf balls while playing on 2,4-D-treated golf courses.[36,37]

There are no publicly available chronic or subchronic laboratory studies of commercial 2,4-D products (containing 2.4-D and "inert" ingredients).

Mutagenicity

A study of herbicide applicators spraying 2,4-D found that white blood cells with multiple nuclei (chromosome-containing structures) were more common in applicators at the end of the spraying season than before the season began. Applicators also had more multiple nuclei than workers not exposed to 2,4-D.[38]

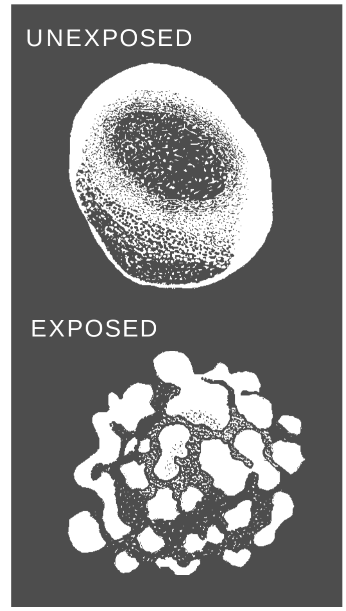
Although government evaluations of laboratory studies of 2,4-D have concluded that it does not cause genetic damage,[12,39] in fact it has been mutagenic in a variety of studies. These include both studies using live animals and studies using cell cultures.

Animals: When administered in rabbits' drinking water, the sodium salt of 2,4-D caused an increase in the number of brain cells with unusual numbers of chromosomes or cells with multiple chromosome sets.[40] Dermally applied 2,4-D caused an increase in the number of abnormalities in the nuclei of hair follicle cells in mice.[41] 2,4-D also increased the frequency of abnormal chromosomes in the bone marrow cells of mice[42] and rats[43] fed 2,4-D.

Human cell cultures: The dimethylamine salt of 2,4-D caused breaks in DNA molecules (genetic material) from human connective tissue.[44] Commercial products containing the amine salt of 2,4-D,[45] and 2,4-D acid,[42] caused chromosome aberrations in cultured human white blood cells. Also in white blood cells, 2,4-D acid caused an increase in sister chromatid exchanges,[46] the exchange of DNA between parts of a duplicating chromosome.[47]

Other cell cultures: When cultured cells from hamster connective tissue were exposed to 2,4-D, the frequency of mu-

Figure 6
Effect of 2,4-D on Red Blood Cells

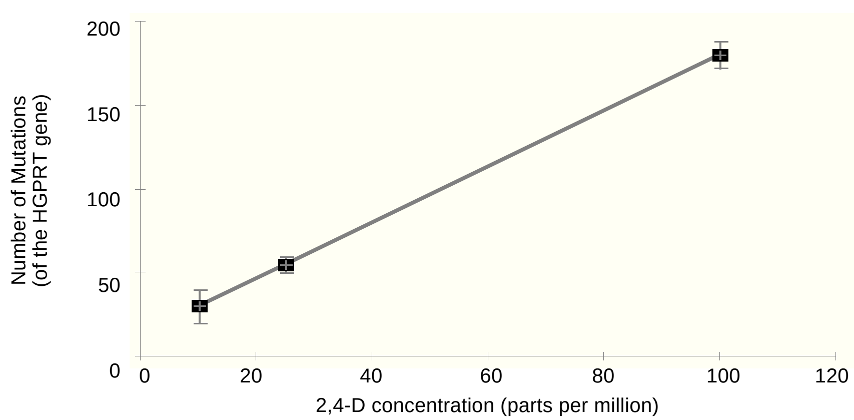


(based on scanning electron micrographs)

Source: Suwalsky, M. et al. 1996. Interaction of 2,4-dichlorophenoxyacetic acid (2,4-D) with cell and model membranes. *Biochim. Biophys. Acta* 1285:267-276.

2,4-D damages the membranes of red blood cells causing them to develop an abnormal shape.

Figure 7
2,4-D Causes Gene Mutations



Pavlica, M., D. Papes and N. Nagy. 1991. 2,4-Dichlorophenoxyacetic acid causes chromatin and chromosome abnormalities in plant cells and mutation in cultured mammalian cells. *Mut. Res.* 263: 77-81.

2,4-D causes mutations in cell cultures of hamster muscle cells.

tations at a particular gene, called the HGPRT locus, increased.[48] (See Figure 7.) In cultures of cow muscle cells, 2,4-D increased the frequency of polyploid cells (those with multiple sets of chromosomes) as well as cells with other chromosome abnormalities.[49]

Simultaneous exposure to several chemicals may increase the genetic damage caused by 2,4-D. In cultures of human connective tissue cells, copper and 2,4-D together caused DNA damage and repair that was not caused by either chemical alone.[50]

References

1. Munro, I.C. et al. 1992. A comprehensive, integrated review and evaluation of the scientific evidence relating to the safety of the herbicide 2,4-D. *J. Amer. Coll. Toxicol.* 5:559-664.
2. Industry Task Force II on 2,4-D Research Data. 1996. Where in the world and the environment are we: A one day status report and briefing on the reregistration of 2,4-D. Seminar sponsored by the Northwest Food & Forest Education Foundation. Portland, OR, July 31.
3. U.S. EPA. Office of Pesticide Programs. 1999. USEPA/OPP chemical ingredients database. www.cdpr.ca.gov/docs/epa/epachem.htm
4. This information came from a survey of labels and material safety data sheets from 56 2,4-D products. They were found at the following web addresses: www.bonideproducts.com; www.dowagro.com; www.monsanto.com/ag; www.appliedbiochemists.com; www.pbigordon.com; www.rp-ag.com; www.riverdalecc.com; www.ortho.com; and www.cdms.net/manuf/.
5. U.S. EPA. Office of Pesticides and Toxic Substances. Office of Pesticide Programs. 1986. Pesticide fact sheet: 2,4-D. Washington, D.C., July.
6. U.S. EPA. Prevention, Pesticides, and Toxic Substances. 1998. Status of pesticides in registration, reregistration, and Special Review. (EPA 738-R-98-002.) Washington, DC, Spr.
7. Aspelin, A.L. 1997. Pesticides industry sales and usage: 1994 and 1995 market estimates. U.S. EPA. Office of Pesticide Programs. Biological and Economic Analysis Division. Washington, D.C., Aug.
8. Whitmore, R.W., J.E. Kelly, and P.L. Reading. 1992. National home and garden pesticide use survey. Final report, volume 1: Executive summary, results, and recommendations. Research Triangle Park NC: Research Triangle Institute.
9. U.S. Dept. of Agriculture. National Agricultural Pesticide Impact Assessment Program. 1996. Biologic and economic assessment of benefits from use of phenoxy herbicides in the United States. 1-PA-96. Washington, D.C.
10. National Toxicology Program. 1991. NTP chemical repository (Radian Corporation, Aug. 29, 1991) 2,4-dichlorophenoxyacetic acid. http//ntp-db.niehs.nih.gov/NTP_Reports/NTP_Chem_H&S/NTP_Chem9/Radian94-75-7.txt.
11. Goldstein, N.P. et al. 1959. Peripheral neuropathy after exposure to an ester of dichlorophenoxyacetic aid. *JAMA* 171:1306-1309
12. World Health Organization. 1997. Pesticide residues in food - 1996: toxicological evaluations. Joint meeting of the Food and Agriculture Organization Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group. Rome, 16-25 Sept.
13. Ramirez, B.U. and M.A. Soza. 1988. Intramuscular pH and endurance are abnormal in skeletal muscles from rats with experimental myotonia. *Exp. Neurol.* 101:347-355.
14. Beasley, V.R. et al. 1991. 2,4-D toxicosis I: A pilot study of 2,4-dichlorophenoxyacetic acid- and dicamba- induced myotonia in experimental dogs. *Vet. Hum. Toxicol.* 33:435-440.
15. Kenigsberg, Y.E. 1968. Myotonia simulation in rats induced by the diethylamine salt of 2,4-dichlorophenoxyacetic acid. *Dokl. Akad. Nauk. Beloruss.* 12:473-475.
16. The Neuropathy Association. 1999. What are your symptoms? www.neuropathy.org/explaining/symptoms.html.
17. Berkley, M.C. and K.M. Magee. 1963. Neuropathy following exposure to dimethylamine salt of 2,4-D. *Arch. Int. Med.* 111:351-352.
18. Oliveira, G.H. and J. Palermo-Neto. 1993. Effects of 2,4-dichlorophenoxyacetic acid (2,4-D) on open-field behavior and neurochemical parameters of rats. *Pharmacol. Toxicol.* 73:79-85.
19. Desi, I. et al. 1962. Nervous system effects of a chemical. *Arch. Environ. Health* 4:101-108.
20. Schulze, G. and J.A. Dougherty. 1988. Neurobehavioral toxicity of 2,4-D-n-butyl ester (2,4-D ester): Tolerance and lack of cross-tolerance. *Neurotoxicol. Teratol.* 10:75-79.
21. Schulze, G.E. 1988. 2,4-D-n-Butyl ester (2,4-D ester) induced ataxia in rats: role for n-butanol formation. *Neurotoxicol. Teratol.*:10:81-84.
22. Rosso, S.B. et al. 1997. Effects of 2,4-dichlorophenoxyacetic acid on central nervous system of developmental rats: Associated changes in ganglioside pattern. *Brain Res.* 769:163-167.
23. Duffard, R. et al. 1996. Central nervous system myelin deficit in rats exposed to 2,4-dichlorophenoxyacetic acid throughout lactation. *Neurotoxicol. Teratol.* 18:691-696.
24. Lakshmana, M.K. and T.R. Raju. 1996. 2,4-Dichloro phenoxy acetic acid alters monoamine levels, acetylcholinesterase activity, and operant learning in rats. *Indian J. Med. Res.* 104:234-239.
25. Evangelista de Duffard, A.M., M.N. de Alderete, and R. Duffard. 1990. Changes in brain serotonin and 5-hydroxyindoacetic acid levels induced by 2,4-dichlorophenoxyacetic butyl ester. *Toxicol.* 64:265-270.
26. Elo, H.A., H. Hervonen, and P. Ylitalo. 1988. Comparative study on cerebrovascular injuries by three chlorophenoxyacetic acids (2,4-D, 2,4,5-T, and MCPA). *Comp. Biochem. Physiol.* 90C:65-68.
27. Oliveira, G.H. and J. Palerma-Neto. 1995. Toxicology of 2,4-dichlorophenoxyacetic acid (2,4-D) and its determination in serum and brain tissue using gas chromotography-electron-capture detection. *J. Anal. Toxicol.* 19: 251-255.
28. Suwalsky, M. et al. 1996. Interaction of 2,4-dichlorophenoxyacetic acid (2,4-D) with cell and model membranes. *Biochim. Biophys. Acta* 1285:267-276.
29. Bukowska, B., E. Reszka, and W. Duda. 1998. Influence of phenoxy herbicides and their metabolites on the form of oxyhemoglobin and deoxyhemoglobin of vertebrates. *Biochem. Mol. Biol. Intern.* 45:47-59.
30. Elo, H.A., T. Luoma, and P. Ylitalo. 1991. Inhibition of human and rabbit platelet aggregation by chlorophenoxyacid herbicides. *Arch. Toxicol.* 65:140-144.
31. Ylitalo, P. et al. 1991. Inhibition of platelet aggregation and thromboxane A2 production by chlorophenoxy acid herbicides. *Arch. Toxicol. Suppl.* 14: 174-178.
32. U.S. EPA. 1992. Integrated risk information system: 2,4-Dichlorophenoxyacetic acid (2,4-D). www.epa.gov/iris/subst/0150.htm.
33. Mustonen, R. et al. 1989. Effects of commercial chlorophenolate, 2,3,7,8-TCDD, and pure phenoxyacetic acids on hepatic peroxisome proliferation, xenobiotic metabolism, and sister chromatid exchange in the rat. *Arch. Toxicol.* 63:203-208.
34. Toyoshima, E., R.F. Mayer, and S.R. Max. 1985. effects of 2,4-dichlorophenoxyacetic acid (2,4-D) on the contractile properties of reinervated rat skeletal muscle. *Exp. Neurol.* 90:601-610.
35. Kuntz, D.J. et al. 1990. Toxicity of mixtures of parathion, toxaphene and/or 2,4-D in mice. *J. Appl. Toxicol.* 10:257-266.
36. Leonard, C, et al. 1997. "Golf ball liver": agent orange hepatitis. *Gut* 40:687-688.
37. Johnston, J., G. McCusker, and T.J. Tobinson. 1998. "Golf ball liver": a cause of chronic hepatitis? *Gut* 42:143-146.
38. Figgs, L. et al. 1998. Occupational exposure to the herbicide 2,4-dichlorophenoxyacetic acid is associated with increased lymphocyte replicative index. *Proc. Am. Assoc. Cancer Res.* 39:337-338. (Abst.) For a definition of "replicative index" see Kolachana, P. and M. Smith. 1994. Induction of kinetochore-positive micronuclei in human lymphocytes by the anti-fungal drug griseofulvin. *Mut. Res.* 322:151-159.
39. U.S. EPA. Office of Prevention, Pesticides and Toxic Substances. 1997. Carcinogenicity peer review (4th) of 2,4-dichlorophenoxyacetic acid (2,4-D). Memo from J. Rowland and E. Rinde, Health Effects Div., to J. Miller, Registration Div. and W. Waldrop, Special Review and Reregistration Div. Washington, D.C., Jan 29.
40. Atanassov, K. 1992. Effect of the herbicide Schpritshormit (Na salt of 2,4-D chlorophenoxyacetic acid) on the karotype of the domesticated rabbit (*Oryctolagus cuniculus*). *Animal Sci.* 29:54-61.
41. Schop, R.N., M.H. Hardy, and M.T. Goldberg. 1990. Comparison of the activity of topically applied pesticides and the herbicide 2,4-D in two short-term in vivo assays of genotoxicity in the mouse. *Fund. Appl. Toxicol.* 15:666-675.
42. Pilinskaya, M.A. 1974. Cytogenetic effect of the herbicide 2,4-D on human and animal chromosomes. *Tsitol. Genet.* 8:202-206.
43. Turkula, T.E. and S.M. Jahal. 1987. Induced clastogenicity in white rats by the herbicide 2,4-D. *Cytologia* 52:275-181.
44. Clausen, M., G. Leier, and I. Witte. 1990. Comparison of the cytotoxicity and DNA-damaging properties of 2,4-D and U 46 D fluid (dimethylammonium salt of 2,4-D). *Arch. Toxicol.* 64:497-501.
45. Mustonen, R. et al. 1986. Effects of phenoxyacetic acids on the induction of chromosome aberrations *in vitro* and *in vivo*. *Mutag.* 1:241-245.
46. Turkula, T.E. and S.M. Jahal. 1985. Increased rates of sister chromatid exchanges induced by the herbicide 2,4-D. *J. Heredity* 76:213-214.
47. U.S. EPA. Prevention, Pesticides, and Toxic Substances. 1996. In vitro sister chromatid exchange assay. Health effects test guidelines. www.epa.gov/docs/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Drafts/870.
48. Pavlica, M., D. Papes and N. Nagy. 1991. 2,4-Dichlorophenoxyacetic acid causes chromatin and chromosome abnormalities in plant cells and mutation in cultured mammalian cells. *Mut. Res.* 263: 77-81.
49. Basrur, S.V., R.A. Fletcher, and P.K. Basrur. 1975. In vitro effects of 2,4-dichlorophenoxy acetic acid (2,4-D) on bovine cells. *Can. J. Comp. Med.* 40:408-415.
50. Jacobi, H. and I. Witte. 1991. Synergistic effects of U46 D Fluid (dimethylammonium salt of 2,4-D) and $CuCl_2$ on cytotoxicity and DNA repair in human fibroblasts. *Toxicol. Lett.* 58:159-167.

● HERBICIDE FACTSHEET

2,4-D: TOXICOLOGY, PART 2

2,4-D is a widely used herbicide in the phenoxy family.

Studies of male farmers exposed to 2,4-D have found that exposed farmers have low-quality sperm. In addition, farmer-applicators in areas of high 2,4-D use have more children with birth defects than unexposed men.

2,4-D exposure has been linked with increased risk of the cancer non-Hodgkin's lymphoma in a series of studies. These include studies of farmers in the U.S. and Canada; workers in 2,4-D manufacturing plants; professional lawn care applicators; and gardeners. In addition, exposure to 2,4-D-treated lawns has been associated with an increased risk of lymphoma in dogs. 2,4-D's ability to cause cancer has been controversial since the first of these studies was published.

2,4-D disrupts the normal functions of hormone systems: it decreases blood concentrations of the metabolic hormone thyroxine and increases production by the testes of estradiol, a female sex hormone.

The U.S. Environmental Protection Agency has reported that 2,4-D is contaminated with dioxins, including the notorious 2,3,7,8-TCDD. TCDD causes a variety of reproductive problems, cancer, and damage to the immune system.

Among the many "inert" ingredients in commercial 2,4-D products are the carcinogen crystalline silica, the neurotoxic solvent xylene, and the teratogen and eye irritant 2-ethyl-1-hexanol.

BY CAROLINE COX

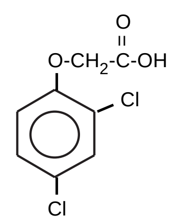
This is the second of two articles discussing the toxicology of the herbicide 2,4-D (see Figure 1), a widely used member of the phenoxy family.[1] This article addresses 2,4-D's effects on reproduction, its carcinogenicity, its effects on the endocrine and immune systems, and the toxicology of contaminants and "inert" ingredients in 2,4-D products.

Effects on Reproduction

Evidence that exposure to 2,4-D can adversely affect reproductive function comes from studies of male farmers. In one study, exposed farmers had lower quality of sperm than did unexposed farmers. Exposed farmers' sperm counts were 52 percent lower, the frequency of abnormal sperm was doubled, and the ability of the sperm to move was reduced compared to sperm from unexposed farmers. Exposure was verified by urine analysis: exposed farmers' urine contained an average of 9 parts per million (ppm) of 2,4-D while none was found in urine from unexposed farmers.[2]

Caroline Cox is JPR's editor.

Figure 1
2,4-D



A 1996 study of private pesticide appliers (farmers licensed to apply restricted-use pesticides on their farms) in Minnesota also associated 2,4-D use with adverse effects on reproduction. The study found that the birth defect rate was higher in children of private appliers than in the general population, and that the birth defect rate for children of appliers was highest in western Minnesota where use of phenoxy herbicides (primarily 2,4-D and MCPA) was highest. The birth defect rate was highest for children of western Minnesota appliers conceived in the spring, when phenoxy herbicides are applied. Finally, corroborating the sperm quality study, the frequency of births among pesticide appliers in high phenoxy herbicide use counties was about half that of the general population in those counties.[3]

Laboratory studies of 2,4-D's effects on reproduction in rats have shown that relatively high doses of 2,4-D cause extra ribs, misalignment and slow bone formation in the backbone, and other rib abnormalities.[4,5]

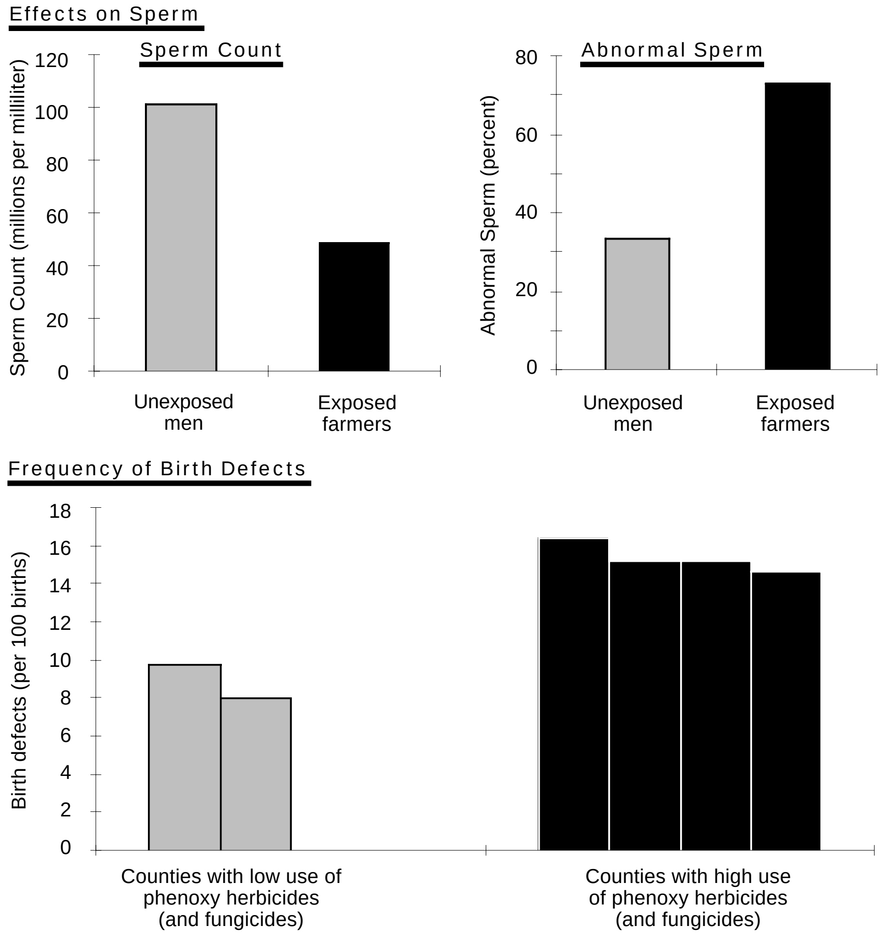
Concerns about 2,4-D's effects on reproduction are increased by laboratory studies showing that 2,4-D is found in the brain and blood of fetuses after dosing of mother animals.[6,7] Repeated exposures increase accumulation of 2,4-D.[8]

There are no publicly available laboratory studies of commercial 2,4-D products' effects on reproduction. However, NCAP's survey of 56 2,4-D products found that four have warnings about damage to the testes.[9]

Gender Differences in Susceptibility to 2,4-D

Laboratory tests have shown that there

Figure 2
2,4-D Use Is Linked with Sperm Damage and Increased Birth Defects



Sources:
Lerda, D. and R. Rizzi. 1991. Study of reproductive function in persons occupationally exposed to 2,4-dichlorophenoxyacetic acid (2,4-D). *Mut. Res.* 262:47-50.
Garry, V.F. et al. 1996. Pesticide appliers, biocides, and birth defects in rural Minnesota. *Environ. Health Persp.* 104:394-399.

Although a review commissioned by the manufacturers of 2,4-D states that reproductive effects of 2,4-D are "not expected at the low levels to which humans may be exposed," several independent studies have documented these kinds of effects in exposed people.

are complex gender differences in the way 2,4-D affects animal physiology. In rats, females are not able to rid the body of 2,4-D as quickly as are males, leading to higher concentrations of 2,4-D in females. In hamsters, the reverse is true.[10] In rats, effects of 2,4-D butyl ester on behavior (the ability to balance on a spinning rod) were observed in males and pregnant females, but not in nonpregnant females. A similar pattern was observed with respect to learning an avoidance behavior.[11]

Carcinogenicity

2,4-D's ability to cause cancer has been controversial since the 1970s when a Swedish oncologist reported that many of his lymphoma patients had been exposed to phenoxy herbicides (including 2,4-D) and the related chlorophenols.[12] Since that time the association between 2,4-D exposure and cancer has been frequently studied. Epidemiologists (scientists who study the causes of disease in actual patients rather than laboratory animals) have focused on non-Hodgkin's lymphoma (NHL), the sixth most common cancer in the U.S. and one whose incidence is increasing.[13]

Studies which have found an association between 2,4-D exposure and NHL include the following:

• The National Cancer Institute (NCI) studied the incidence of NHL among Kansas men, and found that men with NHL were more likely to have used farm herbicides than men without the disease. Looking at just 2,4-D exposure, men with NHL were 2.6 times more likely to have used 2,4-D than men without the disease. The relative risk of NHL increased with the number of days of herbicide exposure per year and the length of time since the first exposure. Frequent herbicide users had a six-fold increase in risk.[14]

• A second study by NCI,[15] conducted in Nebraska, found a similar association between use of 2,4-D and NHL. Overall, the study found "a 50% excess of NHL associated with mixing or applying 2,4-D."[15] As in the Kansas study, risk increased with increasing number of days that 2,4-D was used.

This study, and the Kansas study, were criticized because they obtained information about herbicide exposure from spouses if the farmers themselves had died. NCI then did two studies to compare responses from farmers and spouses and found that agreement was excellent on yes/no questions and adequate on more detailed questions.[16] While the reliability of responses about pesticide use are "lower than desired," the biases this introduces would tend to obscure any association between disease and pesticide use, and thus strengthens the results of the Kansas and Nebraska studies.[17]

• A study by the Laboratory Centre for Disease Control (Canada) found that the risk of NHL in male Saskatchewan farmers increased with the acreage they sprayed with herbicides. Herbicide use by Saskatchewan farmers during the years of the study was 75 - 90 percent 2,4-D.[18]

• A third National Cancer Institute

study found that dogs with canine malignant lymphoma (the canine equivalent of NHL) were more likely than healthy dogs to live in households where owners applied 2,4-D to their lawn or employed lawn care companies to treat their yard for weeds. Risks were highest when both commercial and owner applications were made and in households making four or more applications per year.[19] A task force of 2,4-D manufacturers sponsored a detailed critique of the study,[20] but most of these have been answered by the study's authors.[21] A follow-up study showed that dogs living in homes with 2,4-D treated lawns had high urinary 2,4-D levels.[22]

• A study of workers in a U.S. 2,4-D manufacturing plant found that cancers of the lymph system were 3 times more frequent than expected.[23] The incidence of NHL remained high in a follow-up study.[24] In 4 British factories manufacturing phenoxy herbicides, the incidence of non-Hodgkin's lymphoma was 2 times higher than expected.[25]

• Among employees of the lawn care company ChemLawn, the incidence of NHL for male lawn applicators was about 1.6 times that expected. The incidence among applicators employed over three years was 7 times that expected. More follow-up is planned.[26] A study of golf course superintendents, who often apply herbicides, found that the death rate from non-Hodgkin's lymphoma were approximately double the expected rate.[27]

• Studies of smaller populations have also found an association between 2,4-D use and NHL. In a rural, agriculturally based community in New York where 2,4-D was "widely used" the incidence of NHL was 1.5 times higher than expected.[28] In northern Italy, the incidence of NHL was increased in areas where phenoxy herbicides had been detected in soil or water and increased markedly in areas with the highest contamination levels.[29] Around Milan, Italy, people with NHL were more likely to have been occupationally exposed to herbicides than people without the disease, and researchers found a significant trend with duration of exposure.[30] Among Danish gardeners, the incidence of NHL was twice the expected rate.[31] Agriculture and forestry workers from three Swedish counties who were exposed to phenoxy herbicides were four times more likely to develop malignant lymphomas than unexposed workers.[32]

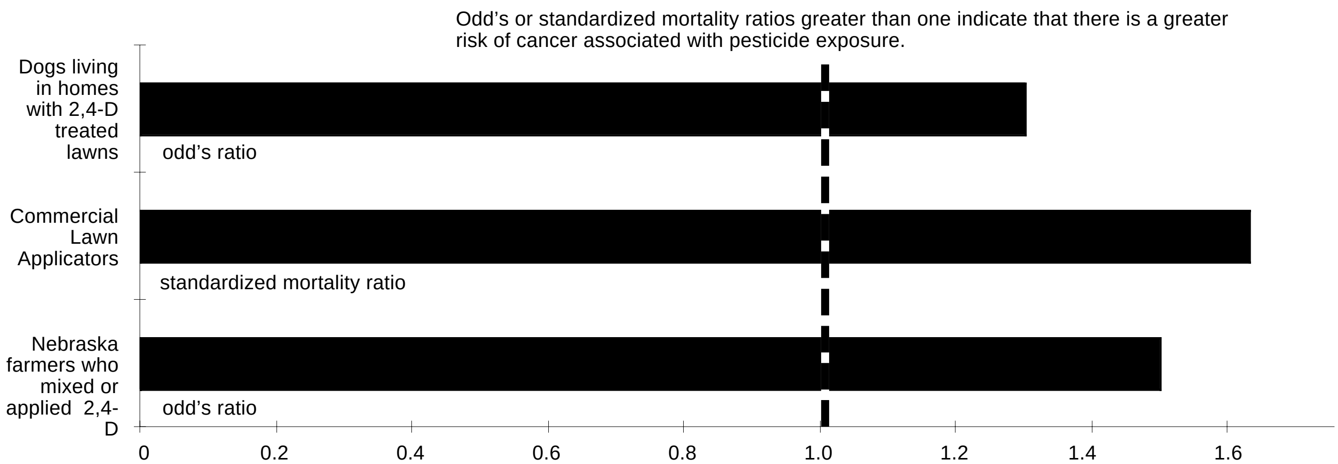
Nine other epidemiological studies have either failed to find a relationship between 2,4-D and NHL, or have found a relationship that was weaker than those summarized above.[33]

Carcinogenicity studies of laboratory animals submitted in support of 2,4-D's registration have not demonstrated increased risks of lymphoma.[33] However, a study conducted by the Food and Drug Administration in 1971 found that the incidence of lymphoma in rats exposed to 2,4-D was 4 percent, while no lymphoma occurred in unexposed rats.[34]

There are no publicly available laboratory studies of the carcinogenicity of commercial 2,4-D products.

The scientists who conducted the NCI studies stated that they "believe that the weight of evidence indicates that the use of 2,4-D in an agricultural setting increases the risk of NHL among persons

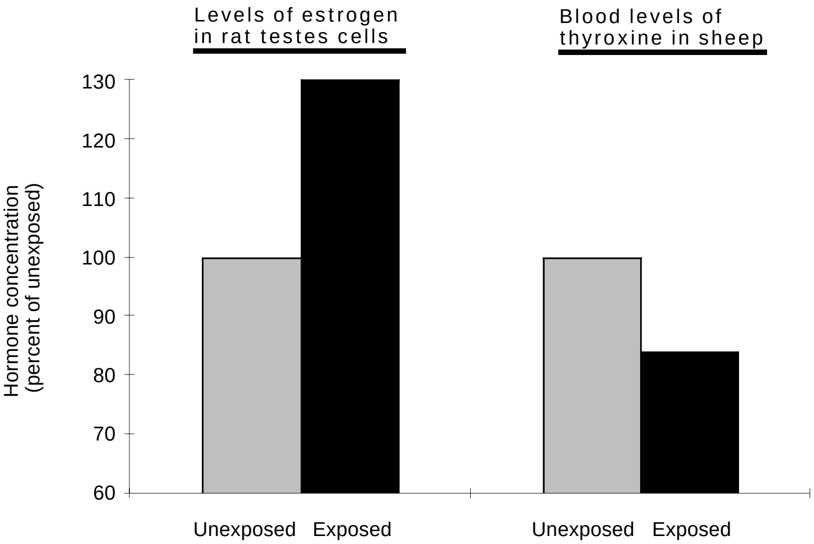
Figure 3
2,4-D Exposure Is Associated with an Increased Risk of Non-Hodgkin's Lymphoma



Sources:
Hayes, H.M. et al. 1991. Case-control study of canine malignant lymphoma: positive association with dog owner's use of 2,4-dichlorophenoxyacetic acid herbicides. *J. Natl. Cancer Inst.* 83:1226-1231.
Zahm, S.H. 1997. Mortality study of pesticide applicators and other employees of a lawn care service company. *J. Occup. Environ. Med.* 39:1055-1067.
Zahm, S.H. et al.1990. A case-control study of non-Hodgkin's lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in eastern Nebraska. *Epidemiol.* 1:349-356.

A series of studies conducted by the National Cancer Institute has found an association between 2,4-D exposure and an increased risk of the cancer non-Hodgkin's lymphoma

Figure 2
2,4-D Alters Hormone Levels



Sources:
Liu, R.C., C. Hahn, and M.E. Hurtt. 1996. The direct effect of hepatic peroxisome proliferators on rat Leydig cell function in vitro. *Fund. Appl. Toxicol.* 30:102-108.
Rawlings, N.C., S.J. Cook, and D. Waldbillig. 1998. Effects of the pesticides carbofuran, chlorpyrifos, dimethoate, lindane, triallate, trifluralin, 2,4-D, and pentachlorophenol on the metabolic endocrine and reproductive endocrine system in ewes. *J. Toxicol. Environ. Health, Pt. A.* 54:21-36.

2,4-D disrupts the normal functioning of hormone systems.

handling the chemical frequently."[15] Yet, government evaluations of 2,4-D's carcinogenicity are less straightforward. 2,4-D is classified as "possibly carcinogenic to humans" (for phenoxy herbicides as a group) by the International Agency for Research on Cancer[35] and as "not classifiable as to human carcinogenicity" by the U.S. Environmental Protection Agency (EPA).[33]

2,4-D presents "a dilemma to the scientific community in how to draw conclusions regarding carcinogenicity when the epidemiologic and experimental data do not agree,"[36] wrote NCI epidemiologist Aaron Blair. "The challenge in the future will be to design studies in the laboratory that mimic the human condition."[36]

In the meantime, although conflicting data are inevitable when studying the complex association between disease and pesticide exposure, eliminating 2,4-D use will protect public health.

Recent research has shown that 2,4-D has biological activity similar to chemicals that stop the growth of human cancer cells and are being considered for use as anticancer drugs.[37] This research does not contradict the research showing that occupational exposure to 2,4-D increases the risk of NHL. Many drugs used to treat, or even prevent cancer, also cause cancer. Examples include tamoxifen, cisplatin, and melphalan.[38]

Endocrine Disruption

Significant research and regulatory resources have been focused recently on chemicals that disrupt the normal functions of the endocrine system, the glands and hormones that regulate the growth and development of animals. Although much research remains to be done, experimental evidence suggests that 2,4-D disrupts animal endocrine systems.

Thyroxine is a hormone produced by the thyroid that is involved in the regulation of metabolism. In rats, 2,4-D "markedly increases" iodine uptake by the thyroid,[39] and decreases the ability of blood to bind with thyroxine.[40] In addition, 2,4-D changes the distribution of thyroxine in the body, so that more is stored in the liver and brain.[39] In sheep, a commercial 2,4-D product decreases blood concentrations of thyroxine.[41]

Leydig cells are testes cells that produce testosterone, often called the male sex hormone as well as estrogens, often called female sex hormones. Rat Leydig cells exposed to 2,4-D increase their production of estradiol, an estrogen.[42]

Effects on the Immune System

A study of ten Italian farmers who used phenoxy herbicides (2,4-D and the related herbicide MCPA) found a variety of changes in the immune system. The study compared blood samples taken prior to herbicide use with samples taken just after herbicide use. The numbers of five kinds of T cells decreased. T cells are white blood cells with immunological activity. In addition, the numbers of natural killer cells and their activity decreased. Natural killer cells are "directly involved in cell mediated immunity to tumors." Finally, the response of the immune system to two foreign chemicals dropped sharply. Two months after exposure, some of the changes persisted.[43]

Contaminants

Dioxins are a family of compounds that include "extremely toxic and potent"[44] chemicals. Dioxins gained notoriety as contaminants of the 2,4-D-containing herbicide Agent Orange used during the Vietnam War.[44] The little testing that has been done shows that current 2,4-D products are contaminated with dioxins,[45] including 2,3,7,8-TCDD,[45] the most toxic dioxin.[44] 2,3,7,8-TCDD was found in 2 of the 8 samples analyzed for EPA by 2,4-D manufacturers.[45] A closely related dioxin (1,2,3,7,8-pentachlorodibenzo-p-dioxin) was found in 3 of the 8 samples tested.[45] The Washington Department of Agriculture recently surveyed fertilizer products, including one 2,4-D-containing product. Their analysis showed that it was contaminated with 2,3,7,8-TCDD and the same pentadioxin found by EPA as well as three related dioxins.[46]

Adverse health effects associated with 2,3,7,8-TCDD and other dioxins include wasting disease (weight loss), chloracne (a severe skin disease), an increased risk of diabetes, weakening of the immune system, decreased fertility, alterations in levels of sex hormones, increased risk of miscarriages, decreased sperm production, increased frequency of severe birth defects, and cancer. Dioxins are persistent and increase in concentration as they move up the food chain.[44]

References

1. Munro, I.C. et al. 1992. A comprehensive, integrated review and evaluation of the scientific evidence relating to the safety of the herbicide 2,4-D. *J. Amer. Coll. Toxicol.* 5:559-664.
2. Lerda, D. and R. Rizzi. 1991. Study of reproductive function in persons occupationally exposed to 2,4-dichlorophenoxyacetic acid (2,4-D). *Mut. Res.* 262:47-50.
3. Garry, V.F. et al. 1996. Pesticide appliers, biocides, and birth defects in rural Minnesota. *Environ. Health Persp.* 104:394-399.
4. U.S. EPA. Office of Prevention, Pesticides, and Toxic Substances. 1996. 2,4-Dichlorophenoxyacetic acid: Review of a chronic toxicity/carcinogenicity study in rats, a carcinogenicity study in mice, and a developmental toxicity study in rats. Memo from J. Rowland, Health Effects Division, to R. Dumas and J. Bloom, Special Review and Reregistration Division. Washington, D.C., May 23.
5. Chernoff, N, et al. 1990. Effects of chemically induced maternal toxicity on prenatal development in the rat. *Teratol.* 42:651-658.
6. Kim, C.S., Z. Binienda, and J.A. Sandberg. 1996. Construction of a physiologically based pharmacokinetic model for 2,4-dichlorophenoxyacetic acid dosimetry in the developing rabbit brain. *Toxicol. Appl. Pharmacol.* 136:250-259.
7. Sandberg, S.A. et al. 1996. Distribution of 2,4-dichlorophenoxyacetic acid (2,4-D) in maternal and fetal rabbits. *J. Toxicol. Environ. Health* 49:497-509.
8. Kim, C., R.F. Keizer, and J.B. Pritchard. 1988. 2,4-Dichlorophenoxyacetic acid intoxication in-

TOXICOLOGY OF "INERTS" IN 2,4-D PRODUCTS

Like most pesticide products, 2,4-D products contain so-called inert ingredients, chemicals added to the pesticide to make the products more effective and easier to use.* Information about the identity of many of these ingredients is not publicly available. Hazards of the ingredients whose identity is available include the following:

• Diethanolamine causes skin and lung irritation and severe eye irritation.[47] It can also cause nausea, headache, and wheezing.[48] It damages the liver, kidney, and blood.[47]

• Dimethylamine is highly flammable. It can cause severe skin burns as well as wheezing and shortness of breath.[49]

• Ethanolamine is corrosive. It burns skin and can permanently damage eyes. It can cause liver and kidney damage, as well as damage to developing fetuses.[50]

• Ethylenediamine tetraacetic acid has stunted fetal growth and caused birth defects and fetal death in laboratory tests. It also causes genetic damage.[51]

*A table listing "inert" ingredients that have been publicly identified as components of specific 2,4-D products is available on NCAP's web page, www.efn.org/~ncap. Some of these ingredients are fertilizers, because the fertilizer components of "weed and feed" products are classified as inert ingredients.

• Ethylene glycol can cause birth defects,[52] reduce fertility, and damage nerves and the kidney. Symptoms of exposure include nausea and headaches.[53]

• Ethylene glycol monobutyl ether (butoxyethanol) causes severe eye irritation and damages blood, nerves, and the kidney. It also reduces fertility.[54]

• 2-Ethyl-1-hexanol causes birth defects[55] and severe eye irritation. It also damages the liver and kidney.[56]

• F.D.&C. Blue No.1 may cause irritation and is a possible carcinogen.[57]

• Glutaraldehyde is extremely destructive to eyes, skin, and the respiratory tract. It causes wheezing, headache, and nausea. In laboratory tests it has caused genetic damage and fetal toxicity.[58]

• 3-Iodo-2-propynyl butylcarbamate is severely irritating to eyes, damaging to the thyroid, and causes skeletal abnormalities in developing fetuses.[59]

• Kerosene burns skin and eyes; irritates the nose, throat, and lungs; and may damage the kidney.[60]

• Methyl alcohol has caused fetal toxicity, birth defects, and genetic damage in laboratory tests. It may cause kidney damage, dizziness, nausea, vomiting, and blindness.[61]

• Methyl pyrrolidinone causes eye irritation.[62] In laboratory tests, it has caused fetal death and reduced fetal weight.[63]

• Naphthalene can cause anemia, liver damage, cataracts, and skin allergies. In laboratory tests it has caused birth defects and genetic damage.[64]

• Poly (dimethylsiloxane) may cause skin and eye irritation and has caused tumors in laboratory tests.[65]

• Silica (crystalline) has been classified as "carcinogenic to humans" by the International Agency for Research on Cancer. It has also caused genetic damage in some laboratory tests.[66]

• Silica (amorphous) causes eye irritation and has caused lung damage, diarrhea, and tumors in laboratory tests.[67]

• Sodium ligninsulfonate may cause eye, skin, and respiratory tract irritation. It has caused drowsiness and weakness in laboratory tests.[68]

• Tetrakis(trimethylsilyoxy)silane is irritating to the eyes, skin, and respiratory system.[69]

• Triisopropanolamine causes severe eye irritation and may cause skin irritation or nausea. Inhalation can be fatal.[70]

•1,2,4-Trimethylbenzene damages the central nervous system and is irritating to eyes, skin, and the upper respiratory tract.[71]

• Xylenes cause eye and skin irritation, headaches, nausea, and confusion. In laboratory tests they have caused kidney damage and fetal death.[72]

creases its accumulation within the brain. *Brain Res.* 440:216-230.
9. This information came from a survey of labels and material safety data sheets from 56 2,4-D products found on manufacturer's web sites at the following addresses: www.bonideproducts.com; www.dowagro.com; www.monsanto.com/ag; www.appliedbiochemists.com; www.pbigordon.com; www.rp-ag.com; www.riverdalecc.com; www.ortho.com; and www.cdms.net/manuf/.
10. Griffin, R.J. et al. 1997. Sex-dependent differences in the disposition of 2,4-dichlorophenoxyacetic acid in Sprague-Dawley rats, B6C3F1 mice, and Syrian hamsters. *Drug Metab. Disp.* 25:1065-1071.
11. A.M. Evangelista de Duffard, C. Orta, and R. Duffard. 1990. Behavioral changes in rats fed a diet containing 2,4-dichlorophenoxyacetic butyl ester. *NeuroToxicol.* 11:563-572.
12. Hardell, L. 1979. Malignant lymphoma of histiocytic type and exposure to phenoxyacetic acids or chlorophenols. *Lancet* 1(8106):55-56.
13. Ballester, O.F. et al. 1993. Non-Hodgkin's lymphoma in the older person: A review. *JAGS* 41:1245-1254.
14. Hoar, S.K. et al. 1986. Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. *JAMA* 256:1141-1147.
15. Zahm, S.H. et al. 1990. A case-control study of non-Hodgkin's lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in eastern Nebraska. *Epidemiol.* 1:349-356.
16. Brown, L.M. et al. 1991. Comparability of data obtained from farmers and surrogate respondents on use of agricultural pesticides. *Am. J. Epidemiol.* 134:348-355.
17. Blair, A. et al. 1995 Comparability of information on pesticide use obtained from farmers and their proxy respondents. *J. Agric. Safety Health* 1:165-176.
18. Wigle, D.T. 1990. Mortality study of Canadian male farm operators; Non-Hodgkin's lymphoma mortality and agricultural practices in Saskatchewan. *J. Natl. Cancer Inst.* 82:575-582.
19. Hayes, H.M. et al. 1991. Case-control study of canine malignant lymphoma: positive association with dog owner's use of 2,4-dichlorophenoxyacetic acid herbicides. *J. Natl. Cancer Inst.* 83:1226-1231.
20. Carlo, G.L. et al. 1992. Review of a study reporting an association between 2,4-dichlorophenoxyacetic acid and canine malignant lymphoma: Report of an expert panel. *Reg. Toxicol. Pharmacol.* 16:245-252.
21. Hayes, H.M., R.E. Tarone, and K.P. Cantor. 1995. On the association between canine malignant lymphoma and opportunity for exposure to 2,4-dichlorophenoxyacetic acid. *Environ. Res.* 70:119-125.
22. Reynolds, P.M. et al. 1994. Canine exposure to herbicide-treated lawns and urinary excretion of 2,4-dichlorophenoxyacetic acid. *Cancer Epidemiol. Biom. Prev.* 3:23-237.
23. Bond, G.G. et al. 1988. Cause specific mortality among employees engaged in the manufacture, formulation, or packaging of 2,4-dichlorophenoxyacetic acid and related salts. *Br. J. Ind. Med.* 45:98-105.
24. Bloeman, L.J. et al. 1993. An update of mortality among chemical workers potentially exposed to the herbicide 2,4-dichlorophenoxyacetic acid and its derivatives. *J. Occup. Med.* 35:1208-1212.
25. Coggon, D., B. Pannett, and P. Winter. 1991. Mortality and incidence of cancer of four factories making phenoxy herbicides. *Br. J. Ind. Med.* 48:173-178.
26. Zahm, S.H. 1997. Mortality study of pesticide applicators and other employees of a lawn care service company. *J. Occup. Environ. Med.* 39:1055-1067.
27. Kross, B.C. et al. 1996. Proportionate mortality of golf course superintendents. *Am. J. Ind. Med.* 29:501-506.
28. Weininger, R.B., G. Davis, and C.D. Hawks. 1987. Herbicides and cancer. *JAMA* 257:2292.
29. Vineis, P. et al. 1991. Incidence rates of lymphomas and soft-tissue sarcomas and environmental measurements of phenoxy herbicides. *J. Natl. Cancer Inst.* 83:362-363.
30. La Vecchia, C. et al. 1989. Occupation and lymphoid neoplasms. *Br. J. Cancer* 60:385-388.
31. Hansen, E.S., H. Hasle, and F. Lander. 1992. A cohort study on cancer incidence among Danish gardeners. *Amer. J. Ind.* Med. 21:651-660.
32. Hardell, L. et al. 1981. Malignant lymphoma and exposure to chemicals, especially organic solvents, chlorophenols and phenoxy acids: A case-control study. *Br. J. Cancer* 43:169-176.
33. U.S. EPA. Office of Prevention, Pesticides and Toxic Substances. 1997. Carcinogenicity peer review (4th) of 2,4-dichlorophenoxyacetic acid (2,4-D). Memo from J. Rowland and E. Rinde, Health Effects Div., to J. Miller, Registration Div. and W. Waldrop, Special Review and Reregistration Div. Washington, D.C., Jan 29.
34. Hansen, W.H. et al. 1971. Chronic toxicity of 2,4-dichlorophenoxyacetic acid in rats and dogs. *Toxicol. Appl. Pharmacol.* 20:122-129.
35. International Agency for Research on Cancer. 1987. IARC monographs on the evaluation of carcinogenic risks to humans. Overall evaluations of carcinogenicity: An updating of IARC monographs. Vols. 1 to 42. Suppl. 7. Lyons, France.
36. Blair, A. Herbicides and non-Hodgkin's lymphoma: New evidence from a study of Saskatchewan farmers. *J Natl. Cancer Inst.* 82:544-545.
37. Pineau, T. et al. 1996. Activation of a human peroxisome proliferator-activated receptor by the antitumor agent phenylacetate and its analogs. *Biochem. Pharmacol.* 52:659-667.
38. International Agency for Research on Cancer. 1999. Overall evaluations of carcinogenicity to humans. http://193.51.164.11/monoeval/crthall.html.
39. Florsheim, W.H. and S.M. Velcoff. 1962. Some effects of 2,4-dichlorophenoxyacetic acid on thyroid function in the rat: Effects on iodine accumulation. *Endocrinol.* 71:1-6.
40. Florsheim, W.H. S.M. Velcoff, and A. D. Williams. 1963. Some effects of 2,4-dichlorophenoxyacetic acid on thyroid function in the rat: Effects on peripheral thyroxine. *Endocrinol.* 72:327-333.
41. Rawlings, N.C., S.J. Cook, and D. Waldbillig. 1998. Effects of the pesticides carbofuran, chlorpyrifos, dimethoate, lindane, triallate, trifluralin, 2,4-D, and pentachlorophenol on the metabolic endocrine and reproductive endocrine system in ewes. *J. Toxicol. Environ. Health, Pt. A.* 54:21-36.
42. Liu, R.C., C. Hahn, and M.E. Hurtt. 1996. The direct effect of hepatic peroxisome proliferators on rat Leydig cell function in vitro. *Fund. Appl. Toxicol.* 30:102-108.
43. Faustini, A. et al. 1996. Immunological changes among farmers exposed to phenoxy herbicides: preliminary observations. *Occup. Environ. Med.* 53:583-585.
44. U.S. Dept. of Health and Human Services. Public Health Service. Agency for Toxic Substances and Disease Registry. 1998. Toxicological profile for chlorinated dibenzo-p-dioxins, Dec.
45. U.S. EPA. Office of Research and Development. 1994. Estimating exposure to dioxin-like compounds. Vol. II: Properties, sources, occurrence and background exposures. Review draft. Washington, D.C., June.
46. Washington Depts. of Ecology, Agriculture, and Health. 1998. Screening survey for metals and dioxins in fertilizers, soil amendments, and soils in Washington State. Olympia WA, Nov.
47. Sigma Chemical Co. 1998. Material safety data sheet: Diethanolamine. St. Louis MO. www.sigma-aldrich.com.
48. New Jersey Dept. of Health and Human Services. 1996. Hazardous substance fact sheet: Diethanolamine. Trenton NJ. www.state.nj.us/health/eoh/odisweb/.
49. New Jersey Dept. of Health and Human Services. 1994. Hazardous substance fact sheet: Dimethylamine. Trenton NJ. www.state.nj.us/health/eoh/odisweb/.
50. New Jersey Dept. of Health and Human Services. 1996. Hazardous substance fact sheet: Ethanolamine. Trenton NJ. www.state.nj.us/health/eoh/odisweb/.
51. Fisher Scientific. 1998. Material safety data sheet: Ethylenediamine tetraacetic acid. Fairlawn NJ. www.fishersci.com.
52. New Jersey Dept. of Health and Human Services. 1996. Hazardous substance fact sheet: Ethylene glycol. Trenton NJ. www.state.nj.us/health/eoh/odisweb/.
53. Sigma Chemical Co. 1998. Material safety data sheet: Ethylene glycol. St. Louis MO. www.sigma-aldrich.com.
54. Sigma Chemical Co. 1998. Material safety data sheet: Ethylene glycol monobutyl ether. St. Louis MO. www.sigma-aldrich.com.
55. Hellwig, J. and R. Jackh. 1997. Differential prenatal toxicity of one straight-chain and five branched-chain primary alcohols in rats. *Food Chem. Toxicol.* 35:489-500.
56. Aldrich Chemical Co, Inc. 1998. Material safety data sheet: 2-Ethyl-1-hexanol. Milwaukee WI. www.sigma-aldrich.com.
57. Sigma Chemical Co. 1998. Material safety data sheet: Erioglaucine. St. Louis MO. www.sigma-aldrich.com.
58. Sigma Chemical Co. 1998. Material safety data sheet: Glutaraldehyde. St. Louis MO. www.sigma-aldrich.com.
59. U.S. EPA. Prevention, Pesticides and Toxic Substances. 1997. Reregistration eligibility decision (RED): 3-Iodo-2-propynyl butylcarbamate (IPBC). Washington, D.C., Mar.
60. New Jersey Dept. of Health and Human Services. 1997. Hazardous substance fact sheet: Kerosene. Trenton NJ. www.state.nj.us/health/eoh/odisweb/
61. Fisher Scientific. 1997. Material safety data sheet: Methyl alcohol. Fairlawn NJ. www.fishersci.com.
62. Fisher Scientific. 1997. Material safety data sheet: 1-Methyl-2-pyrrolidinone. Fairlawn NJ. www.fishersci.com.
63. Hass, U., B.M. Jakobsen, and S.P. Lund. 1995. Developmental toxicity of inhaled n-methylpyrrolidinone in the rat. *Pharm. Toxicol.* 76:406-409.
64. Acros Organics. 1998. Material safety data sheet: Naphthalene 99%. Fairlawn NJ. www.fishersci.com.
65. Aldrich Chemical Co, Inc. 1998. Material safety data sheet: Poly(dimethylsiloxane). Milwaukee WI. www.sigma-aldrich.com.
66. International Agency for Research on Cancer. 1997. Silica. http://193.51.164.11/htdocs/Monographs/Vol68/SILICA.HTM.
67. Sigma Chemical Co. 1998. Material safety data sheet: Silica. St. Louis MO. www.sigma-aldrich.com.
68. Aldrich Chemical Co, Inc. 1998. Material safety data sheet: Lignosulfonic acid, sodium salt. Milwaukee WI. www.sigma-aldrich.com.
69. Aldrich Chemical Co, Inc. 1998. Material safety data sheet: Tetrakis (trimethylsilyoxy)silane. Milwaukee WI. www.sigma-aldrich.com.
70. Acros Organics. 1996. Material safety data sheet: Triisopropanolamine 99%. Fairlawn NJ. www.fishersci.com.
71. Aldrich Chemical Co, Inc. 1998. Material safety data sheet: 1,2,4-Trimethylbenzene. Milwaukee WI. www.sigma-aldrich.com.
72. U.S. Dept. of Health and Human Services. Agency for Toxic Substances and Disease Registry. 1995. Toxicological profile for total xylenes. Atlanta GA.

HERBICIDE FACTSHEET

2,4-D: ECOLOGICAL EFFECTS

2,4-D is a widely used herbicide in the phenoxy family with a startling number of adverse effects on species other than the weeds it is designed to kill.

2,4-D reduces successful hatching of bird eggs, and destroys birds' food and nesting habitat. It is acutely toxic to earthworms and harms beneficial insects. Both 2,4-D (particularly the butoxyethanol ester) and a 2,4-D breakdown product (2,4-dichlorophenol) are acutely toxic to fish.

Increased risk of lymphoma in dogs has been associated with 2,4-D exposure. A National Cancer Institute study found that owners of dogs with lymphoma had treated their lawns with 2,4-D (or hired lawn care companies) more frequently than owners of dogs without the disease.

2,4-D causes genetic damage in barley, wheat, rice, and onions.

2,4-D treatment can increase insect damage by increasing pest insects' ability to reproduce. 2,4-D can also increase the severity of plant diseases, including tomato early blight, tobacco mosaic virus, and corn leaf blight.

Rhizobium is a nitrogen-fixing bacteria found on the roots of legumes. 2,4-D reduces its growth and nitrogen-fixing ability, as well as the growth and nitrogen-fixing ability of several species of blue-green algae.

BY CAROLINE COX

The widely used herbicide 2,4-D (see Figure 1) has a startling variety of impacts on species other than the weeds it is designed to kill. This article summarizes these hazards of 2,4-D for animals and nontarget plants. The toxicology of 2,4-D is summarized in JPR 19(1):14-19 and 19(2)14-19.

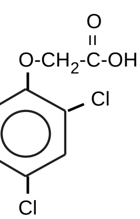
Effects on Birds

Since 2,4-D is an herbicide, its ability to harm birds is surprising. However, both laboratory and field experiments have documented negative impacts.

Hatching of eggs: While some studies conclude that "after normal, or even after excessive, 2,4-D use, there would be no effect on birds' eggs,"[1] other studies have shown serious effects.

Directly injecting 2,4-D acid into chicken eggs causes blood cells to stop dividing.[2] Injection of the dimethylamine salt of 2,4-D lowers hatching success as well as the survival of chicks.[3]

Figure 1
2,4-D



2,4-dichlorophenoxyacetic acid

More realistic types of exposures also damage eggs. Brushing 2,4-D on chicken eggs reduced successful hatching by two-thirds.[4] Spraying 2,4-D on partridge and pheasant eggs resulted in chicks with abnormal genitals: male organs were feminized and females were sterile.[5]

Survival of young: Young birds can also be damaged by 2,4-D. Feeding the dimethylamine salt of 2,4-D to mallard ducks reduced their ducklings' survival by 15 percent.[6] Adding 10 parts per million (ppm) of 2,4-D acid directly to the diet of chicks reduced their growth.[7]

Habitat and food: 2,4-D use also impacts birds indirectly, by disturbing their habitat and food supply. In southwest Kansas, a 2,4-D sagebrush control program decreased the number of birds and bird species. Bobwhites completely disappeared from treated areas.[8] On islands in a Canadian lake, 2,4-D treatment expanded grassy areas, causing birds to nest in unsprayed areas and reducing the numbers of nesting ducks.[9] 2,4-D concentrations of 0.1 ppm in ponds can eliminate water milfoil and sago pondweed, important water fowl food plants.[10]

Effects on Fish

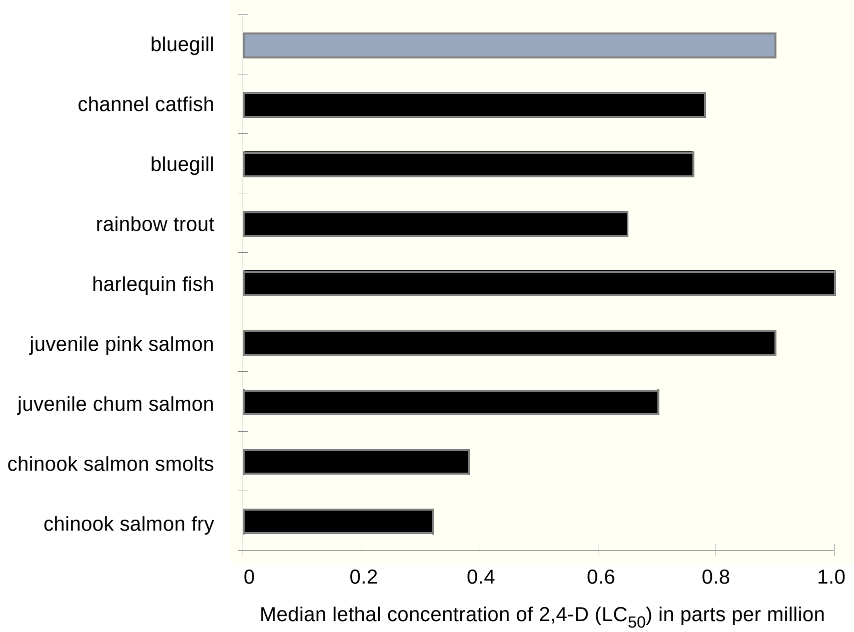
2,4-D's acute toxicity to fish, as summarized by the World Health Organization, "varies widely" depending on species, age, and the form of 2,4-D tested.[1] In general, the butoxyethanol ester is highly toxic to fish, salmon are particularly sensitive, and juveniles are more sensitive than adults. For examples of acute LC_{50}s of 1 ppm or less see Figure 2. (An LC_{50}, median lethal concentration, is the concentration that kills 50 percent of a population of test animals.).

Effects other than death: Sublethal effects of 2,4-D on fish also occur at low

Caroline Cox is JPR's editor.

NORTHWEST COALITION FOR ALTERNATIVES TO PESTICIDES/NCAP
P.O. BOX 1393, EUGENE, OREGON 97440 / (541)344-5044

Figure 2
Acute Toxicity of 2,4-D to Fish



Note: The top bar is 2,4-D acid; all the rest are the butoxyethanol ester of 2,4-D

Sources: Finlayson, B.J. and K.M. Verrue. 1985. Toxicities of butoxyethanol ester and propylene glycol butyl ester formulations of 2,4-dichlorophenoxyacetic acid (2,4-D) to juvenile salmonids. *Arch. Environ. Contam. Toxicol.* 14:153-160.
U.S. EPA. Office of Pesticides and Toxic Substances. Office of Pesticide Programs. 1989. Pesticide factsheet: 2,4-dichlorophenoxyacetic acid. Washington D.C., Sept.
Verschueren, K. 1996. Handbook of environmental data on organic chemicals. Third edition. New York: Van Nostrand Reinhold. p. 715.
Wan, M.T., R.G. Watts, and D.J. Moul. 1990. Acute toxicity to juvenile Pacific salmonids and rainbow trout of butoxyethyl esters of 2,4-D, 2,4-DP and their formulated product: Weedone CB and its carrier. *Bull. Environ. Contam. Toxicol.* 45:604-611.
World Health Organization. 1989. *2.4-dichlorophenoxyacetic acid (2,4-D) - Environmental aspects.* Geneva, Switzerland.

Concentrations of 2,4-D less than 1 part per million can be lethal to fish.

concentrations. Growth of juvenile chinook salmon was reduced by 0.6 ppm of the butoxyethanol ester[11]; physiological stress responses in sockeye salmon occurred at 0.3 ppm[12]; and the ability of rainbow trout to capture food was reduced by 5 ppm of 2,4-D amine.[13]

Bioconcentration: 2,4-D bioconcentrates in fish tissues, meaning that the concentration of 2,4-D in fish is higher than the concentration in the water in which they live. For carp and tilapia, bioconcentration factors (the ratio between the 2,4-D concentration in the fish and the 2,4-D concentration in the water in which it lives) measured in laboratory tests vary between 6 and 45 for carp, and 13 and 23 for tilapia.[14]

Toxicity of Metabolites: 2,4-dichlorophenol, a common breakdown product of 2,4-D in soil,[15] can be acutely toxic to fish. For example, the LC_{50} for bluegill sunfish is 2 ppm.[16]

Synergy: 2,4-D is synergistic with other pesticides in its toxicity towards fish. This means that the combination of two pesticides is more toxic than the sum of the pesticides' individual toxicities. For example, the combination of 2,4-D and the herbicide picloram damages gill cells in catfish, although neither pesticide alone causes similar damage.[17] The insecticide carbaryl increased rainbow trout mortality caused by the butyl ester of 2,4-D[18] by increasing the uptake of 2,4-D.[19]

Effects on Amphibians

2,4-D concentrations of 50 ppm caused liver and kidney damage, followed by death, in male newts. Females were less susceptible, but similar effects occurred at higher concentrations.[20] At lower concentrations, 2,4-D has also disrupted the activity of nerves in frogs' skin.[21]

Effects on Crabs

The acute toxicity of 2,4-D to crabs shows many of the same patterns as that of fish. The butoxyethanol ester is most toxic and juveniles are most susceptible. The LC_{50} of the butoxyethanol ester for the crab *Chasmagnathus granulata* is 0.30 ppm.[22] 2,4-D acid reduced survival of larval Dungeness crabs at 10 ppm, and concentrations of 1 ppm increased the length of its four larval stages.[23]

The butoxyethanol ester of 2,4-D also causes reproductive effects in adult crabs; it inhibited maturation of eggs and decreased the size of the eggs.[24]

Effects on Shellfish

2,4-D exposure has been associated with cancer in shellfish. U.S. Environmental Protection Agency (EPA) scientists studying clams in Maine noticed that sex organ tumors were unusually common at three sites; up to 35 percent of the clams had tumors. Concerned that the tumors were caused by pollutants, they identified possible exposures. Tumor-bearing clams lived close to commercial blueberry and forestry operations or railroad and highway rights-of-way. "Herbicide [including 2,4-D] contamination, the scientists wrote, "is the only common denominator identified at all three sites…"[25]

2,4-D also appears to mimic the sex hormone estrogen in shellfish. Low concentrations of 2,4-D have effects similar to estrogen on mussels' metabolic rate, the rate at which cells break down energy-rich molecules. Both estrogen and 2,4-D increased the metabolic rate.[26]

Other Aquatic Animals

2,4-D is acutely toxic to a variety of

other aquatic animals. The dimethylamine salt is highly toxic to the grass shrimp (LC_{50} 0.2 ppm); the isooctyl ester is highly toxic to the water flea (LC_{50} 0.5 ppm); and the butoxyethanol ester is highly toxic to the side swimmer (LC_{50} 0.4 ppm), the midge (LC_{50} 0.8 ppm)[27], and the scud (LC_{50} 0.4 ppm).[28]

Effects on Earthworms

2,4-D was "very toxic"[29] in studies of acute contact toxicity to the earthworm *Eisenia foetida*.[29] 2,4-D's breakdown product 2,4-dichlorophenol was about 15 times more toxic than 2,4-D itself.[29]

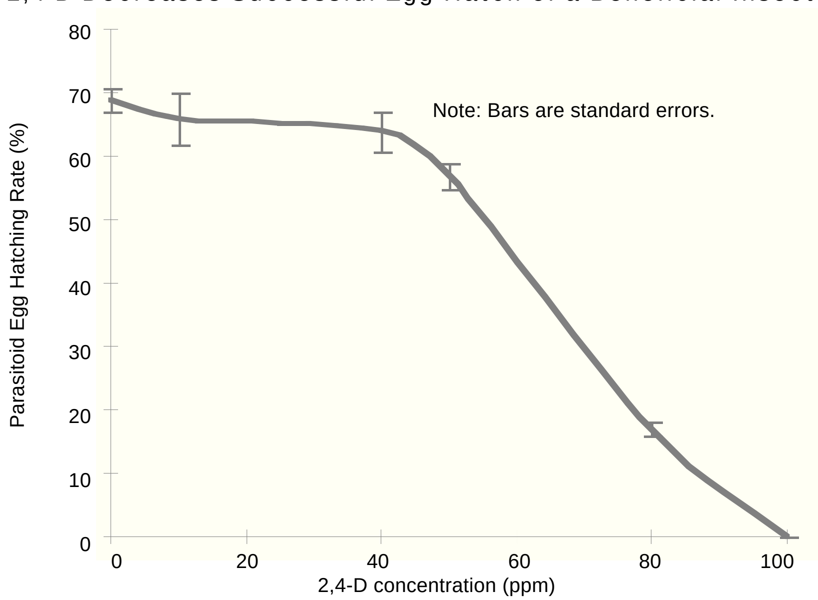
Effects on Insects

Although herbicides are not expected to significantly harm insects, 2,4-D affects them in many ways. Recent laboratory studies of fruit fly larvae have shown that 2,4-D increased the number of mutations (genetic damage): sex-linked lethal mutations,[30,31] wing color and eye color.[31,32] These studies used 2,4-D acid[31,32] and a commercial product containing an amine salt of 2.4-D.[30]

When fed to honey bees, the dimethylamine salt of 2,4-D reduces the production of brood (young bees). The lowest concentration tested (100 ppm) reduced brood 98 percent.[33] Mortality of bees (22 percent) occurred following aerial application of a sodium 2,4-D salt.[34]

Screening tests found that the 2,4-D herbicide Luxan 2,4-D amine killed over 50 percent of a beneficial species of predatory beetle, *Aleochara bilineata*.[35] 2,4-D also caused 50 to 80 percent mortality in a study of two other beetles.[36] Exposure to 2,4-D amine has increased the number of ladybug beetles, also predators, that died as larvae. Oat fields treated with this 2,4-D amine had more aphids than expected because ladybug numbers were reduced.[37,38] Another beneficial species, a parasitoid wasp that develops inside (and kills) moths, is negatively impacted by 2,4-D; exposed wasps laid eggs that were low in glycogen (a molecule that provides energy for the eggs). 2,4-D also reduced successful egg hatch.[39] (See Figure 3.)

Figure 3
2,4-D Decreases Successful Egg Hatch of a Beneficial Insect



Source: Özkan, A. and A. Yanikolu. 1999. Effects of 2,4-D and maleic hydrazide on the glycogen level in the embryonic development of *Pimpla turionellae* (L.) (Hym., Ichneumonidae). *J. Appl. Ent.* 123:211-216.

2,4-D decreases the hatching rate of eggs of *Pimpla turionella*, whose larvae feed on pest moths.

2,4-D can have indirect effects on insects by disrupting the habitat that provides their food and shelter. A study of hedgerows surrounding agricultural fields found that 2,4-D treatments altered the community of carabid beetles living there. Spider communities were also impacted.[40]

Effects on Small Mammals

2,4-D treatment impacts small mammals who depend on vegetation for food. For example, treatment of rangeland in Colorado resulted in almost a 90 percent reduction in pocket gophers. "Drastic changes"[41] in their diet from dandelions and yarrow to grasses were a likely cause.[41]

A study of meadow voles comparing a treated and an untreated area found that in the sprayed area there were fewer voles, a lower survival of female voles, and that the animals were protein deficient.[42]

Effects on Wildlife

Wildlife can be affected by 2,4-D treatments when plants they use as forage are damaged. In British Columbia, plants classified as "high importance" for moose, elk, bighorn sheep, mountain goat, deer, and caribou were "severely" damaged by 2,4-D amines and esters.[43]

Effects on Livestock

2,4-D has caused congested lungs and death in horse toxicity testing.[44] 2,4-D spraying has also injured horses. For example, a county weed control program in Utah sprayed 2,4-D (and other herbicides) on thistles in a corral and nearby creek; 3 horses died and 16 were injured.[45]

2,4-D can also injure livestock by damaging their food. For example, Sudan grass sprayed with 2,4-D had a concentration of hydrocyanic acid (HCN; a compound that is toxic to livestock) 66 percent greater than unsprayed Sudan grass.[46] Cattle seek out recently sprayed ragwort, a toxic weed, because 2,4-D increases the amount of sugar in the plant.[47] Laboratory 2,4-D treatments increase toxic nitrate levels in sugar beets, mustard,[48] and several weeds[49]; poisonings have occurred, some fatal, when cattle ate sugar beets that had been sprayed with 2,4-D.[48,50]

2,4-D also has complex effects on muscle cells in fetal calves. At concentrations of 2 and 20 ppm, it stimulates muscle cells to divide, even when they are fully developed and normally do not divide. Researchers noted that this abnormal division is "usually associated only with pathological conditions including neoplastic [tumorous] growth."[51] In addition, 2,4-D disrupted cell division, causing an increase in the number of cells with multiple chromosome sets.[51]

Effects on Pets

In dogs, large doses of 2,4-D (100 mg/kg of body weight) cause anorexia, leg spasms, inability to stand, and death.[52]

More realistic 2,4-D exposure has been linked to an increased risk of cancer. A study by the National Cancer Institute found that dogs with lymphoma were more likely than healthy dogs to have owners who treated their lawns with 2,4-D (or contracted with lawn care companies). The increased risk of lymphoma doubled for lawns that were treated four times per year.[53] (See Figure 4.)

Genetic Damage to Crops

2,4-D has caused genetic damage in greenhouse and field studies with barley, wheat, rice, and onions. The studies with grains are striking because these are crops on which 2,4-D is often used.[54]

Rice seeds treated with 2,4-D acid, and then grown in field plots, produced plants with more chlorophyll mutations, more pollen mutations, and more sterile pollen than plants grown from untreated seed. 2,4-D caused roughly tenfold increases in each kind of genetic damage.[55]

Treatment of wheat seedlings with 2,4-D ester at a rate of 12 ounces per acre increased the number of abnormal pollen cell chromosomes in mature plants.[56]

Barley plants treated with 2,4-D under similar conditions also developed abnormal chromosomes in pollen cells.[56] In addition, barley seeds treated with 2,4-D and then grown in field plots produced more plants with sterile pollen than plants grown from untreated seed. In the next generation, the number of chlorophyll mutations increased.[57,58]

Onions grown in soil treated with a commercial 2,4-D herbicide had more chromosome abnormalities than onions grown in untreated soil.[59] Another study compared water from an organic farm with water from a farm where 2,4-D (and three other herbicides) was used. Chromosome abnormalities were more common in onions grown with water from the farm using 2,4-D.[60]

Insect Damage to Crops

Laboratory, greenhouse, and field studies have shown that 2,4-D treatment can increase the amount of insect damage on crop plants. In the lab, corn borers reared on plants treated with the triethanolamine salt of 2,4-D were heavier and laid more eggs than borers reared on untreated plants.[61] In the greenhouse, larvae of the rice stem borer grew larger on 2,4-D treated rice plants than on untreated ones[62] and pea aphids reproduced faster on treated beans than on untreated beans.[63] In field studies, corn leaf aphids were twice as numerous on treated corn plants as on untreated ones and corn borer were also more abundant.[61] The likely cause of the increases in insect populations was the increase in protein levels in the plants caused by 2,4-D, causing them to be better insect hosts.[61-63]

Effects on Plant Diseases

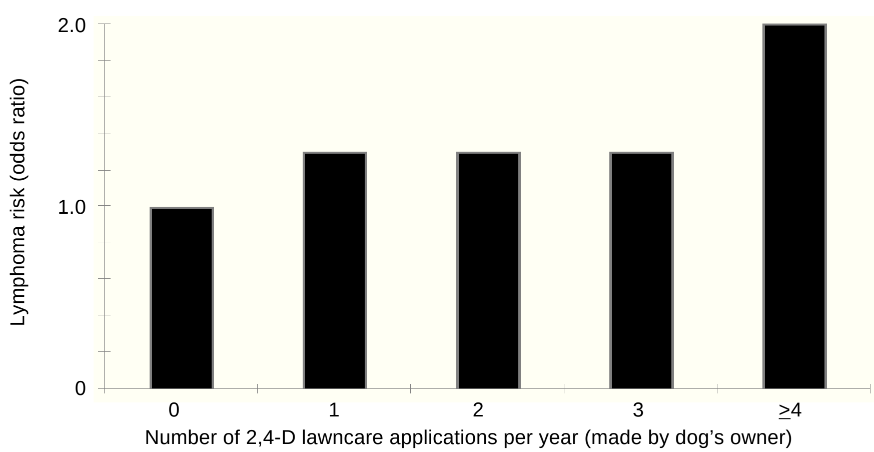
A series of greenhouse studies have shown that 2,4-D treatment can increase the severity of plant diseases. The number of early blight lesions on tomatoes,[64] the size of tobacco mosaic virus (TMV) lesions on tobacco,[65] the growth of leaf spot on bluegrass,[66] the number of southern corn leaf blight lesions on corn,[61] and the number of TMV lesions on cucumbers[67] were all greater on plants treated with 2,4-D than on untreated plants.

A similar relationship between 2,4-D treatment and disease has been found in forest plantations treated with 2,4-D to kill hardwoods. In red pine forests in Wisconsin[68] and spruce forests in Ontario[69] the incidence of the root rot *Armillaria* increased in 2,4-D treated plantations.

Effects on Nitrogen Fixation

Plants need nitrogen in order to grow and reproduce, but they are not able to use nitrogen gas which makes up the bulk of the atmosphere. Instead, plants use

Figure 4
2,4-D Exposure Increases the Risk of Lymphoma in Dogs



Hayes, H.M. et al. 1991. Case-control study of canine malignant lymphoma: Positive association with dog owner's use of 2,4-dichlorophenoxyacetic acid herbicides. *J. Natl. Cancer Inst.* 83:1226-1231.

Risk of lymphoma in dogs is associated with increased use of 2,4-D by their owners.

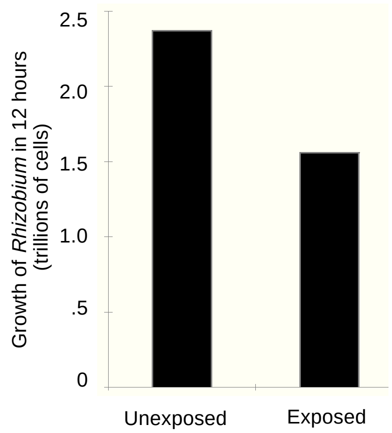
nitrogen that has been transformed into nitrates or other related molecules by algae and bacteria.[70] 2,4-D adversely impacts the nitrogen-fixing bacteria *Rhizobium* as well as several blue-green algae.

Rhizobium bacteria: These bacteria form nitrogen-fixing nodules on legume roots. 2,4-D treatment reduced nitrogen fixation by *Rhizobium* in bean plants[71], caused *Rhizobium* cells to burst[72] and reduced their growth,[73] (see Figure 5) and reduced the number of nodules on clover roots.[74,75] Effects have been measured at concentrations of 1 ppm.[71]

Blue-green algae: 2,4-D decreased the growth of two species of algae that fix nitrogen in rice fields[76] and decreased nitrogen-fixing activity of a third species.[77]

Other parts of the nitrogen cycle are also impacted by 2,4-D. The rate at which ammonia is converted to nitrates in several Iowa soils was reduced by 2,4-D.[78] Also, the activity of an enzyme that converts organic nitrogen to ammonia was reduced by 2,4-D treatment.[79]

Figure 5
2,4-D Reduces Growth of Nitrogen-Fixing Bacteria



Arias, R.N. and A. Fabra de Peretti. 1993. Effects of 2,4-dichlorophenoxyacetic acid on *Rhizobium* sp. growth and characterization of its transport. *Toxicol. Lett.* 68:267-273.

Other Effects on Plant Health

2,4-D affects some species of mycorrhizal fungi, fungi that grow in or near plant roots and increase the plants' ability to take up water and nutrients.[80] A study of three species that grow with forest trees in Canada found that growth of all three species was inhibited by a commercial 2,4-D product. 2,4-D concentrations of 1 ppm inhibited the growth of one of the species.[81]

2,4-D also can increase the susceptibility of plants to nematode attack. In oats, 2,4-D treatment increased the numbers of nematodes in the plants and also increased the susceptibility of a nematode-resistant variety.[82]

The bacteria species *Azospirillum brasilense* lives in association with corn roots, increasing the growth and yield of the corn plants. 2,4-D treatment (simulating field application rates) of corn roots decreased the ability of the bacteria to attach to the roots.[83]

Endangered Species

As a potent herbicide, 2,4-D's impacts on endangered plant species should not be surprising. EPA believes that "certain uses of 2,4-D may jeopardize the continued existence of endangered species...."[27] According to the agency, 2,4-D use jeopardizes 13 endangered species of plants.[84]

Effects on Plant Communities

Although experiments to test the impacts of a pesticide on an entire plant community are rare, one such experiment has been conducted with 2,4-D. This experiment looked at a natural community of winter annuals in Oregon and found that treatment with the isooctyl ester of 2,4-D reduced the total weight of plants in the community. No species was completely eliminated, but 2,4-D treatment simplified the plant community.[85]

References

1. World Health Organization. 1989. *2,4-Dichlorophenoxyacetic acid (2,4-D) - Environmental aspects*. Geneva, Switzerland.
2. Arias, E. 1995. Cytogenetic and cytokinetic effects of 2,4-D on B lymphocytes during development of chick embryo. *In Vitro Technol.* 8:65-69.
3. Gyrd-Hansen, N. and S. Dalgaard-Mikkelsen. 1974. The effect of phenoxy-herbicides on the hatchability of eggs and the viability of the chicks. *Acta Pharmacol. Toxicol.* 35:300-308.
4. Duffard, R., L. Traini, and A. Evangelista de Duffard. 1981. Embryotoxic and teratogenic effects of phenoxy herbicides. *Acta Physiol. Latinoam.* 31:39-42.
5. Lutz, H. and Y. Lutz-Ostertag. 1972. The action of different pesticides on the development of bird embryos. *Adv. Exp. Med. Biol.* 27:127-150.
6. U.S. Food and Drug Administration. 1963. Isopropyl ester of 2,4-D used as a post-harvest fungicide on lemons. Memo from G.E. Whitmore, Division of Pharmacology, to Mr. Stokes, Petition Control Branch, Dec. 5.
7. Whitehead, C.C. 1973. Growth depression of broilers fed on low levels of 2,4-dichlorophenoxyacetic acid. *Br. Poult. Sci.* 14:425-427.
8. Rodgers, R.D. and M.L. Sexson. 1990. Impacts of extensive chemical control of sand sagebrush on breeding birds. *J. Soil Water Cons.* (July-Aug.):494-497.
9. Dwernychuk, L.W. and D.A. Boag. 1973. Effect of herbicide-induced changes in vegetation on nesting ducks. *Can. Field-Natur.* 87:155-165.
10. Forsyth, D.J., P. A. Martin, and G.G. Shaw. 1997. Effects of herbicides on two submerged aquatic macrophytes, *Potamogeton pectinatus* L. and *Myriophyllum sibiricum* Komarov, in a prairie wetland. *Environ. Pollut.* 95: 259-267.
11. FInlayson, B.J. and K.M. Verrue. 1985. Toxicities of butoxyethanol ester and propylene glycol butyl ester formulations of 2,4-dichlorophenoxyacetic acid (2,4-D) to juvenile salmonids. *Arch. Environ. Contam. Toxicol.* 14:153-160.
12. McBride, J.R., H.M. Dye, and E.M. Donaldson. 1981. Stress response of juvenile sockeye salmon (*Oncorhynnchus nerka*) to the butoxyethanol ester of 2,4-dichlorophenoxyacetic acid. *Bull. Environ. Contam. Toxicol.* 27:877-884.
13. Little, E.E. 1990. Behavioral indicators of sublethal toxicity of rainbow trout. *Arch. Environ. Contam. Toxicol.* 19:3880-385.
14. Wang, Y., C. Jaw, and Y. Chen. 1994. Accumulation of 2,4-D and glyphosate in fish and water. *Water Air Soil Pollut.* 74:397-403.
15. Smith, A.E. 1985. Identification of 2,4-dichloroanisole and 2,4-dichlorophenol as soil degradation products of ring-labelled [14C]2,4-D. *Bull. Environ. Contam. Toxicol.* 34:150-157.
16. Buccafusco, R.J., S.J. Ells, and G.A. Blanc. 1981. Acute toxicity of priority pollutants to bluegill (*Lepomis macrochirus*). *Bull Environ. Cont. Toxicol.* 26:446-452.
17. Gallagher, E.P. and R.T. Di Giulio. 1991. Effects of 2,4-dichlorophenoxyacetic acid and picloram on biotransformation, peroxisomal and serum enzyme systems in channel catfish (*Ictalurus punctatus*). *Toxicol. Lett.* 57:65-72.
18. Statham, C.N. and J.J. Lech. 1975. Potentiation of the acute toxicity of several pesticides and herbicides in trout by carbaryl. *Toxicol. Appl. Pharmacol.* 34: 83-87.
19. Statham, C.N. and J.J. Lech. 1976. Studies on the mechanism of potentiation of the acute toxicity of 2,4-D n-butyl ester and 2',5-dichloro-4'-nitrosalicylanide in rainbow trout by carbaryl. *Toxicol. Appl. Pharmacol.* 36: 281-296.
20. Zaffaroni, N.P. et al. 1986. 41:79-87. The toxicity of 2,4-dichlorophenoxyacetic acid to the adult crested newt. *Environ. Res.* 41:79-87.
21. Suwalsky, M. et al. 1999. Toxic action of the herbicide 2,4-D on the neuroepithelial synapse and on the nonstimulated skin of the frog *Caudiverbera caudiverbera*. *Bull. Environ. Contam. Toxicol.* 62:570-577.
22. Rodriguez, E.M. and O.A. Amin. 1991. Acute toxicity of parathion and 2,4-D to larval and juvenile stages of *Chasmagnathus granulata* (Decapoda, Brachyura). *Bull. Environ. Cont. Toxicol.* 47:634-640.
23. Caldwell, R.S. et al. 1979. Toxicity of the herbicides 2,4-D, DEF, propanil and trifluralin to the Dungeness crab, *Cancer magister*. *Arch. Environ. Contam. Toxicol.* 8:383-396.
24. Rodriguez, E.M., M. Schuldt, and L. Romano. 1994. Chronic histopathological effects of par-

athion and 2,4-D on female gonads of *Chasmagnathus granulata* (Decapoda, Brachyura). *Fd. Chem. Toxic.* 32(9):811-818.
25. Gardner, G.R. et al. 1991. Germinomas and teratoid siphon anomalies in softshell clams, *Mya arenaria,* environmentally exposed to herbicides. *Environ. Health Persp.* 90:43-51.
26. Cheney, M.A., R. Fiorillo, and R.S. Criddle. 1997. Herbicide and estrogen effects on the metabolic activity of *Elliptio complanata* measured by calorespirometry. *Comp. Biochem. Physiol.* 118C:159-164.
27. U.S. EPA. Office of Pesticides and Toxic Substances. Office of Pesticide Programs. 1989. Pesticide factsheet: 2,4-dichlorophenoxyacetic acid. Washington D.C., Sept.
28. Sanders, H.O. 1969. Toxicity of pesticides to the crustacean *Gammarus lacustris*. Technical Papers of the Bureau of Sport Fisheries and Wildlife. No. 25. U.S. Dept. of the Interior. Fish and Wildlife Service. Washington D.C., Jan.
29. Roberts, B.L. and H.W. Dorough. 1984. Relative toxicities of chemicals to the earthworm *Eisenia foetida*. *Environ. Toxicol. Chem.* 3:67-78.
30. Kale, P.G. et al. 1995. Mutagenicity testing of nine herbicides and pesticides currently used in agriculture. *Environ. Molec. Mutagen.* 25:148-153.
31. Tripathy, N.K. et al. 1993. Genotoxicity of 2,4-diclorophenoxyacetic acid tested in somatic and germ-line cells of *Drosophila*. *Mut. Res.* 319:237-242.
32. Graf, U. and F.E. Würgler. 1996. The somatic white-ivory eye spot tests does not detect the same spectrum of genotoxic events as the wing somatic mutation and recombination tests in *Drosophila melanogaster*. *Environ. Molec. Mutagen.* 27:219-226.
33. Morton, H.L. and J.O. Moffett. 1972. Ovicidal and larvicidal effects of certain herbicides on honey bees. *Environ. Entomol.* 1:611-614.
34. Palmer-Jones, T. 1964. Effect on honey bees of 2,4-D. *N.Z. J. Agric. Res.* 7:339-342.
35. Hassan, S.A. et al. 1991. Results of the fifth joint pesticide testing programme carried out by the IOBC/WPRS-working group "Pesticides and Beneficial Organisms." *Entomophaga* 36(1):55-67.
36. Muller, G. 1971. Laboratory studies on effects of herbicides on Carabidae. (In German) *Arch. Planzenschutz* 7:351-364. Cited in World Health Organization. 1989. *2,4-Dichlorophenoxyacetic acid (2,4-D) - Environmental aspects.* Geneva, Switzerland. p. 62.
37. Adams, J.B. 1960. Effects of spraying 2,4-D amine on coccinelid larvae. *Can J. Zool.* 38:285-288.
38. Adams, J.B. and M.E. Drew. 1965. Grain aphids in New Brunswick III. Aphid populations in herbicide-treated oat fields. *Can. J. Zool.* 43:789-794.
39. Özkan, A. and A. Yanikolu. 1999. Effects of 2,4-D and maleic hydrazide on the glycogen level in the embryonic development of *Pimpla turionellae* (L.) (Hym., Ichneumonidae). *J. Appl. Ent.* 123:211-216.
40. Asteraki, E.J., C.B. Hanks, and R.O. Clements. 1992. The impact of the chemical removal of the hedge-base flora on the community structure of carabid beetles (Col. Carabidae) and spiders (Araneae) of the field and hedge bottom. *J. Appl. Ent.* 113:398-406.
41. Keith, J.O., R.M. Hansen, and A.L. Ward. 1959. Effect of 2,4-D on abundance and foods of pocket gophers. *J. Wild. Manage.* 23:137-144.
42. Spencer, S.R. and G.W. Barrett. 1980. Meadow vole population responses to vegetational changes resulting from 2,4-D application. *Am. Midl. Nat.* 103:32-46.
43. Balfour, P.M. 1989. Effects of forest herbicides on some important wildlife forage species. Canada. British Columbia. Economic and Regional Development Agreement. Victoria BC, Sept.
44. U.S. EPA. 1979. Weedar 64 Broadleaf Herbicide - EPA Reg. No. 264-2. Memo from Carlo Rodriguez to Willa Garner. Washington, D.C., Apr. 18.
45. Huntsman, R.C. 1993. Notice of claim re: Negligent spraying of Leavitt property. St. George UT, Mar. 1.
46. Swanson, C.R. and W.C. Shaw. 1954. The effect of 2,4-dichlorophenoxyacetic acid on the hydrocyanic acid and nitrate content of Sudan grass. *Agron. J.* 46:418-421.
47. WIllard, C.J. 1950. Indirect effects of pesticides. *Proc. 7th N. Cent. Weed Cont. Conf.* Pp.110-112.
48. Whitehead, E.I., J. Kersten, and D. Jacobsen. 1956. The effect of 2,4-D spray on the nitrate content of sugar beet and mustard plants. *Proc. S.D. Acad. Sci.* 25:105-110.
49. Frank, P.A. and B.H. Grigsby. 1957. Effects of herbicidal sprays on nitrate accumulation in certain weed species. *Weeds* 5:206-217.
50. Stahler, L.M. and E.I. Whitehead. 1950. The effect of 2,4-D on potassium nitrate levels in leaves of sugar beets. *Science* 112:749-751.
51. Basrur, S.V., R.A. Fletcher, and P.K. Basrur. 1975. In vitro effects of 2,4-dichlorophenoxy acetic acid (2,4-D) on bovine cells. *Can. J. Comp. Med.* 40:408-415.
52. Drill, V.A. and T. Hiratzka. 1953. Toxicity of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid. *Ind. Hyg. Occup. Med.* 7;61-67.
53. Hayes, H.M. et al. 1991. Case-control study of canine malignant lymphoma: Positive association with dog owner's use of 2,4-dichlorophenoxyacetic acid herbicides. *J. Natl. Cancer Inst.* 83:1226-1231.
54. Gianessi, L.P. and J.E. Anderson. 1995. Pesticide use in U.S. crop production. National summary report. (Table 18.) Washington, D.C.: National Center for Food and Agricultural Policy.
55. Kumari, T.S. and K. Vaidyanath. 1989. Testing of genotoxic effects of 2,4-dichlorophenoxyacetic acid (2,4-D) using multiple genetic assay systems of plants. *Mut. Res.* 226: 235-238.
56. Unrau, J. and E.N. Larter. 1951. Cytogenetical responses of cereals to 2,4-D: I. A study of meiosis of plants treated at various stages of growth. *Can. J. Bot.* 30:22-27.
57. Khalatar, A.S. and Y.R. Bhargava. 1982. 2,4-Dichlorophenoxyacetic acid - A new environmental mutagen. *Mut. Res.* 103:111-114.
58. Khalatar, A.S. and Y.R. Bhargava. 1985. Mutagenic effects of 2,4-dichlorphenoxyacetic acid alone and with ethyl methane sulfonate in *Hordeum vulgare* L. *Environ. Pollut.* (Ser. A) 38:9-17.
59. Grover, I.S. et al. 1990. Genotoxicity of pesticides and plant systems. In *Mututation and the environment. Pt. E: Environmental genotoxicity, risk, and modulation.* ed. M.L. Mendelsohn and R.J. Albertini. New York NY: WIley-Liss.
60. Kong, M.S. and T.H. Ma. 1999. Genotoxicity of contaminated soil and shallow well water detected by plant bioassays. *Mut. Res.* 426:221-228.
61. Oka, I.N. and D. Pimentel. 1976. Herbicide (2,4-D) increases insect and pathogen pests on corn. *Science* 193:239-240.
62. Ishii, S. and C. Hirano. 1963. Growth responses of larvae of the rice stem borer to rice plants treated with 2,4-D. *Ent. Exp. Appl.* 6:257-262.
63. Maxwell, R.C. and R.F. Harwood. 1960. Increased reproduction of pea aphids on broad beans treated with 2,4-D. *Ann. Entomol. Soc. Amer.* 53:199-205.
64. Richardson, L.T. 1959. Effect of insecticides and herbicides applied to soil on the development of plant diseases. II. Early blight and *Fusarium* wilt of tomato. *Can J. Plant Sci.* 39:30-38.
65. Simons, T.J. and A.F. Ross. 1965. Effect of 2,4-dichlorophenoxyacetic acid on size of tobacco mosaic virus lesions in hypersensitive tobacco. (Abstract.) *Phytopathol.* 55:1076-1077.
66. Hodges, C.F. 1992. Vegetative growth and sporulation of *Bipolaris sorokiniana* on infected leaves of *Poa pratensis* exposed to postemergence herbicides. *Can J. Bot.* 70:568-570.
67. Cheo, P.C. 1969. Effect of 2,4-dichloropheoxyacetic acid on tobacco mosaic virus infection. *Phytopathol.* 59:243-244.
68. Pronos, J, and R.F. Patton. 1977. *Armillaria* root rot of red pine planted on oak sites in Wisconsin. *Plant Dis. Rep.* 61:955-958.
69. Whitney, R.D. 1985. Herbicide-killed roots as inoculum sources of *Armillaria* root rot in Ontario spruce. *Western International Forest Disease Work Conf. Proc.* 16:68-70.
70. Attiwill, P.M. 1995. Nutrient cycling in forests. In *Encyclopedia of Environmental Biology.* Vol. 2, ed. W.A. Nierenberg. San Diego CA: Academic Press. Pp. 627-629.
71, Garten, C.T. 1990. Multispecies methods of testing for toxicity: Use of the *Rhizobium*-legume symbiosis in nitrogen fixation and correlations between responses by algae and terrestrial plants. In *Plants for toxicity assessment.* ASTM STP 1091. ed. Wang, W., J.W. Gorsuch, and W.R. Lower. Philadelphia PA: American Soc. for Testing and Materials. Pp. 69-84.
72. Fabra, A., R. Duffard, and A. Evangelista de Duffard. 1997. Toxicity of 2,4-dichlorophenoxyacetic acid in pure culture. *Bull. Environ. Contam. Toxicol.* 59:645-652.
73. Arias, R.N. and A. Fabra de Peretti. 1993. Effects of 2,4-dichlorophenoxyacetic acid on *Rhizobium* sp. growth and characterization of its transport. *Toxicol. Lett.* 68:267-273.
74. Eberbach, P.L. and L.A. Douglas. 1989. Herbicide effects on the growth and nodulation potential of *Rhizobium trifolii* with *Trifolium subterraneum* L. *Plant and Soil* 119:15-23.
75. Eberbach, P.L. and L.A. Douglas. 1991. Effect of herbicide residues in a sandy loam on the growth , nodulation and nitrogenase activity (C_2H_2/C_2H_4) of *Trifolium subterraneum. Plant and Soil* 131:67-76.
76. Singh, J.B. and S. Singh. 1989. Effect of 2,4-dichlorophenoxyacetic acid and maleic hydrazide on growth of blue-green algae (cyanobacteria) *Anabaena doliolum* and *Anacystis nidulans*. *Sci. Cult.* 55:459-460.
77. Tözüm-Çalgan, S.R.D. and S. Sivaci-Güner. 1993. Effects of 2,4-D and methylparathion on growth and nitrogen fixation in cyanobacterium, *Gloeocapsa*. *Intern. J. Environ. Stud.* 23:307-311.
78. Martens, D.A. and J.M. Bremner. 1993. Influence of herbicides on transformations of urea nitrogen in soil. *J. Environ. Sci. Health* B 28:377-395.
79. Frankenberger, W.T., Jr. and M.A. Tabatabai. 1991. Factors affecting L-asparaginase activity in soils. *Biol. Fert. Soils* 11:1-5.
80. Attiwill, P.M. 1995. Nutrient cycling in forests. In *Encyclopedia of Environmental Biology*. Vol. 2, ed. W.A. Nierenberg. San Diego: Academic Press. Pp. 631-632.
81. Estok, D., B. Freedman, and D. Boyle. 1989. Effects of the herbicides 2,4-D, glyphosate, hexazinone, and triclopyr on the growth of three species of ectomycorrhizal fungi. *Bull. Environ. Contam. Toxicol.* 42:835-839.
82. Webster, J.M. 1967. Some effects of 2,4-dichlorophenoxyacetic acid herbicides on nematode-infested cereals. *Plant Pathol.* 16:23-26.
83. Jofré, E. et al. 1996. 2,4-Dichlorophenoxyacetic acid affects the attachment of *Azospirillum brasilense* Cd to maize roots. *Toxicol.* 107:9-15.
84. U.S. EPA. Office of Pesticide Programs. Endangered Species Protection Program. Undated. County Bulletins. http://www.epa.gov/espp/.
85. Pfleeger, T. and D. Zobel. 1995. Organic pesticide modification of species interactions in annual plant communities. *Ecotoxicol.* 4:15-37.

HERBICIDE FACTSHEET

2,4-D: EXPOSURE

Exposure to the commonly used herbicide 2,4-D is frequent and widespread. Exposure can occur via contaminated air, water, soil, food, and through 2,4-D tracked inside homes.

About 60 percent of air samples collected during national, regional, and local studies are contaminated with 2,4-D. In some cases 2,4-D has moved in air up to 50 miles from the application site.

In a national survey of river basins, the U.S. Geological Survey found 2,4-D in 19 of the 20 basins sampled. Overall, between 10 and 13 percent of the samples collected were contaminated with 2,4-D. It is also found in wells, although not as frequently, and in rain. 2,4-D has been found in water following agricultural, urban, golf course, roadside, and noxious weed treatments.

Persistence of 2,4-D in soils is variable with half-lives (the time required for half of an application of 2,4-D to break down or move away) as short as 2 days and as long as 297 days. One study found 2,4-D in water leaching from an agricultural soil 5 years after 2,4-D treatment.

After a home lawn is treated with 2,4-D, the person who made the application as well as children and pets who live in the home track 2,4-D indoors. Particularly for infants, these exposures are of concern.

People who work with 2,4-D are more highly exposed than the general population. A study of farmers showed that their children's exposures also increase after the farmers make a 2,4-D application.

BY CAROLINE COX

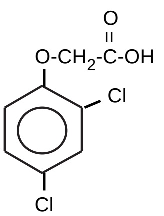
According to its manufacturers, 2,4-D (see Figure 1) is "the most widely used herbicide in the world."[1] This article, the fourth in a series about 2,4-D, summarizes research about exposure to 2,4-D. Earlier articles discussed its toxicology (JPR 19(1):14-19 and 19(2):14-19) and its ecological effects (JPR 19(3):14-19).

Overview of Exposure

Exposure of people to 2,4-D is widespread; a national survey of pesticides in urine samples collected from 1000 adults representing "both sexes and different age groups, races/ethnicities, urban/rural residences, and regions of the country"[2] found that 12 percent of the samples contained 2,4-D.[2]

People and other living things are exposed to 2,4-D in many ways: by breathing contaminated air, drinking and bathing in contaminated water, contacting contaminated soil, eating contaminated food, ingesting contaminated dust, and while working with 2,4-D. While each kind of exposure may involve small amounts of 2,4-D, their cumulative effect is a serious concern. This factsheet summarizes important studies about each of these routes of exposure.

Figure 1
2,4-D



2,4-dichlorophenoxyacetic acid

Air Contamination

One type of exposure to 2,4-D occurs via contaminated air. 2,4-D can move through the air away from the site where it has been applied in three ways: drift occurs when spray droplets move in air currents; volatilization occurs when the pesticide is evaporated from its liquid form to a gaseous form and the resulting vapors move in air currents; and a third type of air contamination occurs when blown soil particles carry pesticides.[3] Since these processes can occur simultaneously and are hard to separate, this section discusses them together.

Any form of 2,4-D can drift or move as airborne soil particles; volatilization is particularly important with certain 2,4-D esters.[4]

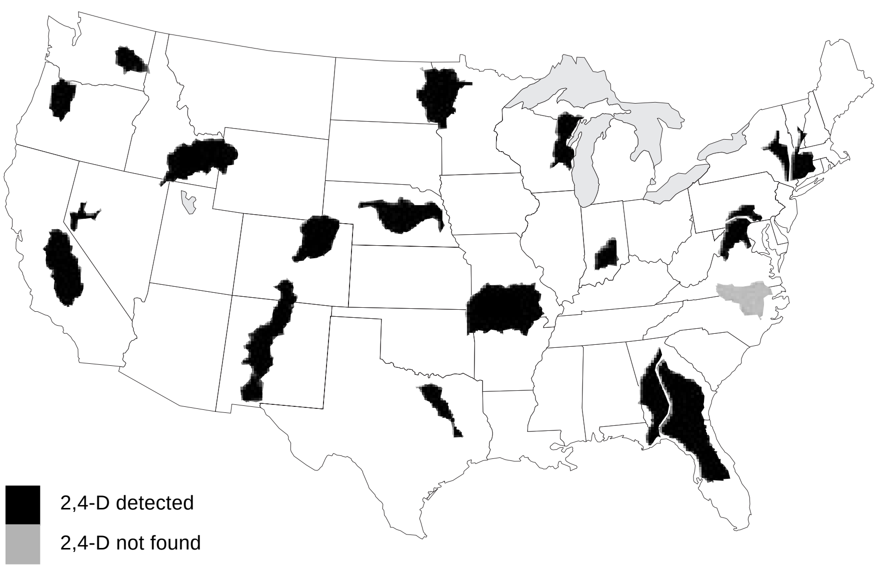
National, regional, and local studies of air contamination show that 2,4-D is commonly found in air. When the U.S. Geological Survey combined data from all available studies, they found that about 60 percent of the air samples tested were contaminated with 2,4-D.[5]

Movement of 2,4-D in air is important because, as expected for a potent herbicide, 2,4-D is phytotoxic in small amounts. This means when 2,4-D moves in air, it has the potential to severely damage nontarget vegetation.

Many experimental studies, dating back to 2,4-D's first use in the 1940s, demonstrate nontarget plants' "extreme sensitivity"[6] to this herbicide. Some of

Caroline Cox is JPR's editor.

Figure 2
Contamination of River Basins with 2,4-D



Sources: U.S. Geological Survey. National Water-Quality Assessment (NAWQA) Program. 1998. Circulars 1144,1150, 1151, 1155-1171. http://water.usgs.gov/pubs/nawqasum/.

The U.S. Geological Survey found 2,4-D in 19 of the 20 river basins the agency sampled across the United States.

these studies are remarkable because of the tiny amounts of 2,4-D that cause damage. For example, as early as the 1960s researchers showed that 2,4-D concentrations of a few parts per billion damaged grapes, geraniums, tomatoes, roses, and a variety of other cultivated plants.[7] Vapors of the butyl ester of 2,4-D in concentrations approximately 10,000 times less than typical field applications damaged tomato and lettuce plants.[8] Similar concentrations of 2,4-D acid[9] and its isooctyl ester[10] also damaged tomatoes and lettuce. One percent of typical agricultural rates damaged cherry, alfalfa, and rose plants.[11] Exposure to 1/900 of a typical application rate of 2,4-D damaged wine grape plants, and symptoms persisted for 120 days.[12]

Drift of 2,4-D can have complex, unexpected results. Simulated drift on sugarbeets, for example, not only decreases sugarbeet yield and sugar content, but also causes the sugarbeets to lose more sugar during storage than the amount lost by unexposed plants.[13]

Widespread plant damage caused by 2,4-D drift has been reported from around the world. In St. Louis, drifting emissions from a 2,4-D manufacturing plant damaged trees up to five miles away.[14] In Natal, South Africa, vegetable crops were damaged by drifting 2,4-D from applications made to sugarcane fields between 10 and 50 kilometers distant.[15,16] In Western Australia, an experimental application of a volatile ester of 2,4-D drifted 10 kilometers from a sprayed wheat field and a 50 kilometer buffer was required to protect a tomato-growing area.[17] In central Washington, 2,4-D applied to wheat fields drifted 10 to 50 miles and damaged vineyards.[18] In Colorado, hot temperatures (above 95°F) during spraying of wheat fields resulted in 2,4-D volatilizing and damaging 30,000 acres of beans. High-, low-, and nonvolatile 2,4-D products travelled up to 10 miles.[19]

A common question is "How far does 2,4-D typically drift?" NCAP's literature review found few experimental measurements of 2,4-D drift using current application equipment. One study of aerial applications (published in 1990) measured 2,4-D drift 1800 feet from the application.[20] Another study measured drift 300 feet from an aerial application.[21]

Contamination of Rivers and Streams

Another source of exposure to 2,4-D is contaminated water. 2,4-D is frequently found in rivers and streams. This is partly due to 2,4-D's wide use; it is also due to 2,4-D's high solubility in water.[22]

The most comprehensive data about water contamination by 2,4-D come from the U.S. Geological Survey's (USGS's) recent study of river basins throughout the U.S. The USGS found 2,4-D in 19 of the 20 basins sampled. (See Figure 2.) 2,4-D was found in 12 percent of agricultural stream samples; 13.5 percent of urban stream samples; and in 9.5 percent of the samples from rivers draining a variety of land uses.[23]

Contamination of rivers and streams has also been directly associated with agricultural, forestry, urban, and golf course applications of 2,4-D. Examples include the following studies:

Agriculture: In an agricultural area in Greece, 2,4-D was found in river and stream water at every sample location tested between May and August. Researchers calculated that 17 percent of applied 2,4-D ended up in the water.[22]

Farm ponds in the Canadian prairies are almost universally contaminated with 2,4-D; over 93 percent of ponds tested in an Agriculture Canada study contained 2,4-D. Highest concentrations were measured in the spring, but unlike other pesticides studied, 2,4-D residues persisted past the end of the growing season.[24]

A study of an agricultural watershed in Manitoba, Canada, found 2,4-D in creek water at highest concentrations during the application season and for a month afterwards. A second peak occurred in the spring when snow melted.[25]

Forestry: The Washington Department of Ecology collected 32 stream samples downstream from a helicopter application of 2,4-D conducted according to the state's "best management practices." 2,4-D was found in all samples collected, in highest concentrations following a rainstorm the day after the spraying.[26]

Urban: In a study from Lousiville, Kentucky, every sample of stormwater runoff contained 2,4-D, occasionally (8 percent) in excess of federal standards.[27] In a Canadian study, 2,4-D was found in all four urban streams sampled, and 70 percent of the samples contaminated with 2,4-D were collected within a few days of nearby 2,4-D applications.[28] In King County, Washington, 2,4-D had high retail sales relative to other pesticides and was found in all samples taken from 10 urban streams.[29] In Essex, United Kingdom, 2,4-D was found in all stormwater samples taken in an urban area.[30]

Golf Courses: Oklahoma State University researchers studied golf course turf that was treated with 2,4-D followed by simulated rainfall. They found 2,4-D in runoff water even when they left buffers of untreated turf. Under conditions of high runoff, concentrations of 2,4-D exceeded the U.S. Environmental Protection Agency's (EPA's) health advisory level for drinking water.[31] Another study found concentrations of 2,4-D in runoff from experimental golf course fairways that were 10 times greater than EPA's drinking water standard. These high concentrations of 2.4-D were measured in the runoff from the first simulated rainfall.[32]

Contamination of Groundwater

2,4-D contaminates wells because of its "very high"[33] mobility in soils, its "weak binding"[33] to soil particles, and its ability to move rapidly through low-density pathways in soils.[34] This means that it is easy for 2,4-D to move after treatment through soil to groundwater. When the USGS tested well water samples for pesticides as part of the national survey mentioned in the previous section, half of one percent of shallow wells in agricultural areas and wells located on major aquifers were contaminated with 2,4-D. One percent of shallow urban wells were also contaminated.[35]

Contamination of ground water has been directly associated with roadside applications of 2,4-D, agricultural treatments, applications for noxious weed control, and 2,4-D manufacturing plants. Examples include the following studies:

Roadsides: Because runoff from a road is typically directed to a roadside ditch, where soil is shallow because some has been removed to make the ditch, rainfall easily moves from roadside ditches to groundwater. This water can carry pesticides. A laboratory study showed that up to 22 percent (depending on soil type) of the 2,4-D applied to a roadside leached to the underlying groundwater.[37]

Agriculture: A three-year study in Alberta, Canada, found 2,4-D in groundwater following a 2,4-D application. 2,4-D persisted in the groundwater long enough that it was found in years when no applications were made.[37]

Noxious weed control: 2,4-D spraying of leafy spurge growing in sandy soils in North Dakota contaminated shallow groundwater. Wells 300-700 feet from treated areas were contaminated, indicating that 2,4-D either drifted or migrated in the groundwater at least that far.[38]

Aquatic weed control: 2,4-D treatment of a Michigan lake contaminated nearby wells for up to 200 days.[39]

Manufacturing Near Perth, Australia, a 2,4-D manufacturing plant contaminated groundwater over an area of 0.7 square kilometers (0.25 square miles).[40]

Contamination of Rain

2,4-D has contaminated rainfall worldwide according to studies from Manitoba,[41] Saskatchewan,[42] and Alberta,[43] Canada; Zurich, Switzerland[44]; Natal, South Africa[15]; and the U.S.[45]

Persistence

Exposure to a pesticide increases if that pesticide persists in the environment. Available data about 2,4-D shows that its persistence is highly variable.

Water: In still water (ponds, lakes, or reservoirs) 2,4-D persists as much as 6 months after application.[46] In streams, one study indicates that microorganisms' ability to breakdown 2,4-D is "very limited"[47] thus 2,4-D will move downstream, but will break down only slowly.

A laboratory study of 2,4-D's persistence in groundwater found that its half life varied between 800 and 1900 days.[48]

Persistence in wastewater treatment plants is a concern because the effluent from these plants is typically discharged into rivers or oceans. A Canadian study found that 2,4-D is "refractory" to treatment in wastewater plants, meaning that most 2,4-D is not biodegraded but is released in the effluent.[49] Additionally, 2,4-D is toxic to many of the bacteria that purify water in a wastewater plant.[50]

Soil: Data collected by 2,4-D manufacturers as part of the reregistration process show that the half-life of 2,4-D (the time it takes for half of an application of 2,4-D to break down or move away) varies from 2 days to 296 days. Longest half-lives occurred for granular 2,4-D ester products used on turf in Ohio.[51] (See Figure 3.)

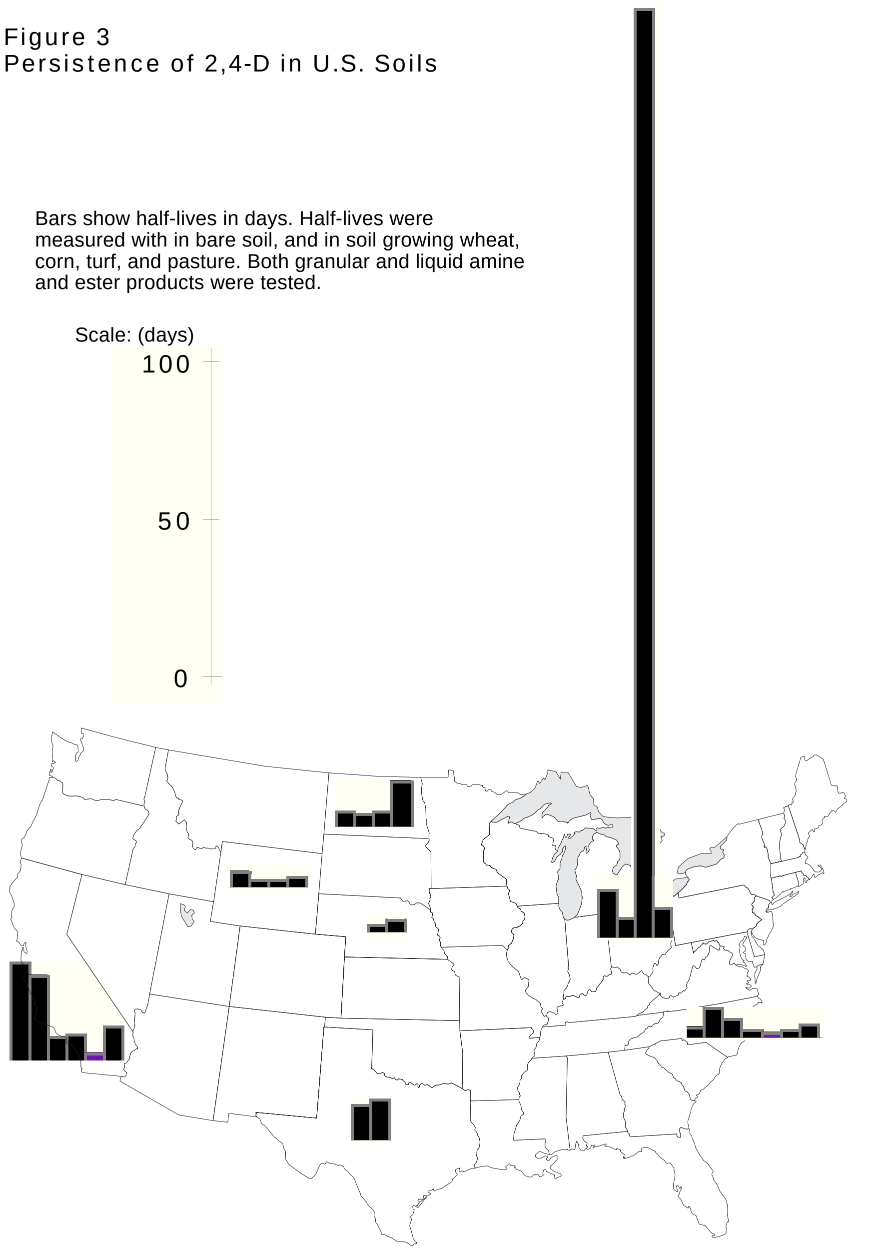
Other data show that 2,4-D can persist for much longer than these half-lives indicate. For example, a Danish study of clay agricultural soils found 2,4-D in water leaching from these soils over 5 years after treatments had been made.[52]

2,4-D is often found in marsh or wetland sediments[30] where it persists longer than in soils with more oxygen.[53]

People who are concerned about 2,4-D contamination often ask if it is possible to speed up the rate at which it breaks down. NCAP's literature review located no studies that specifically address this question. However, a number of studies suggest possible strategies. 2,4-D breaks down more quickly in surface soil (the top 12 inches) than in deeper soil.[54] Moist soils have quicker 2,4-D breakdown than dry soils.[55,56] Nitrogen fertilizers slow break down.[57,58] Therefore, bringing contaminated soil to the soil surface, keeping it damp, and not fertilizing should speed 2,4-D breakdown.

Compost: Since lawns are frequently treated with 2,4-D, one source of exposure is through compost made from grass clippings. A Michigan study found 2,4-D

Figure 3
Persistence of 2,4-D in U.S. Soils



Wilson, R.D., J. Geronimo, and J.A. Armbruster. 1997. 2,4-D dissipation in field soils after applications of 2,4-D dimethylamine salt and 2,4-D 2-ethylhexyl ester. *Environ. Toxicol. Chem.* 16:1239-1246.

Persistence of 2,4-D in soil varies widely, from 2 to 296 days.

in clippings after a year of composting.[59]

Contamination of Food

In the early 1990s the U.S. Department of Agriculture's Pesticide Data Program (PDP) found 2,4-D residues on six fruits and vegetables: potatoes,[60-62] oranges,[60-62] grapefruit,[60,61] apples,[61,62] peaches,[61,62] peas,[62] and grapes.[61,62] Recently, the PDP has not tested fruits and vegetables for 2,4-D.[63-65]

Since 2,4-D is commonly used on pastures,[66] 2,4-D residues in milk are possible. Monitoring studies have been contradictory. In 1996 and 1997, the PDP looked for 2,4-D in milk, but found no detectable residues.[67,68] However, a study from the Connecticut Agricultural Experiment Station found that three-quarters of their milk samples contained 2,4-D.[69]

2,4-D residues in wheat are also to be expected, since wheat is frequently treated with 2,4-D.[66] Neither the PDP nor the Food and Drug Administration test wheat for 2,4-D residues.[65,70] A study by Agriculture Canada, however, found 2,4-D in wheat seeds above the Canadian tolerance level (acceptable contamination level) following late-season 2,4-D treatments.[71]

Indoor Exposure

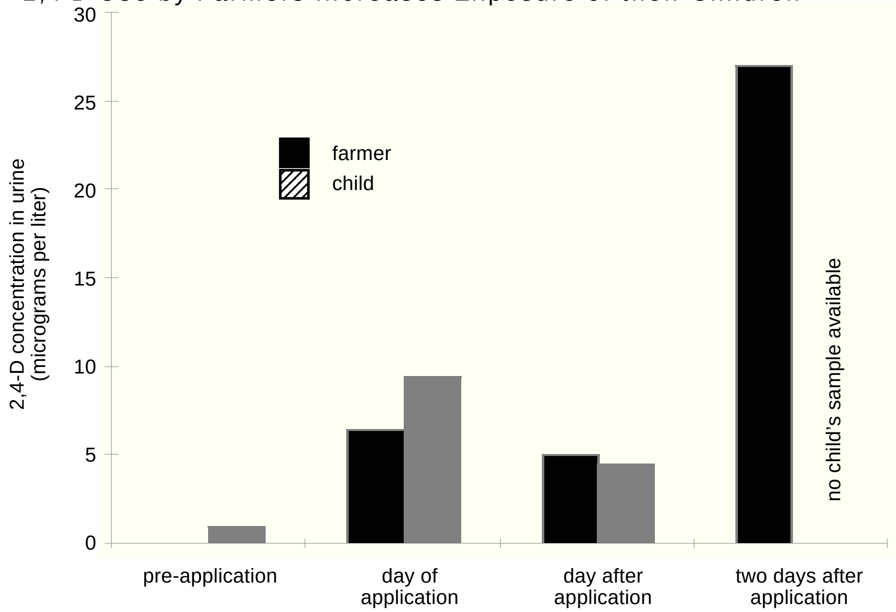
2,4-D is frequently found indoors; a recent study found 2,4-D in carpet dust from 60 percent of the houses sampled.[72] This indoor 2,4-D has "significant implications for human exposure."[73] It does not break down quickly because wind, rain, soil microbes, and sunlight are not available.[73] Because children spend significant time on the floor, indoor residues are an important exposure.

How does 2,4-D end up indoors? Dust carried from outdoors is an important route.[73-75] A simulation study, measuring 2,4-D in dust tracked from an experimental turf plot to a carpet set up next to it, indicated that 2,4-D would be expected in carpet dust up to one year after a lawn application.[73] A subsequent study found 2,4-D in dust from all houses tested. Concentrations were highest in the week following a lawncare 2,4-D application, whether that application was done by a family member or a commercial applicator. This study also showed that highest 2,4-D concentrations occurred in homes with active pets and children.[74] 2,4-D was also found in air samples, and on tables and window sills. The researchers then estimated the exposure of a one-year-old child. The highest estimated exposures were close to EPA's reference dose,[75] the daily exposure "that is likely to be without an appreciable risk of deleterious effects during a lifetime."[76]

Occupational Exposure

Many studies have shown herbicide applicators are more exposed to 2,4-D than the general population. Two recent articles that provide references to many such

Figure 4
2,4-D Use by Farmers Increases Exposure of their Children



Shealy, D.B. et al. 1996. Application of an improved method for the analysis of pesticides and their metabolites in the urine of farmer applicators and their families. *Environ. Intern.* 22:661-675.

Farmers have elevated levels of 2,4-D in their bodies after applying the herbicide. Elevated levels are also found in their children.

studies have been published by the Centers for Disease Control and Prevention.[2,77] Rather than summarizing all of these studies, this factsheet summarizes a few with interesting or unexpected results.

Family exposure: Data from the ongoing federal Agricultural Health Study showed that farmers applying 2,4-D had urinary concentrations of 2,4-D that were approximately 8 times higher than farmers who did not use the herbicide. These elevated levels of 2,4-D persisted for several days after 2,4-D application. At the same time, elevated levels of 2,4-D were measured in the children of the farmers who applied 2,4-D.[77] (See Figure 4.)

Duration of exposure: It is often assumed that when 2,4-D application ends, occupational exposure will cease. However, a study of aerial applicators showed that 2,4-D in their urine samples disappeared slowly after their spray work ended. Further monitoring showed that their living quarters, desks, and work vehicles were contaminated with 2,4-D. 2,4-D was found on all surfaces that were tested, including sinks, counter tops, refrigerators, and truck door handles.[78]

Problems with measurements of occupational exposure: Occupational exposure to 2,4-D is often estimated by providing workers with a cloth patch which they wear during their work day. The patch is then analyzed for 2,4-D. Recent research has shown that if the 2,4-D analysis is not done immediately, but takes place 48 hours after exposure, up to 80 percent of the 2,4-D on the patch is sequestered in the fabric and will be missed by laboratory analysis.[79]

Problems with protective clothing: Protective clothing, particularly gloves, is often recommended to reduce exposure of people applying 2,4-D. However, several problems with 2,4-D and chemically resistant gloves have been documented. Ultraviolet light (from, for example, exposure to sunlight) increases the amount of 2,4-D that penetrates two kinds of rubber gloves.[80] Solvents used as so-called inert ingredients in some 2,4-D products carry 2,4-D through other gloves.[81]

References

1. Industry Task Force on 2,4-D Research Data. 1996. Where in the world and the environment are we: A one day status report and briefing on the reregistration of 2,4-D. Handout titled "2,4-D … Its importance." Seminar sponsored by the Northwest Food and Forest Education Foundation. Portland, OR, July 31.
2. Hill, R.H. et al. 1995. Pesticide residues in urine of adults living in the United States: Reference range concentrations. *Environ. Res.* 71:99-108.
3. Hawthorne, S.B. et al. 196. Atmospheric pollutants and trace gases: Vapor-phase and particulate-associated pesticides and PCB concentrations in eastern North Dakota air samples. *J. Environ. Qual.* 25:594-600.
4. Regehr, D.L. 1999. 1999 chemical weed control for field crops, pastures, rangeland, and noncropland. Kansas State Univ. Agricultural Exp. Station and Cooperative Extension Serv. p.3.
5. Majewski, M.S. and P.D. Capel. 1995. Pesticides in the atmosphere; Distribution, trends, and governing factors. Pesticides in the hydraulic system, Vol. 1. Chelsea MI: Ann Arbor Press, Inc. p.79.
6. Staten, G. 1946. Contamination of cotton fields by 2,4-D or hormone-type weed sprays. *J. Amer. Soc. Agron*. 38: 536-544.
7. Sherwood, C.H., J.L Weigle, and E.L. Denisen. 1970. 2,4-D as an air pollutant: Effects on growth of representative horticultural plants. *HortScience* 5:211-213.
8. van Rensburg, E. and V.G. Breeze. 1990. Uptake and development of phytotoxicity following exposure to vapour of the herbicide ^{14}C 2,4-D butyl by tomato and lettuce plants. *Environ. Exp. Biol. 30:405-414.*
9. Breeze, V.G. and E. van Rensburg. 1991. Vapour of the free acid of the herbicide is toxic to lettuce and tomato plants. *Environ. Pollut.* 72:259-267.
10. Breeze, V.G. and E. van Rensburg. 1992. Uptake of the herbicide [^{14}C] 2,4-D iso-octyl in the vapour phase by tomato and lettuce plants and some effects on growth and phytotoxicity. *Ann. Appl. Biol.* 120:493-500.
11. Washington State Univ. Cooperative Ext. 1993. Herbicide injury symptoms on cherry, grape, alfalfa, and rose. Extension Bulletin No. EB1748.
12. Bhatti, M.A., K. Al-Khatib, and R. Parker. 1996. Wine grape (*Vitus vinifera*) response to repeated exposure of selected sulfonylurea herbicides and 2,4-D. *Weed Technol.* 10:951-956.
13. Schroeder, G.A., D.F. Cole, and A.G. Dexter. 1983. Sugarbeet (*Beta vulgaris* L.) response to simulated herbicide spray drift. *Weed Sci.* 31:831-836.
14. Lamphear, F.O. and O.H. Soule. 1970. Injury to city plants from industrial emissions of herbicides. *HortScience* 5:215-217.
15. Sandmann, E, P.R. de Beer, and L.P. van Dyk. 1991. Atmospheric pollution by auxin-type herbicides in Tala Valley, Natal. *Chemosphere* 22:137-145.
16. de Beer, P.R. C. Smit, and L.P. van Dyk. 1992. Air monitoring for pollution by auxin-type herbicides. *Chemosphere* 24:719-733.
17. Gilbey, D.J. et al. 1984. Airborne 2,4-D and tomato damage at Geraldton, Western Australia. *Australian Weeds* 3:57-60,67-69.
18. Robinson, E. and L.L. Fox. 1978. 2,4-D herbicides in central Washington. A*ir Poll. Cont. Assoc. Journal* 28:1015-1020.
19. Westra, P. and H.F. Schwartz. 1989. Potential

herbicide volatility and drift problems on dry beans. Colorado State Univ. Cooperative Extension Service in Action No. 2.803.
20. Huitink, G., J.T. Walker, and T.L. Lavy. 1990. Downwind deposition of 2,4-dichlorophenoxyacetic acid herbicide (2,4-D) in invert emulsion. *Trans. Amer. Soc. Ag. Eng.* 33:1051-1056.
21. Akesson, N.B. and D.E. Bayer. 1997. Evaluation by air and cotton plant sensors of downwind transfer of rice field herbicides applied by aircraft and ground sprayers. Presentation at the 1997 Annual International Meeting of the Amer. Soc. Ag. Eng. Minneapolis MN, Aug. 10-14.
22. Albanis, T.A. 1992. Herbicide losses in runoff from the agricultural area of Thessaloniki in Thermaikos Gulf, N. Greece. *Sci. Tot. Environ.* 114:59-71.
23. U.S. Geological Survey. National Water-Quality Assessment. Pesticide National Synthesis Project. 1998. Pesticides in surface and ground water of the United States; Summary of results of the National Water Quality Assessment Program (NAWQA). Tables 2-4. http://water.wr.usgs.gov/pnsp/allsum/
24. Grover, R. et al. 1997. Magnitude and persistence of herbicide residues in farm dugouts and ponds in the Canadian prairies. *Environ. Toxicol. Chem.* 16:638-643.
25. Rawn, D.F.K. et al. 1999. A multi-year study of four herbicides in surface water of a small prairie watershed. *J. Environ. Qual.* 28:906-917.
26. Rashin, E. and C. Graber. 1993. Effectiveness of best management practices for aerial application of forest pesticides. Olympia WA: Washington State Dept. of Ecology. Environmental Investigations and Laboratory Services Program. Watershed Assessments Section, Oct. Pp. 23-28.
27. Marsh, J.M. 1993. Assessment of nonpoint source pollution in stormwater runoff in Louisville, (Jefferson County) Kentucky, USA. *Arch. Environ. Contam. Toxicol.* 25:446-455.
28. Hall, J.C. et al. 1993. Enzyme immunoassay based survey of precipitation and surface water for the presence of atrazine, metolachlor and 2,4-D. *J. Environ. Sci. Health, B* 28:577-598.
29. 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, Apr.
30. Fletcher, C.A. et al. 1994. Magnitude and distribution of contaminants in salt marsh sediments of the Essex coast, UK. III. Chlorophenoxy acid and s-triazine herbicides. *Sci. Tot. Environ.* 155:61-74.
31. Cole, J.T. et al. 1997. Influence of buffers on pesticide and nutrient runoff from bermudagrass turf. *J. Environ. Qual.* 26:1589-1598.
32. Smith, A.E. and D.C. Bridges. 1996. Movement of certain herbicides following application to simulated golf course greens and fairways. *Crop Sci.* 36:1439-1445.
33. Cheah, U-B., R.C. Kirkwood, and K-Y. Lum. 1997. Adsorption, desorption and mobility of four commonly used pesticides in Malaysian agricultural soils. *Pestic. Sci.* 50:53-63.
34. Tindall, J.A. and W.K. Vencill. 1995. Transport of atrazine, 2,4-D, and dicamba through preferential flowpaths in an unsaturated claypan soil near Centralia, Missouri. *J. Hydrol.* 166:37-59.
35. Ref. # 23, Tables 7-9.
36. Kohl, R.A., C.G. Carlson, and S.G. Wangemann. 1994. Herbicide leaching potential through road ditches in thin soils over an outwash aquifer. *Appl. Eng. Agric.* 10:497-503.
37. B.D. Hill et al. 1996. Seasonal variation in herbicide levels detected in shallow Alberta groundwater. *Environ. Sci. Health B* 31:883-900.
38. Murphy, E.C. and E.A. Greene. 1992. The occurrence of picloram (Tordon) and 2.4-D in groundwater in the Denbigh Sand Hills, McHenry County, North Dakota. Rep. of Investigation No. 95. North Dakota Geological Survey.
39. Lovato, J.L., B.O. Fisher, and W.E. Brown. 1998. Migration of aquatically applied herbicides from surface water to ground water. 1998 Groundwater Protection Council Annual Conference.
40. Appleyard, S.J. 1993. Resourcing the investigation and management of groundwater contamination in Western Australia — a case study of contamination from herbicide manufacture near Perth. AGSO *J. Aust. Geol. Geophys.* 14:177-181.
41. Rawn, D.F.K. et al. 1999. A multi-year study of four herbicides in air and precipitation from a small prairie watershed. *J. Environ. Qual.* 28:898-906.
42. Waite, D.T. 1995. Atmospheric deposition of pesticides in a small southern Saskatchewan watershed. *Environ. Toxico. Chem.* 14:1171-1175.
43. Lethbridge Research Centre. 1999. Scientists detect herbicides in Southern Alberta rainfall. News release, Feb. 25.
44. Bucheli, T.D. 1997. Simultaneous determination of neutral and acidic pesticides in natural waters at the low nanogram per liter level. *Anal. Chem.* 69:1569-1576.
45. Ref#5, p.159.
46. U.S. EPA. Pesticides and Toxic Substances. 1988. Guidance for the reregistration of pesticide products containing 2,4-dichlorophenoxyacetic acid (2,4-D) as the active ingredient. Washington D.C., Sept. p.18.
47. Palmisano, A.C. et al. 1991. Seasonal changes in mineralization of xenobiotics by stream microbial communities. *Can. J. Microbiol.* 37:939-948.
48. Cavalier, T.C., T.L. Lavy, and J.D. Mattice. 1991. Persistence of selected pesticides in groundwater samples. *Ground Water* 29:225-231.
49. Monteith, H.D. et al. 1995. Modeling the fate of pesticides in municipal wastewater treatment. *Water Environ. Res.* 67:964-970.
50. Milner, C.R. and R. Goulder. 1986. Comparative toxicity of chlorophenols, nitrophenols, and phenoxyalkanoic acids to freshwater bacteria. *Bull. Environ. Contam. Toxicol.* 37:714-718.
51. Wilson, R.D., J. Geronimo, and J.A. Armbruster. 1997. 2,4-D dissipation in field soils after applications of 2,4-D dimethylamine salt and 2,4-D 2-ethylhexyl ester. *Environ. Toxicol. Chem.* 16:1239-1246.
52. Felding, G. 1995. Leaching of phenylalkanoic acid herbicides from farmland. *Sci. Total Environ.* 168:11-18.
53. DeLaune, R.D. et al. 1990. Fate of petroleum hydrocarbons and toxic organics in Louisiana coastal environments. *Estuaries* 13:72-80.
54. Veeh, R.H., W.P. Inskeep, and A.K. Camper. 1996. Soil depth and temperature effects on microbial degradation of 2,4-D. *J. Environ. Qual.* 25:5-12.
55. Ou, L.T. 1984. 2,4-D degradation and 2,4-D degrading microorganisms in soils. *Soil Sci.* 137:100-107.
56. Johnson, W.G., T.L. Lavy, and E.E. Gbur. 1995. Sorption, mobility and degradation of triclopyr and 2,4-D on four soils. *Weed Sci.* 43:678-684.
57. Mulroy, P.T. and L.-T. Ou. 1997. Influence of nitrogen and phosphorus fertilizers on 2,4-D degradation in soil. *J. Environ. Sci. Health B* 32:353-362.
58. Entry, J.A., K.G. Mattson, and W.H. Emmingham. 1993. The influence of nitrogen on atrazine and 2,4-dichlorophenoxyaetic aid mineralization in grassland soils. *Biol. Fertil. Soils* 16;179-182.
59. Vandervoort, C. et al. 1997. Fate of selected pesticides applied to turfgrass: Effect of composting on residues. *Bull. Environ. Contam. Toxicol.* 58:38-45.
60. U.S. Dept. of Agriculture. Agricultural Marketing Service. 1994. Pesticide Data Program (PDP): Summary of 1992 data. Appendix 7. Apr.
61. U.S. Dept. of Agriculture. Agricultural Marketing Service. Science Div. 1995. Pesticide Data Program (PDP): Annual summary calendar year 1993. Appendix B. June.
62. U.S. Dept. of Agriculture. Agricultural Marketing Service. Science & Technology. Undated. Pesticide Data Program (PDP): Annual summary calendar year 1994. Appendix C.
63. U.S. Dept. of Agriculture. Agricultural Marketing Service. Science and Technology Div. 1997. Pesticide Data Program (PDP): Annual summary calendar year 1995, May. Pp. 4-5.
64. U.S. Dept. of Agriculture. Agricultural Marketing Service. Science and Technology. 1998. Pesticide Data Program (PDP): Annual summary calendar year 1996, Feb. Pp. 4-6.
65. U.S. Dept. of Agriculture. Agricultural Marketing Service. Science and Technology. 1998. Pesticide Data Program (PDP): Annual summary calendar year 1997, Feb. Pp. 4-6.
66. National Agricultural Pesticide Impact Assessment Program. 1996. Biologic and economic assessment of benefits from use of phenoxy herbicides in the United States. NAPIAP Rep. 1-PA-96, Nov. Pp. 51-69.
67. Ref. #64. Appendix F.
68. Ref. #65. Appendix H.
69. Pylypiw, H.M. and L. Hankin. 1991. Herbicides in pooled raw milk in Connecticut. *J. Food. Prot.* 54:136-137.
70. U.S. Food and Drug Admin. Undated. Food and Drug Administration pesticide program: Residue monitoring 1998. p.11. http://vm.cfsan.fda.gov/~dms/pesrpts.html.
71. Cessna, A.J. and F.A. Holm. 1994. Residues of 2,4-D in wheat following application after heading. *Can. J. Plant Sci.* 74:199-203.
72. Colt, J.S. 1998. Comparison of pesticides and other compounds in carpet dust samples collected from used vacuum cleaner bags and from a high-volume surface sampler. *Environ. Health Persp.* 106:721-724.
73. Nishioka, M.G. et al. 1996. Measuring transport of lawn-applied herbicide acids from turf to home: Correlation of dislodgeable 2,4-D turf residues with carpet dust and carpet surface residues. *Environ. Sci. Technol.* 30:3313-3320.
74. Nishioka, M.G. et al. 1999. Distribution of 2,4-dichlorophenoxy acetic acid in floor dust throughout homes following homeowner and commercial lawn applications: Quantitative effects of children, pets, and shoes. *Environ. Sci. Technol.* 33:1359-1365.
75. Nishioka, M.G. et al. 1999. Transport of lawn-applied 2,4-D from turf to home: Assessing the relative importance of transport mechanisms and exposure pathways. Research Triangle Park NC: U.S. EPA National Exposure Research Laboratory, Mar.
76. U.S. EPA. 1994. Glossary of risk assessment-related terms. www.epa.gov/iris/glossary.htm.
77. Shealy, D.B. et al. 1996. Application of an improved method for the analysis of pesticides and their metabolites in the urine of farmer applicators and their families. *Environ. Intern.* 22:661-675.
78. Frank, A., R.A. Campbell, and G.J. Sirons. 1985. Forestry workers involved in aerial application of 2,4-dichlorophenoxyacetic acid (2,4-D): Exposure and urinary excretion. *Arch. Environ. Contam. Toxicol.* 14:427-435.
79. Wester, R.C. 1994. Time-response necessary in validation for extraction of pesticides from cloth patches used in field exposure studies. *Arch. Environ. Contam. Toxicol.* 27:276-280.
80. Moody, R.P. 1992. Analysis of 2,4-D glove permeation under controlled environmental conditions. *In* McBriarty, J.P. and N.W. Henry, (eds.) *Performance of protective clothing.* Vol. 4. ASTM STP 1133. Philadelphia PA: Amer. Soc. Test. Mat.
81. Harville, J. and S.S. Que Hee. 1989. Permeation of a 2,4-D isooctyl ester formulation through neoprene, nitrile, and Tyvek protection materials. *Am. Ind. Hyg. Assoc. J.* 50:438-446.