● 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.

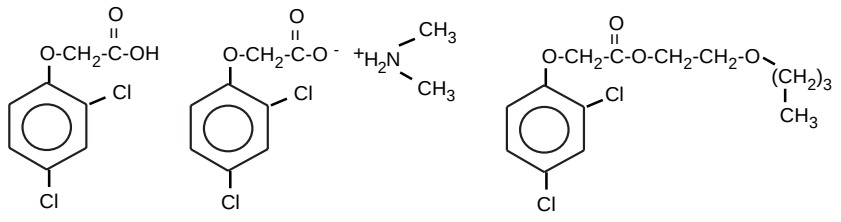
2,4-D has also caused genetic damage in tests using both cell cultures and laboratory animals. It increased the frequency of a gene mutation in hamster muscle cell cultures, increased the frequency of abnormal chromosomes in bone marrow cells of rats and mice, and increased the number of breaks in human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

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]

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

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,

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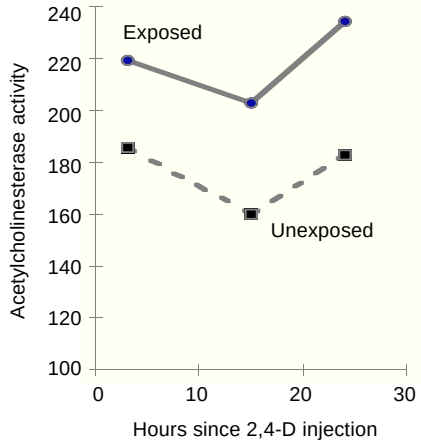
Caroline Cox is JPR's editor. The second part of NCAP's summary of 2,4-D's toxicology will be published in the Summer 1999 issue of JPR.

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.

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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

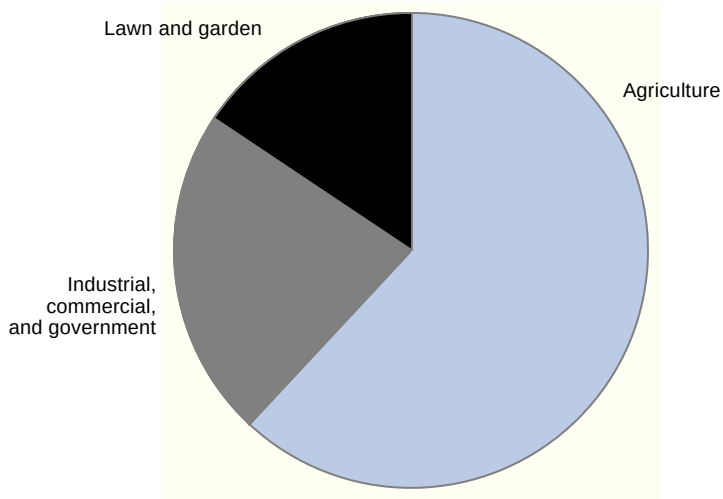
2,4-D and its dimethylamine, diethanolamine, isopropylamine, and triisopropanolamine salts are "severe eye irritants" which cause eye lesions lasting at least 3 days. In long-term feeding studies at relatively high doses, 2,4-D has also caused degeneration of the retina and cataracts.[12]

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

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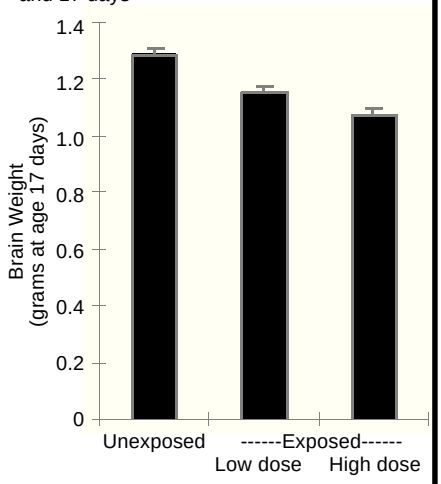
Figure 4
2,4-D Exposure Changes Behavior

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]

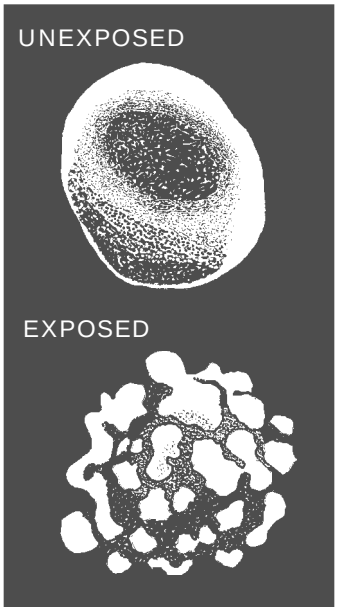
Although government evaluations of laboratory studies of 2,4-D have concluded that it does not cause genetic damage,[12,39] in fact it has been mutagenic in a variety of studies. These include both studies using live animals and studies using cell cultures.

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

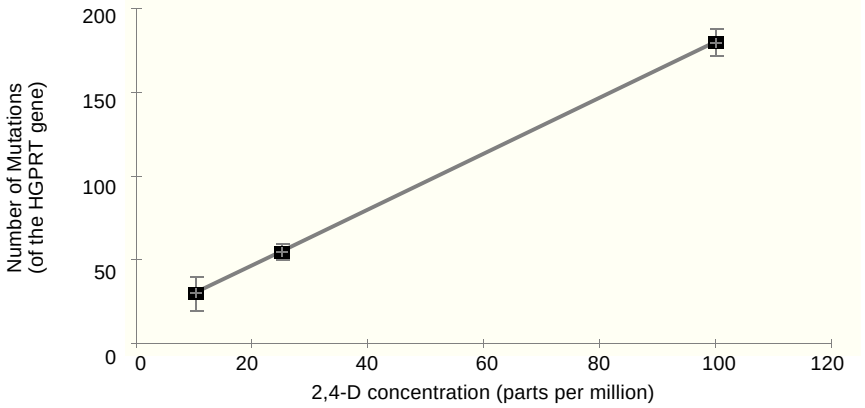


(based on scanning electron micrographs)

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.

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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]

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