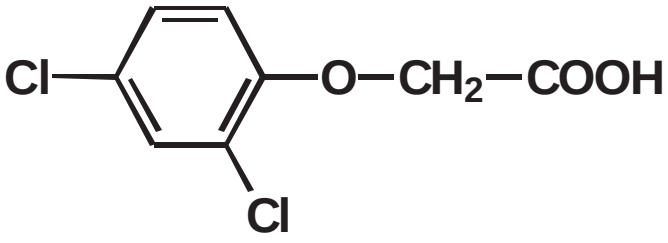
III.3 2,4-D



2,4- DICHLOROPHENOXYACETIC ACID

SUMMARY

2,4-D (2,4-dichlorophenoxyacetic acid) is a somewhat selective, systemic broadleaf herbicide that is used to control a variety of submersed, emersed and floating aquatic plants. 2,4-D exists in the acid form as well as in a variety of chemical forms. There are about 66 different formulations of 2,4-D, most of which are registered for terrestrial use. The 2,4-D acid form of this compound is not generally used for aquatic weed control (Reinert and Rodgers, 1987). The two categories of formulations which have been used most commonly for aquatic weed control include the butoxyethanol esters (2,4-D BEE) and the dimethylamine salts (2,4-D DMA) of 2,4-D. There are also a number of formulations being used for aquatic control containing the 2-ethylhexyl esters (2EHE), also known as 2-isooctyl esters (IOE), of 2,4-D (Reinert and Rodgers, 1987). 2,4-D formulations can exist as either emulsifiable concentrates, granulars, soluble concentrates, ready-to-use or pressurized liquids (DFA, 1988). The physical and chemical properties of 2,4-D are dependent on the chemical form of the active ingredient and vary dramatically.

In general, ester formulations of 2,4-D are more toxic to plants and fish than are amine salts. 2,4-D BEE formulations are generally not very soluble in water whereas 2,4-D DMA formulations have relatively high water solubility. Neither type of formulation is very volatile. Hydrolysis of 2,4-D BEE is a major fate process for this compound whereas it is not expected to be a significant fate process for 2,4-D DMA. Biotransformation and biodegradation are the major aquatic fate processes for both types of formulations. 2,4-D BEE tends to bioconcentrate to some degree in various organisms whereas 2,4-D DMA has a very low potential to bioconcentrate.

Many studies have been conducted with various formulations of 2,4-D addressing both toxicity and environmental fate and persistence. The U.S. Environmental Protection Agency (EPA) issued a registration standard for 2,4-D acid and all its chemical forms in September 1988 (USEPA, 1992). Since that time, the agency has been working with the industry to collect additional information under the mandate of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) amendments of 1988.

REGISTERED PRODUCTS IN MASSACHUSETTS

The current list of aquatic herbicides containing 2,4-D that are registered in Massachusetts can be accessed at http://www.state.ma.us/dfa/pesticides/water/Aquatic/Herbicides.htm

on the Massachusetts Department of Agricultural Resources (DAR) Aquatic Pesticide Website. The DAR updates this list regularly with changes. In addition, the DAR can be contacted directly at (617) 626-1700 for more specific questions regarding these products.

2,4-D USES AND APPLICATION

2,4-D can be used to control submersed, emersed and floating weeds. Liquid formulations of 2,4-D are only registered for the control of floating (e.g., waterhyacinth) and emergent vegetation. Surface applications can be made from a boat or from shore with dilute or concentrated product. Aerial applications can be made by spraying a dilute form of the product. Subsurface applications can be made with weighted trailing hoses from the boat (WSSA, 1983; Aquatic Plant Identification and Herbicide Use Guide, 1988).

Granular 2,4-D formulations can be distributed as either a surface application or as an aerial application using conventional mechanical spreaders or comparable equipment for large areas or a portable spreader for spot treatments (WSSA, 1983).

Treated water should not be used for irrigation, for agricultural sprays, for livestock watering or as a domestic water supply unless an approved assay indicates that the 2,4-D level does not exceed 0.1 mg/l 2,4-D acid-equivalent (Aquatic Plant Identification and Herbicide Use Guide, 1988). Alternatively, 2,4-D should not be used in waters with these uses. No swimming should take place for one day after treatment and fish should not be used from the treated waterbody for 3 days.

The best time to apply 2,4-D is in spring or early summer when young vegetation is actively growing. Application should be made in long strips separated by buffer zones. Application of liquid formulations should not be made during high wind or high water flow conditions. Aerial spraying should not be conducted if wind speed exceeds 8 km/hour. Drift control agents should be used when aerial spraying is conducted (Aquatic Plant Identification and Herbicide Use Guide, 1988).

For application of liquid formulations, especially when used on emersed or floating vegetation, use of invert emulsions or polymeric thickeners is recommended. For application of oil-soluble amine formulations, mixture with kerosene or other oil soluble solvent is recommended (Aquatic Plant Identification and Herbicide Use Guide, 1988).

Application rates of specific products vary due to the variation in the amount of active ingredient. For specific information on recommended application rates for a particular product, the product label should be consulted. The USEPA Office of Pesticide Programs (OPP) has a link to a database of product pesticide labels at http://www.epa.gov/pesticides/pestlabels/. A list of the weeds that these products control, which has been compiled from the Environmental Protection Agency (EPA) registration labels for these products, is contained in Table III.3-1.

Table III 3-1. List of Aquatic Weeds Controlled by 2,4-D

Common Name	Scientific Name
Arrowhead	*Sagittaria* spp.
Bladderwort	*Utricularia* spp.
Bulrush	*Scirpus* spp.
Coontail or Hornwort	*Ceratophyllum demersum*
Creeping Waterprimrose	*Jussiaea repens*
Pickerelweed	*Pontederia* spp.
Spatterdock, Cow Lily, Yellow Water Lily	*Nuphar* spp.
Burreed	*Sparganium* spp.
Waterweed	*Elodea*
Waterchestnut	*Trapa natans*
Watermilfoil	*Myriophyllum* spp.
Water Smartweed	*Polygonum* spp.
White Waterlily	*Nymphaea* spp.
Naiad	*Najas flexilis*
Pondweed	*Potamogeton* spp.
Watershield	*Brasenia* spp.

(Riverdale Chemical Co.)

MECHANISM OF ACTION

2,4-D is readily translocated throughout the plant phloem, especially from foliage to roots, probably along with the products of photosynthesis (Aquatic Plant Identification and Herbicide Use Guide, 1988; Joyce and Ramey, 1986). It is a somewhat selective, systemic growth regulator with hormone-like activity. 2,4-D inhibits cell division of new tissue and stimulates cell division of some mature plant tissue, resulting in inhibition of growth, necrosis of apical growth and eventual total cell disruption and plant death. Low concentrations of 2,4-D may stimulate plant growth (Aquatic Plant Identification and Herbicide Use Guide, 1988). Introduction of saturation levels of artificial auxins (including 2,4-D) into growing plants disrupted the plants' delicate hormonal balance, causing reductions in root uptake of salts and water, phloem transport and photosynthesis, contributing to the death of the plant (White-Stevens, 1971 as cited in HSDB, 1995). 2,4-D also affects plant respiration and food reserves (Joyce and Ramey, 1986). Since 2,4-D produces many toxic responses, the primary mode of action has not been clearly established (Joyce and Ramey, 1986).

ENVIRONMENTAL FATE AND TRANSPORT

The environmental fate and transport of 2,4-D in aquatic environments is determined by the chemical formulation of the 2,4-D and the physical properties of the individual compounds.

2,4-D butoxyethyl esters (BEE) have low water solubility (estimated at approximately 12 mg/l) whereas 2,4-D diethylamines (DMA) have relatively high water solubility (about 3.0×10^6 mg/l) (Tables III 3-2 and III 3-3). The water solubility of the 2,4-D acid ranges from about 600-900 mg/l (Reinert and Rodgers, Aquatic Plant Identification and Herbicide Use Guide, 1988).

The 2,4-D acid has a Henry's Law (H) value of 6.2×10^{-3} indicating that it is somewhat volatile. The relative rate of volatilization is dependent on the formulation. In general, the acid, inorganic salts and amines are less volatile than the esters, which vary from high to low. The oil soluble amines are considered the least volatile (WSSA, 1983). Both 2,4-D BEE and 2,4-D DMA have relatively low volatility (Tables III 3-2 and III 3-3) (Reinert and Rodgers, 1987; Aquatic Plant Identification and Herbicide use Guide, 1988). A volatilization half-life of 895 days was calculated for 2,4-D BEE (Reinert and Rodgers, 1987).

The relatively high octanol:water partitioning coefficient (K_{OW}) and organic carbon partitioning coefficient (K_{oc}) for the BEE form (Table III 3-2) would suggest that this form would likely adhere to soils and or sediments with some organic content. The opposite would hold for the DMA form (Table III 3-3).

Estimates of the typical overall half-life of 2,4-D in water range from 10 to greater than 50 days. The primary fate process of 2,4-D in water is microbial biodegradation. The various chemical formulations of 2,4-D are also subject, to varying degrees, to breakdown via hydrolysis and photolysis.

There are a variety of microorganisms in both fresh and marine waters which are capable of degrading 2,4-D. The rate of biodegradation is dependent on a number of factors including the level of nutrients present, temperature, availability of oxygen and whether/not the water has had a prior history of contamination with 2,4-D or other phenoxyacetic acids. 2,4-D is generally more persistent in oligotrophic waters and in waters with high 2,4-D concentrations. Biodegradation half-lives in clear waters have been estimated to be from 18 to greater than 50 days. In muddy waters, biodegradation half-lives have ranged from 10-25 days (HSDB, 1995).

The significance of hydrolysis as a 2,4-D fate process varies with the chemical formulation. The 2,4-D acid is somewhat subject to hydrolysis. Hydrolysis is a significant fate process for 2,4-D BEE formulations but is not expected to be an important fate process for the 2,4-D DMA formulations (Reinert and Rodgers, 1987; Aquatic Plant Identification and Herbicide Use Guide, 1988).

There are conflicting reports as to the photolysis of 2,4-D and its derivatives in water. The relative significance of this fate pathway is dependent on the chemical formulation of the 2,4-D derivative. There are no available data which show direct photolysis of 2,4-D in the atmosphere upon exposure to natural sunlight. Most photolysis studies of 2,4-D have used high-intensity mercury lamps which emit large amounts of ultraviolet (UV) radiation (DFA, 1988). It has been shown, however, that 2,4-D exhibits an absorption maximum at 288 nm extending to greater than 290 nm. Sunlight reaching the earth is composed of wavelengths greater than 280 nm. These facts suggest that 2,4-D may be susceptible to direct photolysis (HSDB, 1995). Whereas some researchers do not believe that photolysis is a significant fate pathway for 2,4-D BEE (Aly and Faust, 1964 as cited in Reinert and Rodgers, 1987) others have calculated a photolysis half-life for these formulations (see Table III 3-2) (Zepp *et al.*, 1975 as cited in Aquatic Plant Identification and Herbicide Use Guide, 1995). Photolysis is generally not expected to be a

significant fate pathway for 2,4-D DMA formulations (Reinert and Rodgers, 1987; Aquatic Plant Identification and Herbicide Use Guide, 1988).

Table III 3-2. Properties of 2,4-D BEE

CAS #:	1929-73-3
Molecular formula	$C_{14}H_{18}O_4Cl_2$
Molecular weight (g/mole)	321.2
Physical properties	colorless to amber oily liquid
Melting point	NA
Density	NA
Vapor pressure (mm Hg)	1.7×10^{-5} - 4.5×10^{-6}
Volatility [Henry's Law constant (atm m^3/mol)]	10^{-7}-10^{-5}
Photolysis half-life (days)	10-20
Hydrolysis half-life (days)	0.02-26
Biodegradation half-life (days)	0.11-2.3
K_{ow}	3400
K_{oc}	6607-6900 ml/g
BCF	162-408
Water solubility (mg/l)	12

(Reinert and Rodgers, 1987; Aquatic Plant Identification and Herbicide Use Guide, 1988; USEPA, 1988)

The overall fate of the 2,4-D BEE added to surface waters for Milfoil treatment in granular form is one of initial low concentrations of the ester in the water, appearance of the acid form of 2,4-D in the water shortly thereafter and relatively rapid subsequent decreases in concentration. The appearance of the ester in the water overlying sediments with the 2,4-D BEE associated granules is limited by the low water solubility of this compound, its uptake by water weeds and its rapid hydrolysis (Birmingham et al., 1981). Other recognized breakdown forms of the ester in water are butoxyethanol and 2,4-D acetate, and 2,4-dichlorophenol (Hoeppel and Westerdahl, 1983). After granular BEE application at the label rates to artificial ponds, maximum BEE concentrations in water were 0.16 and 0.68 mg/L one day after treatment and at or below detection limits of 0.01 mg/L after 13-15 days. Maximum concentrations of the acid form of 2,4-D occurred within 15 days and decreased 93% over the next 165 days (Birmingham et al., 1981).

Degradation of 2,4-D in aquatic sediments has been characterized as generally rapid (less than one day) and occurs mostly through microbial biodegradation (HSDB, 1995). Greater concentrations (~4x) of 2,4-D acid than ester occurred in the sediments of BEE treated artificial ponds (23 kg a.i./ha). Concentrations of the ester decreased by 94% over 42 days (Figure III 3-1) (Birmingham et al., 1981).

Table III 3-3. Properties of 2,4-D DMA

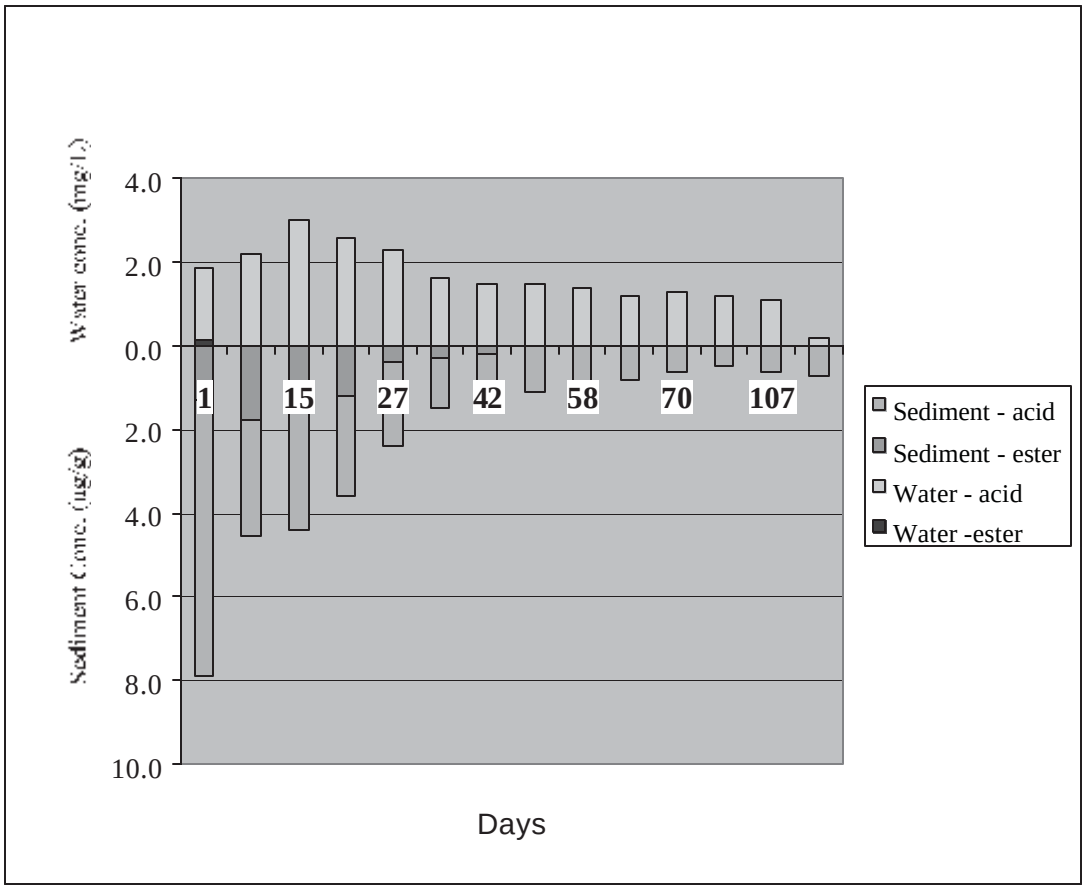
CAS #:	2008-39-1
Molecular formula	$C_{10}H_{13}O_3Cl_2N$
Molecular weight (g/mole)	266.12
Physical properties	white crystalline solid
Melting point (^oC)	85-87
Density	NA
Vapor pressure (mm Hg)	10^{-6} at 28^oC
Volatility [Henry's Law constant (atm m^3/mol)]	insignificant
Photolysis half-life (days)	insignificant
Hydrolysis half-life (days)	insignificant
Biodegradation half-life (days)	3.9-11 (based on overall half-life)
K_{ow}	low
K_p	0.13-0.25
BCF	1-7
Water solubility (mg/l)	3.0×10^6

(Reinert and Rodgers, 1987; Aquatic Plant Identification and Herbicide Use Guide, 1988; USEPA, 1988)

Mobility/Leaching:

A potential route of 2,4-D migration after application to lakes would be from the lake water into sediments and then into groundwater underlying the lake. This section evaluates the likelihood of such a process happening. Areas most likely to be subject to this flux are those where there is active recharge of the groundwater from the lake through the sediments and into underlying groundwaters. This situation may not exist in all lakes, only those that have significant contributions of lake water to groundwaters such as is found in many of the Atlantic Coastal Plain ponds on Cape Cod and possibly in southeastern Massachusetts. Some of these ponds receive the majority of water inflow from groundwater and have little surface water discharge, as Lake Water exits back through more porous pond sediments. Under these flow conditions, the likelihood of 2,4-D in sediments leaving with groundwater will be a function of the form of the 2,4-D, its water solubility and affinity for the sediments, and the organic content of the sediments.

Figure III 3-1. 2,4-D Decay and Partitioning in Experimental Ontario Ponds. Prepared from Data of Birmingham et al. (1981)



Information on water solubilty and soil (not sediment) affinity of 2,4-D can be used to make some conjectures about the likely fate of 2,4-D in situations where it has been applied to surface waters. The BEE form of 2,4-D is fairly water insoluble. The acid form of 2,4-D is quite water insoluble. A number of older studies summarized by Kelty (1980) demonstrated that organic carbon content of soils was a factor controlling leaching in terrestrial soils. Drenge (1969, cited in Kelty, 1980) found that 2,4-D acid or salt easily leached from permeable soils (sandy loam New Mexico soil). Opposing perspectives on leaching potential of 2,4-D acid were highlighted (Weber, 1970 and Liu and Cibes-Viade, 1973 cited in Kelty, 1980). 2,4-D acid movement in subsurface water was negligible (concentrations < 1 ug/L) after application of the acid to a small agricultural watershed in a sandy coastal plain soil. However, the author noted that the potential for losses in subsurface waters was greatest when it rained soon after herbicide application. Weber (1980) only cited one study in which 2,4-D application to ponds adversely affected water quality. The pond, groundwater and rivers in an area where 2,4-D had been used for weed control all had persistent (2.5 mo.) concentrations of 2,4-D. Groundwater concentrations did not appreciably decrease over the monitoring period.

Therefore, leaching from sediments into recharging groundwater would not be predicted based on the low water solubility of 2,4-D BEE and acid. In sandy, low organic content sediments, leaching would be facilitated by the low content and porous material. Field sediment and soil monitoring has documented 2,4-D migration into underlying groundwaters in such situations.

Biological Concentration:

The ability of 2,4-D and its derivatives to bioconcentrate in aquatic organisms is determined by its chemical formulation. One source indicates that there is little evidence that bioconcentration of 2,4-D acid occurs through the food chain. This conclusion was reached after a large-scale monitoring for 2,4-D residues in the many routes of metabolism and degradation that exist in ecosystems (Gray *et al.*, 1983 as cited in HSDB, 1995).

Bioconcentration factors for zooplankton after field exposure to either the butoxyethanol ester or the dimethylamine salt of 2,4-D ranged from 1 to 603 (Reinert and Rodgers, 1987). The maximum BCF in game and nongame fish after application of 2,4-D BEE at 22.5 and 45 kg/ha (~ maximum 0.68 mg/L 2,4-D in water) was approximately 22. Filter-feeding organisms may be more predisposed to accumulating 2,4-D in areas treated with granular BEE, but excretion is rapid and complete in less than 2 weeks (Hoeppel and Westerdahl, 1983).

Whole body 2,4-D BEE nonequilibrium BCF values were found to be very low, ranging from 2-14 in channel catfish (*Ictalurus punctatus*) and 6-12 in bluegill sunfish in aquaria. The ester was quickly hydrolyzed to the acid form and then rapidly excreted (Rodgers and Stalling, 1972 as cited in Reinert and Rodgers, 1987). Nevertheless, several estimates of the bioconcentration factor (BCF) for 2,4-D BEE formulations were made, varying by study and organism, ranging from 162-408 (Reinert and Rodgers, 1987). The bioconcentration potential for 2,4-D DMA formulations has consistently been shown to be low (Reinert and Rodgers, 1987).

MAMMALIAN TOXICITY OF 2,4-D ACID

The following subsections on mammalian toxicity are a summary of the detailed review of the toxic effects of 2,4-D by Harnois (1999). Harnois (1999) includes additional details including citations for the stated effects referenced in this summary.

Overview:

The extensive data from studies on the toxicity of 2,4-dichlorophenoxyacetic acid (2,4-D) and its derivative forms (e.g., salts, amines, esters) have been periodically reviewed by panels of experts. The most recent reviews were made during 1996 and 1997 by the United Nations Food and Agriculture Organization and World Health Organization (FAO/WHO), the United States Environmental Protection Agency (EPA) and the California Environmental Protection Agency (CALEPA).

Noncancer Effects:

In these recent reviews, noncancer effects were found to be similar in all species tested, although dogs were more sensitive for some effects. In almost all cases, derivatives of 2,4-D (amines, salts, esters) produced essentially the same effects as 2,4-D alone. The exception relates to exposure to the butanol portion of 2,4-D n-butyl ester, which was reported to lead to a form of ataxia not seen following exposure to 2,4-D or with other derivatives.

For noncancer effects, the Massachusetts Department of Environmental Protection (DEP) and EPA previously estimated that ingestion of 1 mg/kg/day over a lifetime would be without adverse effects in animals. The more recent reviews by EPA, FAO/WHO and CALEPA support this exposure level as an appropriate No Adverse Effects Level, or NOAEL, for 2,4-D. To derive a recommended human exposure limit protective against noncancer effects, all these agencies have applied a total uncertainty factor of 100 to

this NOAEL to account for differences between humans and animals and for differences in sensitivity between humans. This yields a recommended limit for human daily intake (Reference Dose) of 0.01 mg/kg/day for 2,4-D.

Cancer Effects:

Cancer effects have been studied in laboratory animals in controlled experimental studies using 2,4-D or its derivatives at several dose levels. Potential carcinogenic effects in humans have been investigated by studying populations that either produced or used 2,4-D or its derivatives. In all cases the people studied were also exposed to other potential carcinogens as well. Epidemiology studies now in progress may provide additional insight into any relationships between toxic effects and exposure to 2,4-D alone.

Animal Studies

Experiments on the potential carcinogenic activity of 2,4-D have been performed using mice, rats, and dogs. The results for studies in mice have been consistently negative. A single study in rats (reported in 1986) yielded results suggestive of a 2,4-D dose related increase in the incidence of brain tumors (astrocytomas). A more recent study using the same strain of rats (reported in 1995) and even higher doses showed no increase in total tumors or in tumors of any type. Beagle dogs fed 2,4-D for up to two years showed no indications of cancer effect. Because the normal lifespan of dogs is longer than 2 years, the exposure duration in this study was not adequate to demonstrate that 2,4-D is not carcinogenic in this species.

Based on these data, EPA has concluded that 2,4-D is not carcinogenic in technically acceptable rat and mice studies. No further studies were recommended.

Human Epidemiology Studies

Human epidemiology studies to date have been inadequate to causally relate 2,4-D and cancer effects. This is largely because the populations studied were also likely to have been significantly exposed to other potential carcinogens in addition to 2,4-D.

A potential association between 2,4-D exposure and the development of non-Hodgkins lymphoma has been reported. However, 2,4-D was not identified as the cause of this increase. Additional studies on the use of 2,4-D (and other chemicals) by farmers are in progress and may provide more specific data on chemicals associated with non-Hodgkin's lymphoma and other cancers.

At this time, the epidemiology data are inadequate to support a decision on 2,4-D's carcinogenic potential in humans.

Studies on Effects that May Be Related to Carcinogenicity

Many, but not all, genotoxic chemicals are also carcinogens. Genotoxicity and mutagenicity have been used as a predictor of carcinogenic potential. The results of genotoxicity tests on 2,4-D and derivative compounds vary, depending on the test endpoint. 2,4-D and similar compounds have been inconsistently mutagenic, but have shown more consistent evidence for interference with the distribution of chromosomes in several species. Some of these compounds were able to cause breaks in single strands of deoxyribonucleic acid (DNA) as well. EPA and its Carcinogenicity Peer Review Committee concluded that 2,4-D was not mutagenic but did note that some cytogenetic effects were seen; FAO/WHO concluded that 2,4-D was not genotoxic. None-the-less DEP believes that the current body of data indicate that 2,4-D is genotoxic due to its ability to alter DNA fine structure and composition in cells.

2,4-D is also a peroxisome proliferator. For some chemicals, this characteristic has been associated with a chemical's ability to enhance cellular proliferation and to increase the incidence of tumors in rodents, especially in the liver of mice. 2,4-D can induce peroxisomes in liver, but has not significantly increased the incidence of liver tumors in animal bioassays. It also does not exhibit an ability to transform cultured hamster cells or promote tumor growth in live mice also treated with urethane or naturally infected with mouse leukemia virus. The review panels noted that 2,4-D could enhance peroxisomal proliferation but concluded that the data were inadequate to relate this to carcinogenic potential in humans. Since 2,4-D is unlike the carcinogenic peroxisome proliferators in several respects, 2,4-D should not be considered a potential human carcinogen just because it is a peroxisome proliferator in rodents.

2,4-D has also been tested for ability to affect immune responses. The data from both animal tests and human studies on immune function indicate that there is no consistent effect associated with exposure to 2,4-D, either as a stimulant or as a suppressor. Additional testing for immunotoxic effect is needed since not all endpoints have been evaluated.

Conclusions on Potential for Carcinogenic Effect for Humans

- Technically adequate studies in rats and mice have now completed EPA's requirements for carcinogenicity testing. These studies failed to demonstrate a carcinogenic effect from exposure to 2,4-D.

- Human epidemiology studies have provided evidence that use of herbicides may be associated with increased tumors, but there is inadequate data to show that 2,4-D or its derivative forms are responsible for tumors in humans.

- EPA and WHO have concluded that 2,4-D is currently not classifiable with respect to human carcinogenic effect because of inadequate data from epidemiology studies.

Regulatory Implications

No increase in tumors or noncancer adverse effects were noted when animals were exposed to 100 times the human oral Reference Dose. Average daily intakes at this level are therefore not likely to increase risk of cancer in humans. Based on current information, the Reference Dose is recommended as a basis for deriving environmental guidelines expected to be protective against both noncancer and cancer effects.

MORE DETAILED SUMMARY OF PHARMACOKINETICS AND TOXIC EFFECTS

Data from the various animal tests and from human observations are summarized below (Harnois, 1999). Harnois et al, 1999 provides additional details including reference citations for the stated effects.

Pharmacokinetics:

2,4-D is well absorbed after oral, dermal, or inhalation exposure. 2,4-D is excreted in the original form, as are 2,4-D isopropylamine and 2,4-D triisopropylamine. The sodium salt and simpler amines are excreted as 2,4-D. The butoxyethyl ester and the ethylhexylesters are hydrolyzed to 2,4-D and the respective sidechains. The 2,4-D moiety is assumed to be a biochemically active element in the body since it is not metabolized further. The side chains of the esters are metabolized further and may contribute to the toxic effects seen in some cases.

At low exposure levels, absorbed 2,4-D is bound to carrier proteins (e.g., albumin, thyroxine carrier protein) in the blood; at higher doses, these carriers are saturated. Free 2,4-D is available to pass into the body tissues; in pregnant women it can also pass through the placenta into the fetus. Even at high concentrations, the fetus could still be protected if it had developed far enough to have its own carrier proteins in plasma.

2,4-D is more soluble in water than in oils and fats. The distribution in the body is related to this relative solubility. Two to eight hours after rodents were given an oral dose, 2,4-D was highest in blood, liver and kidneys of rodents of both sexes, and in ovaries of females. Intermediate quantities were present in skin, muscle, and in the male, the testes; fat and brain had the lowest concentrations.

Most of the 2,4-D is excreted through the kidney and in some cases, in perspiration. Lesser amounts are excreted in feces and milk. At doses higher than 50 mg/kg, mice and rats display a biphasic urinary clearance pattern, indicating that the clearance pathway in the kidney is saturable. After an oral exposure, about 50% of the administered dose was cleared in 1 hour (half-life equals 1 hour). In the second phase, the half-life for 2,4-D was 18 hours. At low doses (around 5 mg/kg), about 5% is transferred to bile in the liver and excreted in feces. At doses above 50 mg/kg, when urinary clearance is saturated, 15% or more was transferred to bile and excreted in feces. This biliary excretion pathway may be facilitated by conjugation of the 2,4-D with amino acids in the liver. None of the conjugate forms detected were associated with toxic effects.

At this time, the half-life of 2,4-D ingested by humans is not well defined; the few people tested have shown a high variability in response; the basis for this variability is not yet determined since few people (mostly young adult, physically active men) have been studied. The half-lives range from 4 to 48 hours, depending on the individual and on the administered dose. Even when a relatively low dose of 5 mg/kg was tested, one individual in the three subjects of one test showed a biphasic clearance pattern. The half-life for the first phase was 4 hours; for the second phase, 16 hours. Additional observations are needed if the 2,4-D half-life for humans is to be more accurately defined. The half-life of 2,4-D after dermal exposure (the usual route of human exposure) is even less well defined since it is affected by duration of contact and under working conditions, repeated exposures.

Acute Exposure Effects:

Acute exposures are one-time exposures that take place within a day. Acute exposure tests were used to determine if death or clinical signs could occur within a few weeks after absorption of 2,4-D into the body after ingestion, inhalation, or dermal exposure. Possible effects on skin and eyes from direct contact with 2,4-D were also tested.

Ingestion of 2,4-D results in effects within minutes; these effects include decreased activity, muscular weakness, uncoordinated movements, and possibly excess salivation and urination. At higher doses, the immediate effects can include vomiting, which can decrease the 2,4-D available for absorption into the body. Liver and kidney damage become evident within a few days; death may also occur within a few days. Those animals that do not die recover to function normally in time since 2,4-D is not stored in the body.

Oral doses which kill half the experimental animals in 14 days (LD50s) vary between species and show a broad range even within species. LD50s for beagle dogs ranged from 25 to 250 mg/kg; for rats, the LD50 range was 607 to 980 mg/kg. Human lethal doses vary, depending on the retained dose in suicide attempts. At least one death resulted from ingestion of 80 mg/kg but another person survived a dose of 700 mg/kg (plasma concentration, 392 mg/l) with the help of prompt medical attention, which included induced vomiting and alkaline diuresis therapy. This survivor recovered within 5 weeks and had no observed residual effects.

2,4-D on the skin is less toxic than ingested 2,4-D since absorption through skin is slower than absorption from the stomach. At low skin concentrations, the slower rate of absorption results in a more gradual increase in plasma concentrations of 2,4-D; the rate of removal from plasma could exceed this gradual rate of increase, resulting in no accumulation of 2,4-D in the plasma or body organs. In laboratory animals, the LD50 by this route is greater than 2000 mg/kg. Human exposure to 2,4-D in its various formulations is mainly by dermal exposure when workers are spraying it to control weeds. For humans, toxicity is enhanced when 2,4-D is allowed to remain on the skin or on clothing in contact with the skin. Clinical signs after a dermal exposure are the same as those resulting from ingestion of 2,4-D with the addition of skin irritation and lesions from prolonged skin contact. There are no reports that 2,4-D can cause allergic dermatitis or other types of sensitization.

Eye contact with 2,4-D (acid form) in laboratory animals resulted in corneal opacity, conjunctival edema and redness, ocular discharge, and inflammation of the iris; the effects persisted in rabbits for at least 20 days. Less toxicity and quicker recovery was noted after exposure to esters of 2,4-D.

Few studies are reported for effects after inhalation of 2,4-D. While it is not highly volatile, exposure by that route could occur when the herbicide is sprayed into the air to treat large land areas and in some industrial settings. Field workers applying 2,4-D to control weeds in crops have had symptoms of respiratory irritation (burning sensation in the throat and chest), loss of appetite, weight loss, and weakness.

Subchronic And Chronic Exposure Systemic Effects:

Experimental animal tests showed that 2,4-D is not accumulated or stored in the body but that exposure to low doses over several months can lead to progressive tissue damage, increasing the frequency and severity of effects seen in the liver, kidney, and brain. Additional adverse effects noted in animals included decreased serum thyroxine and hypertrophy of the thyroid, decreased serum glucose, cataracts, and retinal degeneration. Animals seemed to adapt in that the neurotoxic effects decreased with continued exposure. Systemic effects in surviving humans and animals were generally reversed within weeks when the 2,4-D exposure was stopped.

Genotoxic Effects:

Results of tests for genotoxicity were variable. Tests that measured mutations were usually negative; tests that measured breaks in individual DNA strands were positive; tests which detected defective distribution of chromosomes were often positive. Newly reported studies using exposure of larval forms of fruit flies to 2,4-D showed that both the treated insects and their offspring displayed new characteristics resulting from mutations and chromosome rearrangements. Since substantial evidence indicates that 2,4-D can induce chromosome changes in plant, insect, and mammalian cells, 2,4-D should be considered genotoxic.

Immunologic Effects:

Several epidemiology studies addressed possible immunological effects and exposure to 2,4-D. Although some effects were observed between use of herbicides and alterations in immunological parameters, no causal relationship was noted between 2,4-D and either overstimulation or suppression of the immune response. There are only limited data on the immunotoxic potential of 2,4-D and additional studies are needed to complete the current standard battery of immunotoxicity tests.

Neurotoxic Effects:

Observations made on neuromuscular endpoints in animals treated for a year showed that they were less able to relax their muscles after strongly gripping a test object (myotonia). Other signs of neurological

effects included sedation, uncoordinated movement, and decreased locomotor activity; these effects were also observed in subchronic and chronic exposure studies. These effects were reversed after treatment ended. In some studies, rats showed lesser effects as dosing continued, suggesting a possible adaptation.

Rats given 2,4-D-n-butyl ester showed increased foot splay when landing during the functional tests. This effect was also seen when n-butanol was administered, but not when other forms of 2,4-D or other butanols were administered. It was related to dose of n-butanol. This ester could have additional neurotoxic properties in humans not seen with other forms of 2,4-D.

Retinal degeneration was the most severe neurotoxic effect observed when rodents were given lower doses of 2,4-D in their diet for either a subchronic or chronic exposure duration. Females were more susceptible than males.

No human epidemiology studies were found to show a lasting specific 2,4-D effect on the nervous system.

Biochemical Effects:

Consistent effects of 2,4-D on the endocrine system have been reported including decreases in serum glucose and serum thyroxine.

Depletion of detoxifying molecules, such as glutathione and thiols, have also resulted from exposure to 2,4-D. These molecules are involved in the stabilization of cell microstructure by acting alternately as hydrogen donors and acceptors. One of the cell microstructures facilitates the distribution of chromosomes in cell division; interference with its normal function can result in genotoxic effects.

2,4-D stimulates the activity of oxidative enzymes associated with microsomes. It also stimulates activity of other small cytoplasmic particles, the peroxisomes. Increased peroxisome activity leads to a greater release of active oxygen, which can be genotoxic. Chemicals which enhance peroxisome proliferation are not necessarily carcinogens, but those which are carcinogens readily induce liver tumors in rodents. 2,4-D has not induced liver tumors in rodents or shown other activity which can enhance the incidence of tumors: it has not acted as a tumor promoter for pre-treated or virus-infected cells, nor has it stimulated uncontrolled proliferation of cultured normal cells. It is unlikely that 2,4-D would be carcinogenic because it is a peroxisome proliferator.

Reproductive Effects:

Exposure of rats for two generations resulted in no effect on ability to reproduce at doses that were otherwise nontoxic to the animals.

Most of the human epidemiology studies have studied effects in men; these studies have not shown that there were effects specifically from 2,4-D. Studies currently in progress do include significant numbers of women. Results from these studies, if specific to 2,4-D, would provide useful information on the potential of 2,4-D for reproductive effects.

Developmental Effects:

Pregnant rats and rabbits exposed to doses of 2,4-D which did not cause them to be severely ill were able to deliver normal litters, indicating that there were no teratogenic effects directly related to 2,4-D in these tests.

Children born to men who worked as pesticide applicators in the United States were observed for congenital abnormalities. Aborted fetuses and stillborn infants were not observed in the study, so all types of developmental effect were not included in the study. There was an increase in congenital abnormalities in live-borne children of couples residing in the farm area and a greater increase in children of pesticide appliers, especially for those who worked on multiple crops. As with other epidemiology studies, the association between chemical exposure and effects seen could not be attributed to 2,4-D alone.

Cancer Effects:

Animal Studies.

A very early study in mice (Innes, 1969) indicated that 2,4-D, 2,4-D isopropyl ester, 2,4-D butyl ester, 2,4-D isooctyl ester, alpha-(2,4-dichlorophenoxy)propionic acid, and 2-(2-4,-dichlorophenoxy)propionic acid were negative for cancer effect when tested at the maximum tolerable dose. These results for 2,4-D were confirmed in later, more detailed, cancer studies.

Tests in rats and mice on structurally related compounds were included in the review by the EPA Cancer Peer Review Committee (Rowland et al., 1997). 2,4-Dichlorophenoxy butyric acid, 2,4-dichlorophenoxy-2-propionic acid, 4-chloro-2-methylphenoxyacetic acid were not carcinogenic in rats and mice. 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) containing less than 0.05 ppm total dioxins increased the number of tumors in one strain of mice (XVII/G) but not another (C3Hf); dioxin-free 2,4,5-T was not carcinogenic in rats.

Results of a later study (1986) on 2,4-D administered to rats in their diet at doses of 0, 1, 5, 15, and 45 mg/kg/day suggested that the frequency of brain tumors (astrocytomas) was elevated in the high dose males (Table III 3-4). The incidences in males by dose group were 1/50, 0/50, 0/50, 2/50, and 6/50, respectively. There was a significant dose-related trend in the males.

The test was repeated (1995) in the same strain of rat at doses of 0, 5, 75, and 150 mg/kg/day. The frequency of astrocytomas was much lower in this study: 0/50 in male controls and 1/50 in high dose males; 1/50 in control females and 1/50 in high dose females. No tumors were found in the 5 and 75 mg/kg/day animals, but not all animals in the two intermediate groups were examined. The EPA Cancer Peer Review Committee has requested that brain tissue from all animals in the intermediate doses be examined histologically to complete the study. The results of this follow-up are not yet available.

Testing facilities record the observations on control group animals in a database. Observations in controls in subsequent studies are compared to this historical control database to ascertain if the source or lot of animals, or other conditions unrelated to treatment, have influenced the outcome of the study. No data were presented on animals at the testing facilities at which the 2,4-D feeding studies were conducted. However, EPA did compare the control and treated animals to the historical controls in the National Toxicology Program (NTP) database (Table III 3-4). The 2,4-D studies and the NTP studies were all done at different facilities. NTP rats were from various sources, but did not include the Charles River Laboratories, Portage MI, which was the sole source of the animals used in both of the 2,4-D feeding studies. This suggests that the background incidence in NTP rats may not be appropriate for use as historical controls. In this case, comparison should be to the historical control database at the testing facility, which are presently not available.

Adequate tests in two animal species are all EPA requires in its battery of animal toxicology studies used for regulatory purposes. EPA concluded that 2,4-D was not carcinogenic in the technically acceptable rat and mice studies. No further studies were recommended. There was no indication of

potential for cancer induction in the studies before 1986 on 2,4-D and on its derivative forms. The rodent studies thus provided no conclusive evidence that 2,4-D was a carcinogen.

Dogs allowed on lawns freshly treated with 2,4-D could have a relatively high intake of 2,4-D (maximum urinary excretion of 21.3 mg/liter urine two days after lawn treatment) and may be useful environmental sentinels under controlled conditions. Lymphoma frequency in dogs was studied in an effort to test this idea. Owners of dogs brought to veterinary clinics with lymphomas and other diseases (control dogs) were asked about use of pesticides and other environmental factors at their residence. Those dogs with lymphomas were found to have a possibly greater exposure to lawn treatment chemicals than other dogs, but the exposure to 2,4-D could not be separated out from exposure to other chemicals.

In laboratory studies, beagle dogs exposed to only 2,4-D in their diet for 2 years did not show evidence of tumor development.

Table III 3-4. Astrocytomas of the Brain in Rats Fed 2,4-D Acid

Astrocytomas of the brain in rats fed 2,4-D acid										
Sex	Males					Females				
1986 study at Hazleton Laboratories Dose (mg/kg/day)	0	1	5	15	45	0	1	5	15	45
Astrocytomas (after 2 years)	1/50 2%	0/50 0%	0/50 0%	2/48 4%	6/50 12%	0/50 0%	1/50 2%	2/50 4%	1/50 2%	1/50 2%
1995 study at Dow Chemical facility Dose (mg/kg/day)	0		5	75	150	0		5	75	150
Astrocytomas (after 2 years)	0/50 0%		0/26	0/18	1/50 2%	1/50 2%		0/14	0/11	1/50 2%
Background in historical controls (NTP: 3 other laboratories)										
Range	Males: 0-4.4%					Females: 0-4%				
Mean	Males: 0.4%					Females: 0.3				

Human Studies.

Some reports suggest a possible association between exposure to 2,4-D and the development of tumors. Different populations of agricultural workers who applied chemicals, including 2,4-D, to crops showed increased incidence of either soft tissue sarcoma or non-Hodgkin's lymphoma. However, none of the numerous epidemiological studies on the association between 2,4-D and various forms of cancer adequately demonstrate that the association is specific for 2,4-D. The populations studied had significant exposure to other chemicals, either as background exposures over their lifetimes living in rural agricultural areas or at the same time as the 2,4-D exposure. In many cases, increases in tumor incidence were not statistically significant.

Human epidemiology studies have provided evidence that use of herbicides may be associated with increased tumors, but there is inadequate data to show that 2,4-D or its derivative forms is responsible for these tumors. Additional studies are in progress to address deficiencies in those currently available for review.

EPA and WHO have concluded that 2,4-D is currently not classifiable with respect to human carcinogenic effect because of inadequate data from epidemiology studies.

Quantitative Evaluation:

Dose-response for Noncancer Effects:

In chronic exposure tests on animals, effects other than cancer were observed at lower average daily doses than were effects suggestive of cancer. The relationship between average daily dose and the effects seen (dose-response) can be estimated for noncancer effects.

EPA had previously accepted a NOAEL of 1 mg/kg/day 2,4-D (or equivalent) based on absence of liver, kidney, and hematopoietic effects which were seen at higher doses in a subchronic effect study on rats. Their current evaluation is based on a NOAEL of 1 mg/kg/day for absence of systemic effects in a 1-year study in dogs.

The WHO summary shows that the lowest NOAEL of 1 mg/kg/day 2,4-D is based on this same dog study and on absence of noncancer effects (kidney lesions) in the 1986 2-year rat study discussed above.

Both agencies used a total 100-fold adjustment or uncertainty factor to extrapolate to humans and to account for differences sensitivity between people. Based on this approach both agencies estimated that a human reference dose protective against noncancer effects would be 0.01 mg/kg/day 2,4-dichlorophenoxy acetic acid (or equivalent in the salts, amines, or esters).

Evaluation of Carcinogenic Potential:

The available data from animal carcinogenicity studies suggest that 2,4-D would not be a carcinogen for humans. Limited data indicate that several other phenoxy herbicides, including those having 2,4-D as a biologically active moiety, were not carcinogenic when tested in mice and rats.

2,4-D is both a genotoxin and a peroxisome proliferator in animals, but these characteristics are not always related to carcinogenic potential. 2,4-D has not caused a significant increase in the incidence of rodent liver tumors (a characteristic of carcinogenic peroxisome proliferators) or of having the capability to promote the carcinogenic effect of other chemicals or viruses.

Human epidemiological studies have not provided adequate data for evaluation of 2,4-D as a human carcinogen since they include exposure to other chemicals; other studies currently in progress could provide a more accurate characterization of 2,4-D's effect on both men and women. EPA has concluded that 2,4-D is not classifiable as to human carcinogenicity since the human epidemiology data are still being clarified.

Since cancer effects were not observed in animals at even 1 mg/kg/day 2,4-D, exposures at the Reference Dose of 0.01 mg/kg/day are not expected to cause a significant increase in the risk of human cancer.

Available Toxicity Criteria:

The EPA RfD/RfC Workgroup has developed an oral Reference Dose (RfD) of 0.01 mg/kg/day for 2,4-D based upon a 90-day rat oral bioassay and a 1-year interim report from a 2-year rat bioassay. The RfD is an estimate, with uncertainty spanning perhaps an order of magnitude, of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (USEPA, 1992).

The EPA Office of Pesticide Programs (OPP) has developed an RfD of 0.003 mg/kg/day based on a two-year feeding study in rats (USEPA, 1995).

The EPA has developed a Maximum Contaminant Level (MCL) for drinking water for 2,4-D of 0.07 mg/l (USEPA, 1988). This drinking water level has been adopted by Massachusetts as a Massachusetts Maximum Contaminant Level (MMCL).

The EPA has also developed an Ambient Water Quality Criterion (AWQC) for 2,4-D of 0.001 µg/l. This concentration represents an acceptable level of 2,4-D in ambient water for a human who drinks the water and eats fish who inhabit the water (USEPA, 1988).

ECOLOGICAL TOXICITY

Avian:

A number of toxicity tests conducted in birds indicate that 2,4-D is only slightly toxic or practically nontoxic to most test birds. LC50 values (i.e., concentrations lethal to 50% of the test population) for ring-necked pheasant, Japanese quail, Bobwhite quail and mallard ducks to be greater than 5,000 ppm for 2,4-D, the butoxyethanol ester of 2,4-D and the dimethylamine salt of 2,4-D (USDA, 1984). Reported LD50 values for 2,4-D acid include an oral dose of 668 mg/kg for rock doves, an oral dose greater than 2,000 mg/kg for 4 month old mallards and an oral dose of 668 mg/kg for 2 month old mallards (HSDB, 1995). 2,4-D was found to be moderately toxic to 4 month old chukar (partridge) with a reported LD50 value of 200-400 mg/kg (HSDB, 1995).

In terms of reproductive or developmental effects, spraying chicken eggs with 2,4-D amine 29 hours before incubation at rates of 1, 10 and 20 times the recommended rates had no adverse effect on any parameter used to evaluate either incubation or subsequent live performance (USDA, 1984). Two studies indicated that spraying eggs of quail, pheasants and chickens with 2,4-D in concentrations up to 10 times the recommended doses, produced no effect on the hatching rate, body weight, sexual differentiation or reproductive performance (as adults) of number of malformed chicks (GEIR, 1985). In another study in which 2,4-D amine was sprayed at a concentration of 1.1 kg active ingredient per hectare on fertile eggs, 77% of ring-necked pheasant embryos, 43% of red partridge embryos and 77% of grey partridge embryos were dead on the nineteenth day of incubation. Surviving embryos were malformed or partially or completely deformed (GEIR, 1985).

Aquatic Organisms:

The toxicity of 2,4-D has been shown to vary with the formulation, the species of fish, the water quality and the environmental conditions (season and temperature). According to several sources, many of the formulations, especially the esters, are toxic to fish (GEIR, 1985). Hoeppel and Westerdahl (1983) cited numerous studies showing ester formulations of 2,4-D and butoxyethanol to be 50-200 times more toxic to fish than the free acid or dimethylamine salt of 2,4-D. 96-Hour LC50 values for fish are in the

0.6-7 mg/L range. The bluegill is the most sensitive of the species with compiled data and the salmonids are also quite sensitive with LC50s in the lower part of the noted range.

Numerous studies show that the 96-hour LC50s for cutthroat trout fingerlings ranged from greater than 50 ppm for the isooctyl ester down to 0.78 ppm for the butyl ester (USDA, 1984). 96-hour LC50s for the dimethylamine salt were reported as 64 ppm for cutthroat trout, 100 ppm for chinook salmon and 236 ppm for smallmouth bass. A 96-hour LC50 of 1313 ppm for grass carp was also reported for the amine salt (GEIR, 1985). 96-hour fish LC50s for the 2,4-D acid are generally higher, ranging from a slightly toxic 26.7 ppm in banded killifish to a practically nontoxic 358 ppm in rainbow trout.

Table III 3-5 lists the results of a number of 96-hour acute toxicity assays using 2,4-D acid or the butoxy ethyl ester. The acute lethal toxicity (96 hour LC50) of the BEE to a variety of salmonids increases by factors of 2.8-4 times as the water pH decreases (Wan et al., 1990).

Many studies conducted to assess the effects of 2,4-D on lower aquatic organisms suggest that toxicity varies with the different formulations of 2,4-D (GEIR, 1985). Again, it appears that some ester formulations are the most toxic. A 96-hour LC50 was reported as 6.1 ppm for scud and 2.6 ppm for sowbug exposed to the butoxyethanol ester of 2,4-D. Results of other lower aquatic organisms exposed to various 2,4-D esters were similar (USDA, 1984). 2,4-D was only slightly toxic to *Daphnia*, with a 48-hour acute LC50 of 25 ppm (Dow Elanco, 1995). 48-hour TL50s (concentrations at which there is some toxic effect to half the population) of 100 ppm were reported for many crustaceans exposed to the dimethylamine salt (USDA, 1984). A TL50 greater than 100 ppm was reported in crayfish (USDA, 1984).

The relatively rapid conversion of the BEE to the acid form in water makes interpretation of and application of laboratory toxicity study results to field exposures less than straightforward. In static exposure 96 hour tests much of the parent BEE will have changed to the less toxic acid form over the test duration so that recorded mortalities expressed as a nominal concentration of BEE actually reflect decreasing exposures. In study results from flow-through exposures continually delivering fresh BEE to test vessels over a study, mortality results more accurately reflect constant exposures to the nominal concentrations, but will have lessened quantitative relationship to likely exposures in the field.

It should be noted that LC50s are toxicological benchmarks but convey nothing about the sublethal toxicity characteristics of the chemical. Exposure concentrations in the environment that are less than a 96 hour LC50 value may not represent no risk values.

Observed 2,4-D BEE concentrations in the water after application of the 2,4-D BEE to ponds (see section above) peak within a day at less than 0.2 mg/L and decrease within 15 days to 0.01 mg/L (see Section on Environmental Fate and Transport). Both of these concentrations are less than the 96 hour LC50s of the more sensitive aquatic species, but possibly within the range where sublethal effects might occur as the concentration is only a third that of the LC50 of the lowest LC50 value that has been identified.

The acid form of 2,4-D is quickly formed from the ester and is present for much longer at higher concentrations. Highest acid form concentrations over any 96-hour period would be in the range of 3 mg/L. However these types of concentrations (2-3 mg/L) could persist in the water for four to five weeks, representing a considerably longer exposure than that used in standardized aquatic toxicity tests.

This information suggests that there would be the potential for some acute lethal and sublethal toxicity to aquatic species in the days immediately following application, due to the action of the butoxyethyl ester form of 2,4-D. Further lethal toxicity seems unlikely after that because the 2,4-D will have changed to the acid form in concentrations that should be from 10 to 100 times less than the 96 hour

LC50s for aquatic species. However, given that the exposures could persist for many days longer than those in laboratory toxicity tests, the possibility of mortalities or sublethal effects cannot be ruled out. This review has not sought to comprehensively identify the universe of data which may exist on chronic exposures and sublethal toxicity of aquatic species to 2,4-D; therefore the potential for this type of toxicity remains an open question. For comparison, the reproductive performance of female crabs was adversely affected after chronic exposure to 2,4-D isobutoxyethanol ester at a concentration of 50 mg/L which was near the incipient lethal level for this compound and species (Rodriguez et al. (1994). The observed decrease in oocyte sizes is a consistent reflection of the type of outcome which might be associated with the recognized mode of action of this chemical: uncoupling of oxidative phosphorylation in the respiratory chain.

Table III 3-5. 96 Hour LC50 Aquatic Toxicity Tests Using 2,4-D

Form	Species	Conc.(ppm)	Reference
Acid	rainbow trout	358	Dow Elanco, 1995
	fathead minnow	320	Dow Elanco
	american eel	300.6	HSDB, 1995
	bluegill	263	Dow Elanco, 1995
	carp	96.5	HSDB, 1995
	pumpkinseed fish	94.6	HSDB, 1995
	guppy	70.7	HSDB, 1995
	striped bass	70.1	HSDB. 1995
	cutthroat trout	64	USDA, 1995
	white perch	40	HSDB, 1995
	Banded killifish	26.7	HSDB, 1995
	Daphnia	25,36.4	Dow Chemical Co. , 1983a.
BEE	Rainbow trout	2.0	Dow Chemical Co., 1983b
	Bluegill	0.6	Dow Chemical Co., 1983b
	Daphnia	7.2	Dow Chemical Co., 1983b
	Fathead minnow	2.5	Dow Chemical Co., 1983b
	Coho salmon Chinook salmon Chum salmon Pink salmon Sockeye salmon Rainbow trout	0.6-4.3	Wan et al., 1990

Plants:

Treatment of a water body with 2,4-D may cause depletion of dissolved oxygen from decomposition of dead weeds as well as habitat loss (Riverdale Chemical Co., 1988). Benthic macroinvertebrate species diversity was significantly lower in BEE treated experimental ponds than in control ponds after 338 days (Stephenson and Mackie, 1986).

2,4-D References

Aly, O.M. and Faust, S.D. 1964. Studies on the fate of 2,4-D and ester derivatives in natural surface waters. J Agric Food Chem. 12:541-546.

Aquatic Plant Identification and Herbicide Use Guide. November, 1988. Volume I: Aquatic Herbicides and Application Equipment. Howard E. Westerdahl and Kurt D. Getsinger, eds. Environmental Laboratory. Department of the Army. Vicksburg, Mississippi.

Birmingham, B.C., M. Thorndyke and B. Colman. 1981. The dynamics and persistence of the herbicide Aqua-Kleen in small artificial ponds and its impact on non-target aquatic microflora and microfauna. p. 12-23. In, N.K. Kaushik and K.R. solomon, eds. Proceedings of the Eighth Annual Aquatic Toxicity Workshop: November 2-4, 1981, Guelph, Ontario. Can. Tech. Rep. Fish. Aquat. Sci. No. 1151.

Bjorklund, N. and K. Erne. 1966. Toxicological studies of phenoxyacetic herbicides in animals. Acta. Vet. Scand. 7:364-390.

Blakley, B.R.. 1986. The effect of oral exposure to the n-butylester of 2,4-dichlorophenoxyacetic acid on the immune response in mice. Int. J. Immunopharmacol. 8(1):93-99.

Bloom, Jill. 1996. Office of Pesticide Programs. U.S. Environmental Protection Agency.

Collins, T.F.X and C.H. Williams. 1971. Teratogenic studies with 2,4,5-T and 2,4-D in the hamster. Bull. Environ. Contam. Toxicol. 6(6): 559-567.

Corte-Real, Lee. 1995. Personal communication. Massachusetts Department of Food and Agriculture. Pesticide Bureau.

Courtney, K.D. 1977. Prenatal effects of herbicides: Evaluation by the prenatal development index. Arch. Environ. Contam. Toxicol. 6:33-46.

DFA (Department of Food and Agriculture). December 14, 1988. State Individual Review on 2,4-D. Division of Regulatory Services. Pesticide Bureau.

Diener, Robert M., (ed.). A Comprehensive, Integrated Review and Evaluation of the Scientific Evidence Relating to the Safety of the Herbicide 2,4-D. Journal of the American College of Toxicology. Mary Ann Liebert, Inc., publishers.

Dow Chemical USA. 1983a. The acute lethal toxicity of (2,4-dichlorophenoxy) acetic acid to representative aquatic organisms. Tech. Rep. ES-DR-0002-2297-4 by Envtl. Sciences Research Laboratory. Midland, MI.

Dow Chemical USA. 1983b. The acute toxicity of 2-butoxyethyl (2,4-dichlorophenoxy) acetate to representative aquatic organisms. Tech. Rep. ES-DR-0007-2833-2 by Envtl. Sciences Research Laboratory. Midland, MI.

Dow Elanco. 1995. 2,4-D: 45 Years of Effective Use. Down To Earth. 50(2).

GEIR (Generic Environmental Impact Report). 1985. Control of Vegetation of Utilities and Railroad Rights-of-way. published by Harrison Biotec. Cambridge, MA.

Gorzinski, S.J., Kociba, R.J., Campbell, R.A., Smith, F.A., Nolan, R.J. and Eisenbrandt, D.L. 1987. Acute, pharmacokinetic and subchronic toxicological studies of 2,4-dichlorophenoxyacetic acid. Fund. Appl. Toxicol. 9:423-435.

Gray, DA *et al*. 1983. Update and Revision of Multimedia Criteria Document of 2,4-Dichlorophenoxyacetic acid. Syracuse Res Corp. Syracuse, NY. SRC-TR83-721.

Hammond, Larry. 1995. Industry Task Force Chairman. personal communication.

Hansen, W.H., M.L. Quaife, R.T. Habermann and O.G. Fitzhugh. 1971. Chronic toxicity of 2,4-dichlorophenoxyacetic acid in rats and dogs. Toxicol. Appl. Pharmacol. 20(1): 122-129.

Harnois, M.C. 1999. Toxic effects of 2,4-dichlorophenoxyacetic acid (2,4-D). Draft Report. April, 1999. Massachusetts Department of Environmental Protection. Office of Research and Standards. Boston, MA.

Hazelton Laboratories. 1983. Document Accession Number 251473. U.S. EPA. Office of Pesticide Programs. Washington, DC. Contact Disk Mountford (202/557-1830).

HSDB (Hazardous Substances Database). 1995. Hazardous Substances Database. U.S. Environmental Protection Agency.

IARC (International Agency for Research on Cancer). 1977. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. Some Fumigants, the Herbicides 2,4-D and 2,4,5-T, Chlorinated Dibenzodioxins and Miscellaneous Industrial Chemicals. Vol. 15.

ITF (Industry Task Force). 1986. Combined Chronic Toxicity and Oncogenicity Study in Rats. Industry Task Force on 2,4-D Research Data. Hazleton Laboratories America Inc.: Vienna, Virginia.

ITF (Industry Task Force). 1987. Oncogenicity Study in Mice with 2,4-Dichlorophenoxyacetic Acid. Industry Task Force on 2,4-D Research Data. Hazleton Laboratories America Inc.: Vienna, Virginia.

ITF (Industry Task Force II). 1992. Toxicological Summaries. Correspondence from L. Hammond. June 1, 1992.

Joyce, Joseph C. And Ramey, Victor. August, 1986. Aquatic Herbicide Residue Literature Review. Center for Aquatic Weeds. Institute of Food and Agricultural Sciences. University of Florida.

Kelty, M. P. 1980. 2,4-D: A literature review and statement of position. Unpublished report. O.M.Scott. Scott and Sons Co. Marysville, OH.

Khanna, S. and S.C. Fang. 1966. Metabolism of C-14 labeled 2,4-dichorophenoxyacetic acid in rats. J. Agric. Food Chem. 14(5): 500-503.

Khera, K.S. and W.P McKinley. 1972. Pre- and postnatal studies on 2,4,5-trichlorophenoxyacetic acid, 2,4-dichorophenoxyacetic acid and their derivatives in rats. Toxicol. Appl. Pharmacol. 22:14-28.

Kitchen and Brown. 1988. Toxicol Environ Chem. 16:165.

Konstantinova, T.K., L.P. Ephimenko and T.A. Antonenko. 1976. The embryotrophic effect of the dissociation products of herbicides based on 2,4-D. Gig. Sanit. 11:102-105. (Translation for U.S. EPA by Literature Research Co.)

Lehman, 1952 (cited in Gehring and Betso, 1978 (Ecol Bull 27:122-133))

Miller, Joanne. U.S. Environmental Protection Agency. Office of Pesticide Programs. 1995. personal communication.

Pelletier, O. Ritter, L., Caron, J., and Somers, D. (1989). Disposition of 2,4-dichlorophenoxy acetic acid dimethylamine salt by Fischer 344 rats dosed orally and dermally. J. Toxicol. Environ. Health. 28:221-234.

Reinert, K.H. and J.H. Rodgers. 1987. Fate and persistence of aquatic herbicides. Rev. Envtl. Contam. Toxicol. 98:61-98.

Riverdale Chemical Company. - registration labels for 2,4-D Granules
and Weedestroy AM-40 Amine Salt

Rodgers, C.A. and Stalling, D.L. 1972. Dynamics of an ester of 2,4-D in organs of three fish species. Weed Sci. 20:101-105.

Rowe, V.K. and T.A. Hymas. 1954. Summary of toxicological information on 2,4-D and 2,4,5-T type herbicides and an evaluation of the hazards to livestock associated with their use. Am. J. Vet. Res. 15:622-629.
Santolucito, J. 1975. The use of quantitative EEG for detecting low-level prolonged exposure to pesticides. Proc. Int. Symp. Recent Adv. Assess. Health Eff. Environ. Pollut. 4: 2387-2394.

Schwetz, B., G.L. Sparschu and P.J. Gehring. 1971. The effect of 2,4-D and esters of 2,4-D on rat embryonal, fetal and neonatal growth and development. Food Cosmet. Toxicol. 9: 801-817.

Schulze, G.E., Blake, J.W. and Dougherty, J.A. 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.

Unger, T. M., J. Kliethermes, D. Van Goethem and R.D. Short. 1981. Teratology and postnatal studies in rats of the propylene glycol butyl ether and isooctyl esters of 2,4-dichlorophenoxyacetic acid. Environ. Pollut. Control. 20 p. NTIS PB 81-191140.

USDA (United States Dairy Association). August 1984. Pesticide Background Statements: USDA Forest Service Agriculture Handbook. #633. vol. 1.

USEPA (U.S. Environmental Protection Agency). March, 1988. Final Drinking Water Criteria Document for 2,4-D. Environmental Criteria and Assessment Office. Office of Health and Environmental Assessment. Office of Drinking Water. Washington, D.C.

USEPA (U.S. Environmental Protection Agency). 1989. Pesticide Fact Sheet, 2,4-Dichlorophenoxyacetic Acid (2,4-D). U.S. Environmental Protection Agency, Office of Pesticides and Toxic Substances, Office of Pesticide Programs: Washington, DC, pp. 1-10.

USEPA (U.S. Environmental Protection Agency). 1992. Integrated Risk Information System (IRIS).

USEPA (U.S. Environmental Protection Agency). 1994. An SAB report: Assessment of potential 2,4-D carcinogenicity: Review of the epidemiological and other data on potential carcinogenicity of 2,4-D by the SAB/SAP Joint Committee. Science Advisory March 1994. EPA-SAB-EHC-94-005 Environmental Protection Agency. Washington, DC.

USEPA (U.S. Environmental Protection Agency). 9/10/95. Office of Pesticide Programs Reference Dose Tracking Report.

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, ,4-DP and their formulated product: Weedone CB and its carrier. Bull. Envtl. Contam. Toxicol. 45:604-611.

WSSA (Weed Science Society of America). 1983. Herbicide Handbook. Weed Science Society of America. Champaign, Illinois.

White-Stevens, R. (ed.). 1971. Pesticides in the Environment: Volume 1, Part 1, Part 2. New York: Marcel Dekker, Inc. 43.

Ylitalo, P. Narhi, U., and Elo, H.A. (1990). Increase in the acute toxicity and brain concentrations of chlorophenoxyacetic acids by probenicid in rats. Gen. Pharmacol. 21(5):811-814.

Zepp, R.G., Wolfe, N.L., Gordon, J.A., and Baughman, G.L. 1975. Dynamics of 2,4-D esters in surface waters; hydrolysis, photolysis and vaporization. Environmental Science and Technology. 9:1144-1149.