Herbicide Risk Assessment for the Aquatic Plant Management Final Supplemental Environmental Impact Statement

Appendix C
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WASHINGTON STATE DEPARTMENT OF ECOLOGY

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2,4-D

Volume 3, Section 1

LABEL DESCRIPTION & HISTORY

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1.0 2,4-D AS AN AQUATIC HERBICIDE

1.1 REGISTRATION REQUIREMENTS

In order to register a pesticide with the EPA for use in the United States, the active ingredient and its formulations must be tested for mammalian toxicity, physical chemistry, environmental fate, effects on ground water, and eco-tox effects. Work must also be done to demonstrate the expected magnitude of residue in and on edible products and residues in water. Once this data is generated, it is submitted to various branches of EPA for review. If EPA finds that the product does not pose significant risk to man, livestock, or wildlife, and has a favorable environmental persistence and degradation profile, a registration will be granted. With that registration, the manufacturer has permission to sell the product in the United States. However, individual states may also have their own separate registration processes.

Studies conducted since 1987 to support EPA registration must be (or have been) conducted in compliance with Good Laboratory Practice (GLP) regulations as specified in 40 CFR 160. These regulations were designed to improve the quality of records keeping and prevent fraud. They specify what records must be kept and how long they must be kept. They also specify how long analytical standards must be kept, how often they must be re-characterized and what storage conditions they must be stored under. Furthermore, they provide guidelines on how long neat and formulated organic and inorganic reagents, solvents and biological samples can be kept and under what conditions they should be stored. GLPs also provide guidance on how the integrity of these biological samples can be determined and how often they should be determined while the samples are in storage. For practical purposes, GLPs insure the integrity of the data. They allow for the reconstruction and consistent interpretation of the data within the study.

Washington State's registration procedure follows the EPA procedure: It requires that the applicant submit a copy of the EPA approved label and a copy of the confidential statement of formula. The Washington State Department of Agriculture reviews these submittals for compliance with state and Federal requirements. If these requirements are filled, the product will usually be registered by the state unless it presents an unusual hazard to the environment.

Three active ingredients of 2,4-D have been approved by the Washington State Department of Agriculture according to the PICOL Database for the control of aquatic weeds. However, only two of these active ingredients, 2,4-D butoxyethyl ester (BEE) and 2,4-D Dimethlyamine salt (DMA) are available for aquatic weed control in lakes and ponds. Both the EPA and Washington Sate have approved several labeled products with these active ingredients, and those are listed on the Washington State University PICOL database.

1.2 1992 ENVIRONMENTAL IMPACT STATEMENT AND EFFECTS OF STATE SENATE BILL 5424

In the State of Washington, most applications of aquatic herbicides and algaecides are performed under a state permit system. Ecology manages this system and uses a 1992 Environmental Impact Statement (EIS) for endothall, copper compounds, glyphosate, diquat and fluridone as well as manual, mechanical and biocontrol methods as its basis for writing permits for aquatic weed and algae control (Ecology, 1992). The permitting system is a result of six agencies working together to develop a statewide integrated pest management system for aquatic plants and noxious emergent vegetation. The goal is to ensure that the most effective and least environmentally damaging management alternatives will be used.

Ecology is responsible for issuing short-term modifications (STMs) to the water quality standards. These STMs are required for management activities such as use of pesticides, or mechanical or other control methods that might cause excess turbidity or violate other provisions of the water quality standards. Ecology is also responsible for ensuring proposals for control comply with rules and regulations designed to protect groundwater, shorelands, wetlands, air quality, and other elements of the environment.

In 1999, the Washington State Legislature passed legislation (ESBB 5424) requiring an update to the 1992 EIS. From 1992 to present, there has been a considerable amount of research work done to support the continuing registration of aquatic herbicides and algaecides containing 2,4-D. As such, the most current data for these materials has not been considered or used in the issuance of permits to perform aquatic weed and algae control in Washington State (Resource Management, Inc., 1999).

The 2,4-D BEE formulations found in Aqua-Kleen® and Navigate® are effective granular aquatic herbicides that control *Myriophyllum spp.* (watermilfoil), *Heteranthera dubia* (water stargrass), *Uticularia spp*. (Bladderwort*), Nymphaea spp*. (fragrant water lily), *Nuphar spp.* (spatterdock), *Brasenia spp.* (water shield), *Trapa natans* (water chestnut) and *Ceratophyllum demersum* (coontail). 2,4-D butoxyethyl ester is relatively toxic to environmentally relevant species of fish (LC50 = 0.30 to 5.6 mg a.i./L = 0.20 to 3.9 mg a.e./L) (Martens et al 1981 in Ecology, 1989 and Mount & Stephans, 1969 in Ecology, 1989). However, the acid form of 2,4-D is considered to be more representative of these formulations functional toxicity because the ester is essentially insoluble in water. The ester is released gradually from the granules and is rapidly hydrolyzed (within one day) to the acid (Aqua-Kleen® MSDS and Zepp et al, 1975 in JMPR, 1997). 2,4-D acid has a much reduced toxicity to environmentally relevant fish (2.5 to 358 mg a.e./L) (Rewoldt et al, 1977 in JMPR, 1997 & FWS, 1986 in Brian database, 1999).

Government entities in Washington State received legislative permission to use 2,4-D for the control of Eurasian water milfoil under SSB 5424 effective May 10, 1999. (ESSB 5424). There are several provisions that must be followed before government entities can use 2,4-D to control milfoil infestations including the following:

- The milfoil infestation must be recently documented or remaining after the application of other control measures, and must be limited to twenty percent or less of the littoral zone of a lake.

- Pesticide applications must meet all label requirements for the product and

- All public notice and posting requirements must be met, including: The government entity must provide at least twenty-one days notice to Ecology, Washington Department of Fish and Wildlife, Washington Department of Agriculture and Washington Department of Health all lake residents. All public boat access must be posted and informational buoys must be placed around the treatment area.

- WDFW may impose timing restrictions on the use of 2,4-D to protect salmon and other fish and wildlife.

- Ecology may prohibit the use of 2,4-D if the products contain dioxin in excess of the standard allowed by US EPA.

- Government entities using 2,4-D shall consider development of long-term control strategies for eradication and control of Eurasian watermilfoil.

Formal reports to the EPA by the registrant (Dow AgroScience), peer- reviewed literature and EPA databases were reviewed in order to prepare this risk assessment: 1) The documents used by the registrant to support registration were those submitted to EPA in the course of the registration and re-registration process as for 2,4-D. The studies were conducted according to the EPA's current pesticide assessment guidelines and, if conducted after 1987, were also conducted under Good Laboratory Practice Regulations (40 CFR 160). 2) The published articles were found in literature searches for peer reviewed articles written since 1989 using DIALOG OneSearch. 3) A large portion of the toxicity data was collected from EPA's Brian Database or the EPA's ECOTOX Database, which are compilations of ecotoxicology data currently in use at EPA to generate and support ecological risk assessments. Information collected on work done before 1989 was collected from general review articles on the toxicity and environmental fate of endothall such as Halter (1980), Ecology (1980 and 1989) and Ebasco (1993).

Recent history of 2,4-D use in the United States

In the United States, 2,4-D BEE is the most common herbicide used to control aquatic weeds. More 2,4-D was used in 1993 than all other aquatic herbicides combined; 2,4-D accounted for 66% of the total pounds of active ingredient sold for aquatic weed control and 56% of the total aquatic acreage treated. In 1993, Federal, State and local agencies treated a total 4,652 acres with 2,4-D to control aquatic weeds. 3,252 acres were treated with 2,4-D BEE granules and 1,400 acres were treated with 2,4-D DMA liquid (Lembi, 1996). Under the current labels, 2,4-D BEE granules are likely to be used by governmental entities in Washington State for the control of Eurasian watermilfoil if the infestation is recent or is remaining after application of other control measures. 2,4-D DMA is not currently used in the state of Washington, since water hyacinth is not currently a control problem and 2,4-D DMA is registered for use only for Eurasian watermilfoil programs conducted by the Tennessee Valley Authority in dams and reservoirs of the TVA system. However, since 2,4-D DMA (like 2,4-D BEE) is rapidly converted to 2,4-D acid, the two products should be equally effective in controlling Eurasian watermilfoil. Supporting factors for the use of both 2,4-D BEE and 2,4-D DMA are: 1) the similarity of limnological parameters such as pH associated with Eurasian watermilfoil infestations; 2) the influence of the physical site variables associated with

the duration of residues – wind and water movement, water depth and biomass density; 3) the relatively few instances of minor adverse effects on phytoplankton and benthic invertebrates as well as fish and non-target macrophytes and; 4) the fact that when legally permitted, 2,4-D DMA has been the material of choice by state and federal agencies for their waterhyacinth and Eurasian watermilfoil control programs. The multiple studies that review the actual residues in water, fish and sediment show residues to be of short duration and at extremely low levels, well below the approved registration standard. Rhône-Poulenc has pursued a label change to permit the use of 2,4-D DMA (Weedar® 64) and to remove text from the label that states "For Eurasian water milfoil (EWM) in programs conducted by the Tennessee Valley Authority in dams and reservoirs of the TVA system (Gallagher, 1992)."

- **History of 2,4-D use in the State of Washington**

  In 1977, Ecology requested the Seattle District of the US Army Corps of Engineers to assist in establishing a statewide program to prevent the spread of Eurasian watermilfoil in Washington State. In 1979, the Waterways Experiment Station began a large-scale operational management study to test prevention techniques. When pioneer milfoil colonies began to show up in Lake Osoyoos, each new area was treated with the granular formulation of 2,4-D BEE. Treatment was done with a small hand spreader. By 1979, milfoil had moved into the upper reaches of the Okanogan River. The Waterways Experiment Station tested standard granular formulations and liquid formulations applied with adjuvants to restrict drift. In 1981, the first milfoil colonies were discovered in the Columbia River, but the water flow was too great and the herbicide drift unacceptable. (Rawson from Proceedings of the 1st International Symposium on Watermilfoil (*Myriophyllum spicatum*) and related Halogragaceae Species. July 23 and 24, 1995, Vancouver Canada).

  In the Pend Oreille River, the long term management program for control of milfoil began in 1984. That year, 80 acres were treated with 2,4-D DMA and a polymer adjuvant. (Gibbons and Gibbons, 1985). In 1986, EPA refused to allow the use of 2,4-D in flowing water. This put an end to the use of 2,4-D in the Pend Oreille River. About the same time, an environmental group in Okanogan County filed an injunction against the use of 2,4-D in Lake Osoyoos. These events pretty much ended 2,4-D use in Washington, until 1998 when the state legislature directed Ecology to conduct a demonstration project using 2,4-D to control pioneering colonies of milfoil in Loon Lake. Loon Lake results were encouraging since Eurosian watermilfoil appeared to be controlled for the season while most native macrophytes were not affected by the use of 2,4-D (Parsons, 1999 in press). But a slight rebound in watermilfoil biomass after treatment indicates that additional measures may need to be taken to maintain reduced biomass. This could include more than one application of 2,4-D BEE to Eurasian watermilfoil.

1.3 RISK ASSESSMENT (SEE SECTION 4 FOR MORE DETAILS)

Herbicides used for aquatic weed control fall into one or more general categories: 1) Contact herbicides are chemicals that control weeds by direct contact with the foliage and destroy only those portions of the plant; generally the roots survive and regrow. 2) Systematic herbicides are applied to the foliage and/or stems of the plant and translocated to the roots or other portions of the plant, eventually resulting in the death of the entire plant. 3) Broad spectrum herbicides will kill most if not all plants if the dosage is appropriate. 4) Broadleaf herbicides will generally kill dicot plants with broad leaves but there may be exceptions; i.e. 2,4-D can kill monocots with broad leaf morphology and certain "narrowleaf" dicots are not harmed at concentrations of 2,4-D that typically kill broadleaf plants. 5) Submerged (submersed), emerged (emersed) or floating indicates the way the plant typically grows. i.e., below the water line (submerged), from below the water line to above the waterline (emerged) and on the surface of the water and often un-rooted (floating). Pre-emergent and Post-emergent weed control refers to whether control measures are taken prior to or after germination or first growth of the plant.

The Risk Assessment in Section 4 indicates that 2,4 D BEE may be used safely when most species of fish and invertebrates are present. The residue levels in British Columbia lakes drops below 0.100 mg/L in 2 to 6 days. Therefore, the acute expected environmental concentrations is ~ 0.100 mg/L for a 4-day exposure. 2,4-D BEE has sufficient laboratory toxicity to exceed the level of concern (0.1) for both rainbow trout (fish RQ = 0.33 = 0.1 ppm a.i./0.300 ppm a.i. and *Gammarus lacustris* (invertebrate RQ = 0.23 = 0.100 ppm a.i./0.44 ppm a.i.) and raise concerns for the protection of the resident biota. However, field studies with both fish and invertebrates indicates that there are few if any direct permanent effects on the biota due to 2,4-D BEE exposure [Bain and Boltz (1992), Marshal and Rutschy (1974) and Shearer and Halter (1980 citing Smith and Ison, 1967, Whitney et al, 1973, Gangstad, (1978), Pierce, 1960 & 1961 and Lim and Lozoway, 1978 and Brooker, 1974)]. This is believed to be due to the low solubility of 2,4-D BEE and rapid conversion (within a few hours to a day) of 2,4-D BEE to 2,4-D acid (JMPR, 1997). Conversion is postulated to be so rapid as to prevent significant exposure of fish and sediment organisms to 2,4-D BEE. However, this is a consensus opinion based more on observation of the field effects of 2,4-D BEE than empirical laboratory work. Once conversion to the acid has occurred, acute risk assessment indicates that the level of concern for protection of the biota is not exceeded. The risk quotient for the most sensitive species (common carp and *Gamarrus fasciatus*) is 0.05 (RQ = 0.100 ppm a.i./20 ppm a.i.) and 0.031 (RQ = 0.100 ppm a.i./0.3.2 ppm a.i.), respectively (Tables 22 and 23 in Section 4).

Since the long term residue levels of 2,4-D in British Columbia lakes treated with 2,4-D BEE drops below 0.001 mg/L within 5 to 22 days, the functional chronic expected environmental concentration can be considered to be the geometric mean of the acute EEC (< 0.100 mg/L) and the chronic EEC (<0.001 mg/L), which would be 0.01 mg/L. For chronic exposure, the estimated NOEC (0.017 to 0.024 mg/L for rainbow trout and *Gammarus spp.*) is higher than the functional EEC (0.01 mg/L), which leads to the conclusion that the chronic level of concern (1.0) for protection of both fish and invertebrate biota will not be exceeded.

For both acute and chronic risk assessment the levels of concern for protection of free swimming biota are not exceeded for the intoxicating agent (2,4-D acid for acute exposure and 2,4-D BEE and 2,4-D acid for chronic exposure. Therefore, it should be possible to use granular 2,4-D BEE according to the label without significant acute or chronic risk to aquatic animals.

However, the above statement applies only to organisms within the water column. Animals that live in the sediment may be exposed to 2,4-D concentrations that are many times higher than those in the water column. The higher concentrations of 2,4-D acid found in the sediment lead to a risk quotient that is equal to or exceeds the level of concern for protection of the biota. For example, the acute risk quotient for benthic organisms within sediment may be as high as 0.14 (RQ = 0.46 ppm a.i./3.2 ppm a.i.) and this value exceeds the acute level of concern (0.10). The chronic risk quotient for benthic organisms within sediment equals the level of concern (1.0) under a worst case scenario (RQ = 0.18 ppm a.i./0.18 ppm a.i = 1.0) and therefore may or may not represent a chronic risk to the benthic biota. Although these values indicate a possible risk to the benthic biota from exposure to 2,4-D acid due to treatment with 2,4-D BEE, fieldwork indicates that the benthic biota are not greatly affected by the direct effects of 2,4-D BEE. However, secondary effects such as oxygen depletion may cause a shift in the dominant species within a biota without affecting total numbers or overall diversity (Marshall and Rutschky, 1974). Sarkar (1991) and Patnaik and Das (1991) also found that benthic organism or zooplankton populations can be enhanced by exposure to commercial preparations of 2,4-D or 2,4-D sodium salt, respectively.

Older, fairly extensive research indicates that the smoltification process seems to be unaffected by the exposure of several species of salmon to sublethal concentrations of 2,4-D. For example, smolting Coho salmon survive exposure and seawater challenges after exposure to up to 200 mg/L of 2,4-D DMA and smolting Coho, pink or Sockeye salmon exposed to 1 mg/L of 2,4-D BEE for 24 hours survived subsequent seawater challenge tests for at least 96 hours. This indicates that interference with the smoltification process of salmon is unlikely to be a serious problem (Shearer and Halter, 1980).

Granular 2,4-D butoxyethyl ester (Aqua-Kleen® and Navigate®) is a post-emergent systemic herbicide used primarily to control watermilfoil and water stargrass. The other 2,4-D product used primarily around aquatic sites is 2,4-D Dimethlyamine salt (2,4-D DMA). This product is primarily used for control of water hyacinth and brush control along ditchbanks. Another 2,4-D product registered in Washington for the control of noxious weeds is 2,4-D 2-Ethylhexyl ester (2,4-D 2-EHE). 2,4-D 2-EHE is not registered for control of aquatic weeds but is typically used to control purple loosestrife (*Lythrum salicaria*) and brush along ditchbanks. Species other than those listed on the labels may also be controlled fully or in part by application of these products. However, the distributor makes no efficacy claims for control of weed species not listed on the label.

1.4 REGISTRATION LABELS

1.4.1 Current Labels

There are currently over 30 2,4-D formulations registered for aquatic weed control in the United States. The Washington State University PICOL Database lists only three of the active ingredients as being registered for use in the State of Washington for aquatic weed control. They are 2,4-D butoxyethyl ester, 2,4-D dimethylamine salt and 2,4-D 2-ethylhexyl ester. However, according to Ecology, only 2,4-D butoxyethyl is permitted for aquatic weed control in the State of Washington. There are two labeled products that contain this active ingredient registered in Washington. They are Aqua-Kleen® distributed by Nufarm and Navigate® distributed by Applied Biochemists. The labels for Aqua-Kleen® and Navigate® are attached in Appendix 1.

1.4.2 Historical Labels

For the purpose of historical significance, a label from approximately ten years ago is located in Appendix 2. This historical label indicates that the formulations, recommended uses and use rates have not changed significantly for Aqua-Kleen®. Label restrictions and labeled uses described in this section are given in the specimen labels for Aqua-Kleen® (1999), and Navigate® (Still in force on January 27, 2000). The labels and permits that govern the uses and restrictions imposed on this herbicide may be periodically changed based on new information submitted to EPA and Ecology. Label restrictions and labeled uses described in this section are given in the specimen labels Aqua-Kleen® (1999), Navigate® (in force as of January 28, 2000).

1.4.3 Label Restrictions

Information in this section is presented for guidance only. The most recent label should be consulted for current restrictions. The language in this section is from January 1999 Label. Application of Aqua-Kleen® or Navigate® should normally be limited to a portion of the water body at any one time because decaying vegetation can deplete the dissolved oxygen content of the water and aquatic organisms need oxygen to survive.

Water containing heavy vegetation should be treated in lanes leaving a buffer strip between each treated lane. Buffer strips must be present to prevent suffocation of fish and other aquatic animals. Each treated lane should be 50 to 100 feet wide. The treatment lanes and buffer strips should be of equal width and each buffer strip may be treated to control weeds in that area when the weeds in the previously treated lanes have died and decomposed. Decomposition of treated foliage will typically take two to three weeks.

Aqua-Kleen® and Navigate® time release small amounts of 2,4-D butoxyethyl ester which is rapidly converted to the less toxic 2,4-D acid. Therefore, the likelihood of a fish kill due to 2,4-D BEE treatment for aquatic weed control is low. Waterways lightly infested with weeds may be treated in their entirety for control of these plants. For actual area sizes recommended for treatment or other restrictions, consult the label and the permit.

Many species of fish are tolerant to the 2,4-D acid generated from the slow release of 2,4-D butoxyethyl ester contained in Aqua-Kleen® or Navigate® granules. If exotic tropical and marine fish not relevant to the northwestern United States are excluded, the acute toxicity (LC50s) of 2,4-D acid ranges from 25 mg a.e./L for cutthroat trout (Rewolt et al, 1977 in JMPR, 1997) to 358 mg a.e./L for rainbow trout FWS, 1986 in Brian, 1999). These toxicity values place 2,4-D acid in the US EPA's Ecotoxicological Category of slightly toxic (LC50 = >10 to 100 mg/L) to practically non-toxic (LC50 = >100 mg/L) (EPA, 1982 and Ebasco, 1993). It is noteworthy that in the tests evaluated that common carp was the most sensitive species to the effects of 2,4-D acid (LC50 = 20 mg a.e./L) (Vardia and Durve, 1981 in JMPR, 1997).

Most species of fish are acutely affected by 2,4-D butoxyethyl ester, which is the active ingredient of Aqua-Kleen® and Navigate®, at relatively low doses. The acute toxicity (LC50s) of 2,4-D-butoxyethyl ester ranges from 0.30mg a.i./L (0.20 mg a.e./L) for rainbow trout fry (Martens, 1980 in Ecology, 1989) to 3.7 mg a.i./L (2.5 mg a.e./L) for rainbow trout smolts (Finlayson & Verue,1985 in JMPR, 1997). These toxicity values place 2,4-D butoxyethyl ester in EPA's ecotoxicological category of highly toxic (0.1 to 1 mg/L to moderately toxic (> 1 to 10 mg/L). However, the likelihood of fish being exposed to lethal dosages of 2,4-D butoxyethyl ester is small because Aqua-Kleen® and Navigate® are slow release formulations in which the released 2,4-D butoxyethyl ester is rapidly degraded to the less toxic 2,4-D acid (within approximately one day) (Aqua-Kleen® MSDS and JMPR, 1997). However, these products are toxic to fish and should not be applied to water except as specified on the label.

Aqua-Kleen® and Navigate® should not be applied to waters used for irrigation, agricultural sprays, watering dairy animals or domestic water supplies. There are no set back restrictions (i.e. areas around water intake valves that should not be treated) mentioned in the labels. However, 2,4-D applications are generally permitted in waters if the people using water for the above purposes agree to suspend use until water in the treated area reaches the Federal Drinking water standard for 2,4-D; currently this standard is 0.07 mg/L. This concentration is generally obtained 3 to 5 days after treatment. Nevertheless, people who drink lake water and request alternate sources of drinking water be supplied for a month after treatment have had their requests honored.

Drift from these products may injure susceptible plants. Therefore, drift of dust to susceptible plants must be avoided.

Follow all additional precautionary statements, storage and disposal instructions given in the label and permit if available. See Table 1 for additional label restrictions and precautions.

1.4.4 Labeled Use

Aqua-Kleen® and Navigate® are labeled for use in lakes and ponds by the US EPA. When large areas are being treated, granular formulations of Aqua-Kleen® or Navigate® should be scattered as evenly as possible over the treatment area with a Gerber® seeder, Gandy® seeder, or similar device. All equipment should be calibrated carefully to be sure of spreading the proper amount of herbicide.

When small areas (around docks or isolated patches of weeds) are being treated, granular formulations of Aqua-Kleen® or Navigate® should be scattered as evenly as possible

over the areas to be treated with a Cyclone® seeder or similar device. Measure or estimate the area to be treated. Weigh out the amount of material needed and spread uniformly over the area. For best results, split the dosage amount in two and cover the area twice, applying the second half at a right angle to the first.

Application rates are dependent on the resistance of the weed species to the chemical, density of weed mass at time of treatment, stage of growth, water depth and rate of water flow through the treated area (flushing rate). Use the higher rate for dense weeds, when water is more than eight feet deep and where there is a large water volume turnover. The application rate varies from 100 to 200 pounds per acre. For exact application rates, please review the label. Practical experience from local applicators indicates that an application rate of 90 to 100 pounds/acre may be more effective than rates of 200 pounds/acre due to a change in the plants physiology at higher rates (McNabb, 1999, Personal Communications).

1.4.5 Effectiveness Controlling Specific Aquatic Plant Species

Aqua-Kleen® and Navigate® products are systemic, granular broadleaf, post-emergent herbicides with greatest effectiveness against various milfoil species *(Myriophyllum spp.)* and water stargrass *(Heteranthera dubia)*. At higher rates Aqua-Kleen® and Navigate® are also effective against *Utricularia spp*. (bladderwort), *Nymphaea spp.* (White water lily), *Nuphar spp*. (spatterdock or yellow water lily), *Brasenia spp*. (water shield), *Trapa natans* (water chestnut) and *Ceratophyllum demersum* (coontail). Spatterdock and coontail are often difficult to control and multiple treatments, separated by a period of time specified in the label or permit, may be necessary to achieve full control.

Certain aquatic and wetland species are of particular interest to Ecology. They are *Myriophyllum spicatum* (Eurasian watermilfoil), *Lythrum salicaria,* (purple loosestrife), *Egeria densa* (Brazilian elodea), *Myriophyllum aquaticum* (parrotsfeather*), Cabomba caroliniana* (fanwort), *Hydrilla vertcillata* (hydrilla), *Tamarix ramosissima* (saltcedar), *Amorpha fruticosa* (indigobush), *Polygonum sachalinense,* (giant hogweed or giant knotweed), *Polygonum cuspidatum* (Japanese knotweed), *Lysimachia vulgaris* (garden loosestrife) and *Phalaris arundinacea* (reed canarygrass). Of these plants, the label only specifies control for Eurasian watermilfoil and parrotsfeather. See Table 2.

There are several species of aquatic plants of great concern in the northern tier of states. They are Eurasian watermilfoil, purple loosestrife, curly leaf pondweed, Brazilian elodea, Monoesius Hydrilla, *Spartina altternaflora* (smooth cordgrass*), Phragmites australis* (common reed), *Nuphar spp*. and *Nymphaea spp.* (water lilies), and water chestnut (*Trapa natans*). Except for water chestnut, all of these weed species currently can be found in the waterways of Washington State. If these species expand further in Washington waters, they have the potential to cause additional serious aquatic weed problems. Of these introduced and potentially problematic weeds, only Eurasian watermilfoil, water lilies and water chestnut are controlled effectively with Aqua-Kleen® and Navigate®. For further detail, a more complete listing of weeds, and degree of control by Aqua-Kleen® and Navigate®, see Table 2.

Use of these products to control weeds not listed on the label is not recommended. However, other weeds may be controlled incidentally as a result of application of Aqua-Kleen® or Navigate® for the control of species listed on the label.

1.5 MAINTAINING THE CURRENT REGISTRATION

Since the last Supplemental Environmental Impact Statement for 2,4-D (Ebasco, 1993 and Ecology, 1989), a number of additional studies that are compliant with the EPA's FIFRA Pesticide Assessment Guidelines and Good Laboratory Practice Standards have been completed and submitted to the US EPA for review. Studies that are compliant with current regulations add to the database. These compliant studies also increase the confidence of regulatory organizations, elected officials and the general public that the data supports the most recent risk assessment (JMPR, 1997) and the Supplementary Environmental Impact Statements (State of Washington 1980, 1989 & 1992 and Ebasco, 1993). These studies may result in the addition or removal of certain use restrictions depending upon their outcome. The changes brought by the development of new data will be assessed in later sections of this document. However, although many of the studies evaluated here do not meet core requirements for EPA studies, those judged supplemental are used by EPA in risk assessment to augment the limited number of "core" studies.

1.6 INTERVIEWS WITH APPLICATORS REGARDING TYPICAL PRACTICES IN WASHINGTON STATE

A set of questions was developed based on specific points of interest outlined by Ecology. The items addressed were those that the applicators (Doug Dorling of Allied Aquatics, Inc. and Terry McNabb of Resource Management, Inc.) would have direct knowledge of. Their input was incorporated in the main body of Sections 1 and 4. The original questions and answers given by the applicators are presented in Appendices 3 and 4 of Section 1 of the "Endothall Risk Assessments in Support of Updates to the 1991 Aquatic Plant Management Supplemental Environmental Impact Statement". Prior to finalization of the interviews, the respondents were requested to review them, correct any errors and elaborate on points of particular interest or concern to them.

1.7 RATE TECHNOLOGIES

The same set of applicator questions was also asked of Kurt Getsinger of the Army Corp of Engineers. Dr. Getsinger heads the chemical Technologies Research Unit at Waterways Experiment Station. Dr. Getsinger is a leading expert in chemical control technologies. He is the author of many scientific papers in this field and co-author with Howard Westerdahl of the "Aquatic Plant Identification and Herbicide Use Guide (1988)." Dr. Getsinger was also asked to discuss his research in rate reduction technology including hardware, products and methods used. Dr. Getsinger's input was incorporated in the main body of Section 1 and in the assessments and recommendations portions of this document. The original questions and answers given by Dr. Getsinger are presented in Appendix 5 of Section 1 of the "Endothall Risk Assessments in Support of Updates to the 1991 Aquatic Plant Management Supplemental Environmental Impact Statement. Prior to finalization of the interview, the respondent was requested to review the document, correct any errors and elaborate on points of particular interest or concern to him.

REFERENCES (2,4-D BBE)

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4. ECOTOX Database, 1999. Online EPA Database that Summarizes Ecotoxicological collected from Reviewed Literature as well as the Unreviewed Brian Database.

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Table 1: Comments and Label Restrictions for 2,4-D Butoxyethyl Ester (AquaKleen® and Navigate®) Formulations

	Comments & Label Restrictions
1.	Aqua-Kleen® or Navigate® should be applied in spring and early summer during the time when weeds first start to grow.
2.	The active ingredient in Aqua-Kleen® and Navigate® (2,4-D Butoxyethyl ester) is hydrolyzed to 2,4-D acid within one-day of release from the granules (Aqua-Kleen® MSDS, 1999, Zepp, 1975 in JMPR, 1977).
3.	The product (2,4-D butoxyethyl ester) is toxic to fish. LC50 values for environmentally relevant species ranges from 0.3 mg a.i./L (0.23 mg a.e./L) for rainbow trout fry to 3.7 mg a.i./L (2.5 mg a.e./L) for rainbow trout smolts. However, the low solubility of 2,4-D BEE and its rapid hydrolysis to 2,4-D acid makes extensive contact with 2,4-D BEE unlikely.
4.	The product, after being released slowly and being rapidly hydrolyzed to 2,4-D acid, has a fairly safe toxicity profile to fish. LC50s of 2,4-D acid range 20 mg a.e../L for common carp to 358 mg a.e./L for rainbow trout, which places this product in EPA's Ecotoxicological Risk Categories ranging from slightly toxic (>10 to 100 mg/L) to practically non-toxic (>100 mg/L). For further discussion of Fish Toxicity See Section 4.1.1.2
5.	Although the label states that Aqua-Kleen® and Navigate® should not be applied to waters used for irrigation, agricultural sprays, watering dairy animals or for domestic water supplies, Ecology and the Washington Department of Agriculture have interpreted this to mean that these products may be applied to areas used for the listed purpose under certain conditions. There are no exceptions for set back restrictions mentioned in the labels. Water should not be utilized for the above purposes until the 2,4-D concentration drops to or below the Federal drinking water standard of 0.07 mg/L.
6.	Drift from these products may injure susceptible plants. Therefore, drift of dust to susceptible plants must be avoided.
7.	Follow all additional conditions, procedures, restrictions, precautionary statements and instructions listed in the label and permit if available.
8.	The comments and label restrictions discussed here reflect the instructions in the Aqua-Kleen® label dated 03/99 and the Navigate® label as of 1/27/2000.

Table 2: Species Controlled with Aqua-Kleen® and Navigate®, Effectiveness of Control and Registration Status for Control of Listed Species

Species Controlled	Effectiveness of Control or Labeled Use	
	Aqua-Kleen®	**Navigate®**
Potamogeton spp. Pondweed	No Efficacy Claimed[7]	No Efficacy Claimed
Ceratophyllum spp. Coontail	Labeled Use Fair Control[2]	Labeled Use Fair Control[2]
Hydrilla verticillata Hydrilla	No Efficacy Claimed	No Efficacy Claimed
Myriophyllum spicatum Eurasian watermilfoil	Labeled Use Excellent Control[2]	Labeled Use Excellent Control[2]
Myriophyllum spp. Milfoil	Labeled Use Excellent Control[1]	Labeled Use Excellent Control[1]
Myriophyllum hetrophyllum Variable leaf milfoil	Labeled Use Excellent Control[1]	Labeled Use Excellent Control[1]
Brasenia spp. Watershield	Labeled Use Excellent Control[2]	Labeled Use Excellent Control[2]
Uricularia spp. Bladderwort	Labeled Use Fair Control[6] Good Control[3]	Labeled Use Fair Control[6] Good Control[3]
Heteranthera spp. Water stargrass	Labeled Use	Labeled Use
Sparganium spp. Bur reed	No Efficacy Claimed	No Efficacy Claimed
Hygrophila polysperma Hygrophila	No Efficacy Claimed	No Efficacy Claimed
Lythrum salicaria Purple loosestrife	No Efficacy Claimed	No Efficacy Claimed
Egeria densa Brazilian elodea	No Efficacy Claimed	No Efficacy Claimed
Myriophyllum aquaticum Parrotsfeather	Labeled Use Excellent Control[1,2]	Labeled Use Excellent Control[1,2]
Cabomba caroliniana Fanwort	Fair Control[4] No Efficacy Claimed	Fair Control[4] No Efficacy claimed
Tamarix ramosissima Saltcedar	No Efficacy Claimed	No Efficacy Claimed
Amorpha fruitcosa Indigobush	No Efficacy Claimed	No Efficacy Claimed
Polygonum sacalinense Giant knotweed	No Efficacy Claimed	No Efficacy Claimed
Polygonum cuspidatum Japanese knotweed	No Efficacy Claimed	No Efficacy Claimed

Table 2: Species Controlled, Effectiveness of Control and Registration Status for Control of Listed Species (Continued)

Species Controlled	Effectiveness of Control or Labeled Use	
	Aqua-Kleen®	**Navigate®**
Lysimachia vulgaris Garden loosestrife	No Efficacy Claimed	No Efficacy Claimed
Phalaris arundinacea Reed canarygrass	No Efficacy Claimed	No Efficacy Claimed Labeled Use
Typha Spp. Cattail	Labeled Use Fair Control[5] Good Control[4]	Fair Control[5] Good Control[4]
Elodea canadensis American waterweed	No Efficacy Claimed	No Efficacy Claimed
Nuphar spp. Spadderdock	Labeled Use Fair Control[5] Excellent Control[2]	Labeled Use Fair Control[5] Excellent Control[2]
Nymphaea spp. Fragrant water lilies	Labeled Use Good Control[3] Excellent Control[2]	Labeled Use Good Control[3] Excellent Control[2]
Hydrilla	No Efficacy Claimed	No Efficacy Claimed
Spartina Smooth cordgrass	No Efficacy Claimed	No Efficacy Claimed
Phragmites australis. Common reed	No Efficacy Claimed	No Efficacy Claimed
Trapa natans Water chestnut	Labeled Use Good Control[4]	Labeled Use Good Control[4]
Algae species	No Efficacy Claimed	No Efficacy Claimed

1 (Robinette, 1998-1999)
2 (Westerdahl et al., 1988)
3 (Robinette, 1998-1999)
4 (Westerdahl et al., 1988)
5 (Robinette, 1998-1999)
6 (Westerdahl et al., 1988)
7 No Efficacy Claimed = The indicated formulation has not been shown to control this species. Not listed as a controlled species on the label.

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APPENDIX 1: Current Labels

APPENDIX 2: Historical Labels

2,4-D

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2.0 2,4-D

2,4-D (2,4-Dicholorophenoxy acetic acid) is the active component in a variety of herbicide products used for both terrestrial and aquatic application sites. 2,4-D is a selective plant hormone type product that is translocated within the plant to the susceptible sites. Its mode of action is primarily as an auxin, or stimulant of plant stem elongation. 2,4-D stimulates nucleic acid and protein synthesis and affects enzyme activity, respiration, and cell division. It is absorbed by plant leaves, stems, and roots and moves throughout the plant. It accumulates in growing tips. Its primary use is as a post-emergent herbicide.

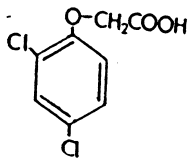
2,4-D is formulated in a multitude of forms, however only two active ingredient forms are currently being supported by the manufacturers for use in aquatic sites. These are the dimethylamine salt and the butoxyethyl ester (See Section 1.0 above for a discussion of salts and esters). The butoxyethyl ester is the active ingredient in the two products used in the State of Washington. These products are Aqua-Kleen® (Nufarm, Inc.) and Navigate® (Applied Biochemists) and are available from several manufacturers. Typical products and their labels may be found in Section 1.0.

Because these are the primary products for use in aquatic sites, the physical chemical characteristics and data reported are limited to the pure acid active ingredient (and technical product), the dimethylamine salt and the butoxyethyl ester. The majority of the data was obtained from a recent Food and Agricultural Organization (FAO) document. This document was extensively peer reviewed and for the purposes of chemical and physical properties, is relatively complete and up to date.

2.1 COMPOSITION

- **Active ingredient**

Common name:	2,4-Dichlorophenoxy acetic acid (2,4-D)
CAS Registry No.:	94-75-7 (acid)
Chemical name:	(2,4-Dichlorophenoxy)acetic acid (2,4-D)
Empirical formula:	$C_8H_6Cl_2O_3$
Molecular weight:	221.04
Structure:	



- **Impurities**

There are no known impurities identified by the manufacturers or the USEPA, which are expected to be of toxicological concern. There may exist low levels of 2,4-

dichlorophenol, a starting material in the synthesis of the compound. It should be noted that the low levels of impurities such as 2,4-dichlorophenol are determined and quantitated in the technical grade active ingredient at its manufacture. The technical grade active ingredient is used to produce an end-use product by the addition of solvents, diluents, surface active agents, antifoams, etc. Therefore, the level of any impurities which may occur in the technical grade active ingredient will be further reduced during the manufacture of the end-use product which contains only 27.6 weight percent a. i.

Additionally, when used in typical aquatic applications, only 1 ppm 2,4-D is required for efficacy. Therefore, the concentration of any ingredient other than the active will be further reduced. With this significant reduction in concentration, comes a reduction in risk to both humans and the environment.

There has been concern expressed over the association of 2,4-D products with low levels of chlorinated dioxins and furans. Extensive investigation by US manufacturers of technical grade 2,4-D has shown that there are no halogenated dioxins and furans which exceed the limits of quantitation (LOQs) expressed in the June 15, 1987 USEPA Data Call-In Notice for dioxins and furans in 2,4-D products. These limits of quantitation vary based on the specific congener, but are based on safety margins estimated for the most toxic congener, 2,3,7,8-TCDD, which has a required quantitation level of 0.1 parts per billion (ppb). Other quantitation limits range up to 100 ppb based on toxicological equivalence to 2,3,7,8-TCDD (Hammond, 1999). The quantitation limits discussed in the Data-Call-In remain the USEPA guidelines for reporting of these contaminants. While there may be occasional apparent detections of individual dioxin or furan compounds below these quantitation limits, they are not considered to be toxicologically significant.

There have been several reports of contamination of 2,4-D products produced outside the US (particularly in Russia), however, these products are not registered for use in the United States and therefore have no impact on the current discussion.

Past concerns have been fueled by the finding of dioxins/furans in “Agent Orange”, a mixture of 2,4-D and a related herbicide 2,4,5-T, which was used extensively in Vietnam. Subsequent work, such as that described above, has shown that 2,4-D is not contaminated, but that the 2,4,5-T component was significantly contaminated, which has resulted in its being banned for use in the US.

- **Intentionally added inert ingredients**

Intentionally added inert or “other” ingredients in 2,4-D formulations include dimethylamine used to produce the amine salt in some formulations and butoxyethanol to produce the butoxyethyl ester in others. Other formulation ingredients included in the end-use products have been reviewed by the USEPA and approved when used for their intended purpose, however, these are not reported, as they are confidential manufacturing information.

The USEPA has established a category listing system for the “other” (inert) compounds used in pesticide formulations. The lists are designated 1, 2, 3, 4a and 4b. List 1 contains eight compounds, which, due to their toxicological profile, require special labeling if

used in a pesticide formulation. There are no List 1 compounds in the 2,4-D formulations (Aqua-Kleen® and Navigate®) used in the State of Washington. List 2 compounds are those for which USEPA has not yet determined a full profile but is reviewing existing information. At the completion of their evaluation, it is expected that the compounds still in use in pesticide formulations will be moved to List 1 or to List 4. List 3 contains those compounds which have not been fully evaluated, but which have profiles of lesser concern in the USEPA evaluation scheme. It is expected that most of these compounds will be moved to List 4. List 4 is divided into two categories. List 4A contains compounds generally regarded as safe for use in pesticide formulations and includes such compounds as corn cobs and cookie crumbs. List 4B contains those compounds that have sufficient data on file at EPA to substantiate that they can be used safely in pesticide products. In the case of each list, if USEPA determines that a compound is no longer used in any pesticide formulation, it will be removed from the list.

There are compounds from Inerts Lists 2, 3 and 4 in Aqua-Kleen®. The levels of these compounds are relatively low as the clay carrier makes up the bulk of the formulation and the active ingredient accounts for 27.6% of the weight and the majority of the balance of the formulation.

In addition to the above-mentioned review by the USEPA, all registered pesticidal end-use products (the products actually applied to the environment to control weeds or pests) must undergo a series of toxicological tests to establish their safety. Because these tests are performed on the actual end-use formulation, the effects of the "other" ingredients are effectively tested simultaneously. This toxicological screen of the "other" compounds affords an additional opportunity to examine comparative data on the active ingredient versus the end-use product to determine if there is a need to test each of them in a complete testing battery.

2.2 COLOR

Color is an end-point observation of the product used to assist in identification.

	Color	**Citation**
2,4-D Acid	White to light brown	FAO, 1996
Dimethylamine, 2,4-D Salt	Amber	FAO, 1996
Butoxyethyl Ester, 2,4-D	Tan	FAO, 1996

2.3 PHYSICAL STATE

Physical state is an end-point observation of the product, solid, liquid or gaseous used to assist in identification. The formulated product may be either a liquid or a solid depending on the formulation.

	Physical State	**Citation**
2,4-D Acid	Solid	FAO, 1996
Dimethylamine, 2,4-D Salt	Liquid or Solid	FAO, 1996
Butoxyethyl Ester, 2,4-D	Liquid or Solid	FAO, 1996

2.4 ODOR

Odor is an end-point observation of the product used to assist in identification. Odor may also serve as a warning in cases where odorants are added as a safety factor.

	Odor	Citation
2,4-D Acid	Phenolic	Mahlburg, 2000
Dimethylamine, 2,4-D Salt	Strong Ammonia	FAO, 1996
Butoxyethyl Ester, 2,4-D	Phenolic	Mahlburg, 2000

2.5 MELTING POINT

The melting point is a physical end point observation used for identification of pure compounds and may provide some indication of thermal stability. Melting point is not applicable to the formulations because they are either liquids or impregnated clay granules.

	Melting Point (°C)	Citation
2,4-D Acid	140.5	FAO, 1996
Dimethylamine, 2,4-D Salt	NA	NA
Butoxyethyl Ester, 2,4-D	NA	NA

2.6 BOILING POINT

The boiling point is a physical end point observation for identification of pure compounds. The boiling point for the pure acid active ingredient is undefined. (A solid at room temperature.) The boiling points for the liquid formulations are undefined, as they are solids.

2.7 DENSITY, BULK DENSITY OR SPECIFIC GRAVITY

Bulk density is a measure of the weight per unit volume of the product and is useful for physical identification or differentiation of two similar products. The value may also be needed in the calculation of application rates in some instances. Density is typically reported as grams per cubic centimeter at 20°C.

	Density (g/cc)	Citation
2,4-D Acid	1.56	
Dimethylamine, 2,4-D Salt	1.24	FAO, 1996
Butoxyethyl Ester, 2,4-D	1.20	FAO, 1996

2.8 SOLUBILITY

Solubility is a physical end point useful for understanding potential environmental impact. High water solubility is frequently associated with mobility and affects distribution in water and soil. This endpoint is determined for the active ingredient in a product and is typically reported as grams per 100 ml water at 25°C (Reported values are for pH 7).

	Solubility (g/100 ml)	**Citation**
2,4-D Acid	2.3	FAO, 1996
Dimethylamine, 2,4-D Salt	72.9	FAO, 1996
Butoxyethyl Ester, 2,4-D	Insoluble	Nufarm, 1999

2.9 VAPOR PRESSURE

Vapor pressure is a physical end point useful for understanding the distribution of the active ingredient between water/soil and air. High volatility is an indication of potential impact in the air compartment. This endpoint is determined for the active ingredient in a product and is typically reported as mm mercury (Hg) at a specified temperature.

	Vapor Pressure (mm Hg)	**Citation**
2,4-D Acid	1.4×10^{-7}	FAO, 1996
Dimethylamine, 2,4-D Salt	$<1 \times 10^{-7}$	FAO, 1996
Butoxyethyl Ester, 2,4-D	2.4×10^{-7}	FAO, 1996

2.10 DISASSOCIATION CONSTANT

Disassociation constant is a physical end point used to assess the distribution of the product in aqueous media. The reported pH values indicate the environmental pH at which the active ingredient molecule will dissociate to its ionic form. In the case of 2,4-D, there is only one dissociable functional group.

	Dissociation Constant (pKa)	**Citation**
2,4-D Acid	2.78	FAO, 1996
Dimethylamine, 2,4-D Salt	NA	NA
Butoxyethyl Ester, 2,4-D	NA	NA

2.11 OCTANOL/WATER PARTIITON COEFFICIENT

Octanol/Water partition coefficient is a physical end point used to assess the potential of a compound to bioaccumulate in the environment. The value represents the ratio of product in octanol versus water at equilibrium at 25°C. Values less than 10 indicate little or no likelihood of bioaccumulation.

	Octanol/Water Coefficient (Kow)	**Bioconcentration Factor (BCF)**	**Citation**
2,4-D Acid	2.81	32	FAO, 1996
Dimethylamine, 2,4-D Salt	2.81	21	FAO, 1996
Butoxyethyl Ester, 2,4-D	4.17	740	FAO, 1996

2.12 pH

pH is a physical end point used to identify the product and to assess the potential effect of the equilibrium in the environment. For 2,4-D amine, the pH is reported for the product.

	(pH)	**Citation**
2,4-D Acid	NA	NA
Dimethylamine, 2,4-D Salt	6.8-9	FAO, 1996
Butoxyethyl Ester, 2,4-D	NA	NA

2.13 STABILITY

Stability is a chemical evaluation of the product to assess the potential effect of heat, light, metals and metal ions on the active ingredient. This data is not required for the formulated products.

2.14 OXIDIZING OR REDUCING ACTION

Oxidizing or reducing action is an assessment of the potential for a compound to react with common oxidizers or reducers. In the case of 2,4-D and its formulated products, there is little likelihood of such reactions occurring.

2.15 FLAMMABILITY

Determination of flammability is measurement of the temperature that will sustain a flame and is used to classify the product for hazard in storage and shipping. Determination of flammability is not required for technical grade products. The formulated products are clay based and will not support combustion.

2.16 EXPLODABILITY

Determination of explodability is measurement of the potential for a compound to explode when exposed to physical or thermal shock. Determination of explodability is not required for technical grade products. The formulated products are clay based and are not explosion hazards. Additionally, the 2,4-D molecule contains no explodable functional groups.

2.17 STORAGE STABILITY

Storage stability is the physical determination of the stability of the active ingredient when stored in its commercial packaging over extended time periods, usually one to two years or more. 2,4-D products have been shown to be stable under normal storage conditions for periods of at least two years when stored in sealed containers. (FAO, 1996).

2.18 VISCOSITY

Viscosity is a physical end-point measurement used to identify the product and to assess the ability of the product to be poured or pumped. The measurement is not required on technical grade products or on solid products.

2.19 MISCIBILITY

Miscibility is a physical assessment of the ability of a formulated product to mix with spray oils for use during application. Since the 2-4,D aquatic products are not labeled for application in oil, this data requirement is not applicable.

2.20 CORROSION CHARACTERISTICS

Corrosion characteristics require the physical observation/measurement of the effects of the product on the commercial packaging. Measurements of the weight, deformation, strength of the packaging, etc. are reported. For the 2,4-D formulations, no significant changes were noted in the packaging.

2.21 DIELECTRIC BREAKDOWN VOLTAGE

Dielectric breakdown voltage is the physical measurement of the effect of an electric arc on the stability of the formulated product. This requirement applies only to formulations that are applied around electrical equipment or apparatus. As there is no likelihood of open electrical apparatus in the aquatic environment, this test is not applicable.

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2,4-D

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3.0 2-4-D

2,4-D has been used as an herbicide for a number of years, primarily for terrestrial weed control. Due to its high profile and an increasing use as an aquatic herbicide, there have been a number of studies conducted since the original 2,4-D Environmental Impact Statement (EIS) was issued in 1993, to determine the fate and behavior of 2,4-D in aquatic environments. For ease of reference, this update incorporates data from the earlier EIS in addition to information from pre-1990 sources not cited in that document, and from references published since 1990. In addition, several "registration" studies performed by and for registrants are cited.

The Washington State Department of Ecology is only considering the butoxyethyl ester (BEE) of 2,4-D for use in aquatic weed management. Comparatively less information was located on BEE in the literature. Most work has been conducted on either 2,4-D acid, the 2-ethylhexyl ester (2-EHE), which is also called the isooctal ester, or on the dimethyl amine (DMA) formulation. Attention should be paid to the differences in hydrolysis, photolysis, and other results in this Section, and 2-EHE and DMA data should be interpolated with caution when predicting BEE behavior in the environment.

3.1 VOLATILIZATION

No test data were found regarding BEE volatilization. The following quote from Washington State (1993) summarizes the volatility issue well as follows.

"Available data indicate that neither the ester (2,4-D BEE) or the amine salt (2,4-D DMA) formulations of 2,4-D are highly volatile. At 25°C, the vapor pressure of 2,4-D BEE is 4.5×10^{-6} mm mercury (Zepp *et al.* 1975). Henry's Law constant for 2,4-D BEE is reported at 10^{-6} to 10^{-7} atm cubic meter/mole (Hunter *et al.* 1984; Thibideaux 1979). given these characteristics, the volatilization half-life for 2,4-D BEE in an aquatic system at 25°C and 1 meter depth as been estimated at 895 days (Zepp *et al.* 1975). Similarly, 2,4-D DMA which has a low vapor pressure (Klingman *et al.* 1975) and high solubility in water, would also exhibit low volatility.

2,4-D acid with a vapor pressure of 8.0×10^{-6} mm Hg and a Henry's Law constant of 2.5×10^{-10} atm cubic meter/mole is considered nonvolatile (Reinert and Rodgers 1987)."

No additional studies were found regarding the environmental fate of 2,4-D through volatilization of the acid or any of the esters or salts.

3.2 HYDROLYSIS

<u>Summary</u>: Breakdown of the BEE form of 2,4-D by hydrolysis in sterile water is pH dependent. Half-lives were reported as 196 days at pH 5, about 26 days at pH 6, 74 hours at pH 7, and 35 to 55 minutes at pH 9. In unsterilized well water at pH 7, the BEE half-life ranged from 24 hours to 1.6 days. Sterile water hydrolysis of the 2-EHE form displays similar pH dependency. Half-lives range from 99.7 days at pH 5 to 37 hours and 52 hours at pH 9. In unsterilized water, half-lives of 1.25 and 1.45 hours were reported at pH 6.9 and at an unreported pH. Both of the esters mentioned yield 2,4-D acid as the primary product. No data were found regarding a specific time for the acid half-life, but it is generally regarded as stable to hydrolysis. Since the pH of most natural waters

ranges from 6 to 9, with higher values during higher bioproductivity in summer, BEE can be expected to hydrolyze rapidly when used in lakes and ponds.

Hydrolysis refers to the chemical interaction of the agrochemical with water as a mechanism of agrochemical breakdown. While aqueous or aquatic (the terms are synonymous in this review) persistence studies are sometimes conducted in natural water bodies, true hydrolysis studies are conducted in laboratories using sterile distilled or deionized water so that the chemical effects of an aqueous environment can be isolated from biological, sunlight, or sediment interactions. Aquatic persistence in natural water is addressed in Section 3.5.

Laboratory hydrolysis studies for EPA submission are typically performed with radioactive ^{14}C 2,4-D at three pH values (pH 5, pH 7, pH 9 corresponding to slightly acid, neutral, and mildly alkaline, respectively) in sterile water for a period of 30 days at 25°C. Sampling for breakdown products and the remaining concentration of parent material occurs at frequent intervals.

BEE and 2-EHE are not appreciably soluble in water. They must first be hydrolyzed into 2,4-D acid in order to go into water solution and be available for weed control. Therefore, the shorter the hydrolysis half-life, the sooner the herbicide is available for use.

3.2.1 Half-life

Shearer and Halter (1980) reviewed 2,4-D environmental fate literature. They cited early references indicating that 2,4-D acid is stable in water in the absence of "enzyme systems" (*e.g.* those found in 2,4-D degrading microflora) or energy inputs such as ultraviolet light. DeMarco, *et al.* (1967) found that 2,4-D acid at 50 ppm in sterilized water had not degraded after 100 days. Aly and Faust (1964) saw no breakdown of 2,4-D acid in lighted flasks containing 3 ppm 2,4-D in aerobic lake water after 120 days. Zepp *et al.* (1975) reported an acid hydrolysis half-life of 26 days.

Table 3.1 illustrates the pH-dependency of BEE and 2-EHE. Half-lives for BEE range from 196 days at pH 5, through 74 hours at pH 7, to 55 minutes at pH 9 (Shepler *et al.*, 1990). Zepp *et al.* (1975), as reported in Washington State (1993), determined that the hydrolysis half-life of BEE can be predicted by the following equation:

$$T(1/2) = 0.693/(K_b)[OH]$$

where K_{bg} = hydrolysis rate constant (mol_{-1} sec_{-1})
[OH] = hydroxyl ion concentration

The hydrolysis rate constant varies from 30.2 mol_{-1} sec_{-1} at 28°C to 235 mol_{-1} sec_{-1} at 47°C, indicating that the hydrolysis rate is temperature dependent, as are nearly all chemical reactions. Zepp's equation was used to calculate BEE half-lives at 28°C of 26 days and 0.6 hours at pH 6 and 9, respectively. Since the pH of most natural waters is approximately 6 to 9, particularly during the summer months, BEE can be expected to degrade fairly rapidly when applied to lakes. Reinert and Rodgers (1987) calculated a half-life in well water of 1.6 days at pH 7.0 to 7.2 from data reported elsewhere. Rodgers and Stalling (1972) found that in laboratory aquaria containing pH 7 water from a deep well, BEE broke down in 24 hours or less if the aquaria contained fish, while 90% degradation required 90 hours in aquaria without fish. It is possible that the excreta of the

fish contained bacteria able to degrade BEE, although 24 hours is very short time for bacterial dispersion and degradation of BEE to have occurred from this source.

An unusual type of hydrolysis study was conducted by Racke (1989) in a clay/water slurry spiked with BEE. This type of study might be used to partially predict the fate of BEE in turbid water, but the results are a combination of hydrolysis, microbial degradation, and probably adsorption to suspended soil particles. He found that BEE broke down rapidly, with a half- life of less than 20 minutes in the slurry.

While not used in Washington State for aquatic weed control, 2-EHE data are included in Table 3.1 for comparison. At pH 5, the 2-EHE half-life was about half of the BEE half-life. However, at pH 7 and 9 the 2-EHE half-lives were considerably longer than those of BEE by factors of 15 to 90 (26). Zepp's equation was also used to calculate hydrolysis half-lives of 1500 days at pH 6 and 37 hours at pH 9 (99). The figure of 1500 days is considerably at odds with the values for pH 5, 7, and 9, and is probably due to some unique circumstances of the test. In soil/water slurries, 2-EHE displayed the same accelerated breakdown as BEE (24).

Two reports of 2,4-D DMA hydrolysis in sterile water at unspecified pH values stated that complete breakdown to 2,4-D occurred in about 30 minutes (31) and in less than one minute (71).

3.2.2 Degradation products

The major product of BEE hydrolysis at pH 5, 7, and 9 was 2,4-D acid (80). The major product of 2-EHE hydrolysis at the same three pH's was also 2,4-D acid (24, 26). 2-4-D acid was also reported as the hydrolysis product of the DMA salt (31). No data were located regarding the specific hydrolysis half-life of 2,4-D acid or its hydrolysis products. From the structure of the molecule, little hydrolysis is expected in the pH ranges found in natural waters before the acid is degraded by other mechanisms such as photolysis and microbial metabolism.

Table 3.2: Hydrolysis of 2,4-D (Laboratory Studies)

Matrix	Compound	pH	Temp	Half-life (DT_{50})	Reference
Sterile water*	BEE ca. 1.0 ppm	5	24°C	196 D[1]	(80) Shepler *et al.* 1990
Sterile water	BEE	6	28°C	ca.[2] 26 D (calculated)	(103) Zepp *et al.*, 1975 in (99)
Sterile water*	BEE ca. 1.0 ppm	7	24°C	74 H[1]	(80) Shepler *et al.*, 1990
Mahoon clay/water slurry	BEE	7	n.r. (probably ca. 25°C)	< 20 Min	(70) Racke, 1989

Table 3.2: Hydrolysis of 2,4-D (Laboratory Studies) (Continued)

Matrix	Compound	pH	Temp	Half-life (DT_{50})	Reference
Deep well water	BEE	7	n.r. (probably ca. 25°C)	≤ 24 H (fish present) DT_{90} 90 H (no fish)	(75) Rodgers and Stalling (1972) in (79)
Missouri well water	BEE	7.0-7.2	n.r. (probably ca. 25°C)	1.6 D (calculated)	(72) Reinert and Rodgers, 1987
Sterile water*	BEE ca. 1.0 ppm	9	24°C	55 Min[1]	(80) Shepler *et al.*, 1990
Sterile water	BEE	9	28°C	ca. 0.6 H (calculated)	(103) Zepp *et al.*, 1975 in (99)
Sterile water*	2-EHE 0.03 ppm	5	25°C	99.7 D	(26) Concha *et al.*, 1993c
Sterile water	2-EHE	6	28°C	1500 D (calculated)	(103) Zepp *et al.*, 1975 in (99)
Sterile water*	2-EHE 0.03 ppm	7	25°C	48.3 D	(26) Concha *et al.*, 1993c
Sterile water*	2-EHE 0.03 ppm	9	25°C	52.2 H	(26) Concha *et al.*, 1993c
Sterile water	2-EHE	9	28 °C	37 H (calculated)	(103) Zepp *et al.*, 1975 in (99)
Caitlin silty clay/water slurry	2-EHE	6.9	25°C	1.25 H	(24) Concha *et al.*, 1993a
Hanford sandy loam/water slurry	2-EHE	n.r.[3]	25°C	1.45 H	(24) Concha *et al.*, 1993a
Sterile water	DMA	n.r.	n.r.	DMA "dissociates in 27-36 minutes"	(31) Dynamac, 1988
Sterile water	DMA	n.r.	n.r.	< 1 minute	(71) Reim, 1989

1 D/H/Min = Days, Hours, Minutes
2 ca. = approximately
3 n.r. = not reported
* EPA guideline study

3.3 AQUEOUS PHOTOLYSIS

Summary: Only one report of BEE photolysis was found. In that study, no significant breakdown of BEE in sterile pH 5 water was observed at up to 30 days of light exposure. (Photolysis of BEE vapor in air was found to occur with a half-life of 13-20 days.) Photolytic degradation of 2,4-D acid was found at pH 3.5, 6.8, and 8.9 with a half-life of about 70 minutes. However, another study at pH 6 found no significant degradation of 2,4-D acid after 8 hours.

The major product of BEE photolysis is 2,4-D acid. When the acid is photolyzed, the primary product is probably 2,4-dichlorophenol, which breaks down further under light to smaller amounts various intermediates, with the final products appearing to be humic acids.

As with hydrolysis, photolysis testing is carried out in a laboratory. Vessels containing solutions of the herbicide in sterile distilled or deionized water are irradiated with either a mercury vapor lamp or natural sunlight. Identical vessels are kept in the dark for the duration of the study and also sampled in order to compensate for the effects of any hydrolysis occurring. Testing is usually carried out at 25°C, at pH 5, 7 and 9, but this is not always the case, particularly with very early studies. Other photolysis testing, such as photolysis of a pesticide on the surface of a soil, is also required by the EPA for products that might be incidentally applied to soil, as is the case for 2,4-D.

The purpose of photolysis experiments is to isolate the effect of sunlight, specifically the ultraviolet and near-ultraviolet part of the spectrum, on the degradation of an herbicide without biological or chemical interactions. Natural sunlight's visible spectrum covers wavelengths from about 800 nm (deep red) to about 300 nm (deep violet). Generally speaking, only light in the violet and ultraviolet end of the spectrum has enough energy to initiate or influence chemical reactions ("photochemical reactions"). Air, as well as ozone, strongly filters near-ultraviolet and ultraviolet radiation, and cuts off nearly all radiation below 290 nm wavelength. Water is transparent to radiation down to approximately 180 nm (far ultraviolet), assuming that there are no suspended solids or dissolved colored material such as humic acids to impair passage of the light.

3.3.1 Half-life

Table 3.2 summarizes photolysis data for 2,4-D. Photolysis testing is normally carried out on 2,4-D acid, and only one study was found that addressed BEE photolysis. Marx and Shepler (1990) reported no significant photodegradation of 2,4-D BEE in a pH 5 buffered water solution over a 30 day period.

In contrast, several studies using 2,4-D acid reported photolytic half-lives ranging from approximately 70 minutes to 13 days (10, 12, 14, 17, 62). Chamarro and Esplugas (1993) reported the shortest half-lives of approximately 70 minutes at pH's of 3.5, 6.8, and 8.9, with degradation occurring somewhat more rapidly at pH 3.5. Two studies found no significant degradation of the acid at pH 6 after 8 hours (40), or very slow degradation in drops of water on a glass slide (96). The reason for the disparity between the 70 minute and 8 hour half-lives at pH 6 and 6.8 is not known. CHMR (1989b) found no significant degradation of 2,4-D acid on a sterile soil surface in 30 days.

Zepp *et al.* (1975) measured photolytic breakdown of BEE vapor in air and estimated a half-life of 13 to 20 days. While BEE is volatile, the relatively long vapor half-life and application to water as granules means that photolysis in air would not be a significant degradation mechanism in aquatic plant-control applications.

A principal degradate is 2,4-dichlorophenol, which is discussed below. Aly and Faust (1961) reported a 50% photolytic loss of 2,4-dichlorophenol in 5 minutes at pH 7.0.

3.3.2 Degradation Products

Marx and Shepler (1998) found that the major product produced during photolysis of BEE was 2,4-D acid. However, they attributed the presence of the acid to hydrolysis in the aqueous solution since the dark control produced 2,4-D acid in a comparable manner. No other data were found dealing with BEE photolysis.

Since the major hydrolysis product of BEE is 2,4-D acid, the identity of the acid photolysis products is relevant. Boval and Smith (1973) identified 2,4-dichlorophenol (2,4-DCP) as one of the photolysis products of 2,4-D acid. CO_2 was the final oxidation product. Harrison and Venkatesh (1999) also identified 2,4-DCP as a primary 2,4-D acid photoproduct, with higher concentrations in a more acidic system (pH 4.5) then under neutral or basic conditions. Crosby and Tutass (1966) postulated a multiple pathway photodegradation scheme for 2,4-D that involves stepwise dechlorination as well as hydrolysis of the ether linkage (Figure 3.2.3). Their postulated degradation steps include 2,4-DCP as well as 4-chlorocatechol, and 2-hydroxy-4-chlorophenoxyacetic acid, and 1,2,4-benzenetriol, with the final products being mixed humic acids. They found no difference in photolytic products and 2,4-D between exposure to natural or artificial light. 1-chloro-4-hydroxyphenoxyacetic acid may also be a product (18). It is of interest that in a wet-soil-surface photolysis study, none of the above compounds were found, or were found at a concentration of 1.1% of the initial dose or less.

Zepp *et al.* (1975) found that photolysis of BEE vapor in air produced dehalogenation products and 2,4-dichlorophenol with 2- and 4-chlorophenoxyacetic acid esters appearing at higher BEE concentrations.

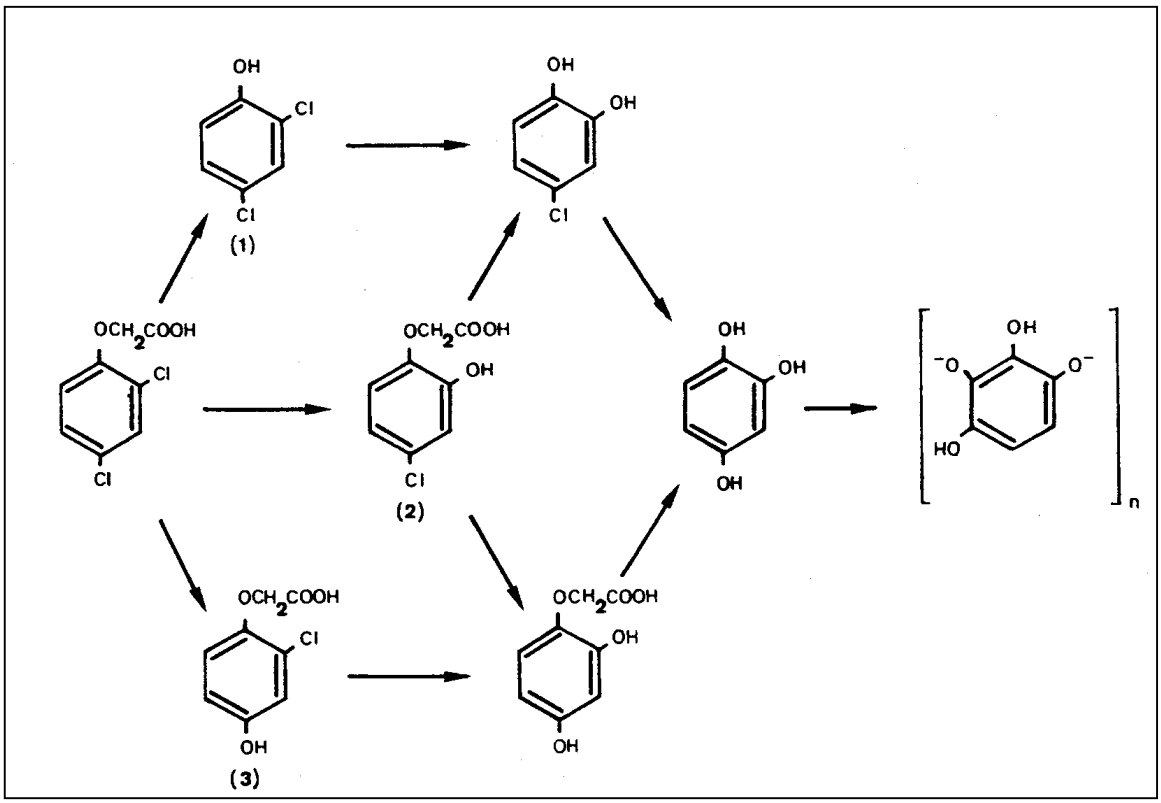
Table 3.3: Photolysis of 2,4-D (Laboratory Studies)

Matrix	Compound	Initial Conc	pH	Temp (°C)	Half-life (DT_{50})	Reference
Sterile water (natural CA sunlight)	BEE	0.96 ppm	5	25°C	n.s.d.[1] in 30 days	(63) Marx and Shepler, 1990
Air	BEE	< 1 mg/L	n/a[2]	n.r.[3]	ca.[4] 13-20 D[5]	(103) Zepp *et al.*, 1975 in (99)
Sterile water	2,4-D acid	n.r.	3.5, 6.8, 8.9	33°C	ca. 70 Min[5]	(17) Chamarro & Esplugas, 1993
Sterile water	2,4-D acid	100 mg/L	6	28°C	n.s.d. in 8 hours	(40) Harrison & Venkatesh (1999)
Sterile water drops on glass slides	2,4-D acid	100 mg/L	6	n.r.	(93% 2,4-D remaining after 11 hours)	(96) Venkatesh & Harrison (1999)
Sterile water	2,4-D acid	ca. 5 ppm	7	25°C	13 D	(14) CHMR, 1989a
Sterile water	2,4-D acid	5-50 ppm	n.r.	26.5°C	ca. 10-13 H[5] (estimated)	(10) Boval & Smith, 1979
Sterile water	2,4-D acid	30-100 ppm	n.r.	25°C	6-14 H	(12) Cabrera et al, 1997 (62) Martin *et al.*, 1997
Sterile loam soil	2,4-D acid	4.35 ppm	n.r.	25°C	n.s.d. in 30 days	(15) CHMR, 1989b

Note: Unless otherwise stated, all experiments utilized artificial light, usually mercury vapor lamps on an approximately 12 hours light/12 hours dark cycle.

1 n.s.d. = no significant degradation
2 n/a = not applicable
3 n.r. = not reported
4 ca = approximately
5 D/H/Min = Days, Hours, Minutes

Figure 3.3: Proposed Photolytic Degradation Pathway of 2,4-D



From Crosby and Tutass, 1966

3.4 DEGRADATION AND PERSISTENCE - SOIL

Summary: No data were found pertaining to BEE half-life in terrestrial soil. Half-lives of 2,4-D acid in soil generally ranged from 2 days to about 12 days at 17-25°C, with 2,4-D from granular applications being on the higher end of that range. A half-life of 39 days was reported for 2,4-D acid when a forest was treated with the DMA salt of 2,4-D. Reduction of soil moisture to about 50% of capacity or less increased half-lives, in some cases dramatically. Temperature was shown to be a factor in the length of persistence, with lower temperatures increasing half-lives. In one study, acid half-lives were much longer in soils taken from 2 to 4 foot depths compared with those from the top foot of soil. These results illustrated the contribution to increased persistence of sparser soil microorganism populations and less organic carbon in the lower depths. 2-EHE half-lives ranged from 1 day to 12.8 days in near-surface soil. One survey of 30 field soil dissipation studies gave a 2-EHE half-life range of 9.9 to 84 days when granular formulations were used. In field dissipation studies, DMA salt half lives ranged from 2.1 to 7.5 days for liquid formulations and 4.0 to 15.5 days for granulars.

A major metabolite of 2,4-D in soil is CO_2. Substantial amounts of soil humic and fulvic acids have been reported as metabolic products in soil studies, as have traces of 2,4-dichlorophenol and 2,4-dichloroanisole. In pure culture metabolism tests of 2,4-D acid using soil microorganisms, numerous other related compounds have been seen. Much of the carbon in the 2,4-D molecule is taken up by soil microorganisms and used to build cell tissues or used in their metabolic processes like carbon from any other source. The

small amounts of numerous compounds seen are likely intermediate compounds caught in a "snapshot" of the metabolic process.

Although only the aquatic uses of 2,4-D are considered in this document, the compound is registered for terrestrial applications, which account for the largest use. Data regarding 2,4-D persistence in soil are therefore required to be submitted to the EPA. This information has a relevance to accidental terrestrial overspray on lake or stream shorelines, and peripherally as an indication of possible fate on near-shore lake bottoms exposed by drought or drawdown following a 2,4-D application. Donald and Syrgiannis, 1995, postulated wind erosion and aerobic decomposition in dry exposed lake bottoms as the cause for very low water lake residues in Saskatchewan prairie lakes. Soil persistence data also can give an indication of the behavior of 2,4-D that may escape a lake basin by seepage into the surrounding soil.

3.4.1 Half-life

No soil degradation BEE half-life data were found during this review, though information may exist in agrochemical company and EPA files. The data summarized in Table 3.4 are for 2,4-D acid, 2-EHE, DMA, and for studies where the form of 2,4-D was not specified in the report or abstract. Broadly summarizing the data, persistence of 2,4-D acid, 2-EHE, and DMA formulations are fairly short under conditions expected in most aerobic natural soils. Half-lives range from about 2 to 12 days with occasional longer times in isolated circumstances.

Laboratory aerobic metabolism studies conducted at 25°C found 2,4-D acid to have a short half-life ranging from 1.7 to 8.5 days, with disappearance from six soils in 5.9 to 25 days (22, 64). Such studies are conducted at 75% field moisture capacity (FMC - a measure of the maximum amount of water that a soil can hold at saturation). Smith (1989) measured a 2,4-D acid half-life of 5 to 10 days in clay soil at 65-100% FMC, but decreasing the soil moisture to 50% FMC increased the half-life to 45 days, suggesting that hydrolysis may have occurred and/or that higher moisture content favored the growth of 2,4-D degrading microflora and the accessibility of the chemical to the microorganisms.

Veeh *et al.* (1996) reported 2,4-D acid degradation in two laboratory flask aerobic soil studies conducted at three temperatures using soil taken from 0-12 inch and 3-4 foot depths. Half-lives in soils from 0-12 inches at 10°C and 17°C ranged from 7 to 11 days. At 24°C half-lives were only 2 and 3 days in the two surface soils. In soils taken from a depth of 2 to 4 feet the half lives were dramatically increased. At 10°C, half lives were 593 and 1691 days (1.6 and 4.6 years). At 17°C and 24°C, half-lives ranged from 10 to 31 days. The authors demonstrated that half-lives were strongly correlated with soil temperature. They also concluded that shorter half-lives were strongly correlated with higher soil organic carbon content and greater bacterial plate counts in the shallow soil layers. No relationship of half-life to soil moisture could be established since moisture was not measured.

Smith *et al.* (1989) monitored neutral-pH Indian Head clay soil plots in Saskatchewan that were treated yearly in springtime with 2,4-D "amine and ester formulations" starting in 1947. Since 1969, 2-EHE and DMA have been used at rates of 0.38 and 1.0 lb/acre. Samples were taken in September, 1987. No residues of 2,4-D were detected in any soil samples taken to a depth of one foot.

A half-life of 25 days in a volcanic ash/clay soil was reported by Kuwatsuka and Miwa (1989), but the formulation used was not specified. In Arkansas silt loam and silty clay, Johnson *et al.* (1995) reported short half-lives of 4 and 5 days respectively, with disappearance of 95% of the residues in 25 days.

In a review of 30 soil (field) dissipation studies using 2-EHE and DMA formulations, Wilson *et al.* (1997) reported 2,4-D acid half-lives of 1.1-5.7 days for liquid formulations and 3.6-11.6 days for granular formulations, reflecting the lag necessary for granular formulations to release the active ingredient to the environment. 2-EHE half lives were reported as 1 to 12.8 days for liquids and 9.9 to 84 days for granulars.

Barney (1996) reported a field dissipation half-life for 2-EHE of 1 day, with a follow-on half-life for the resulting 2,4-D acid of 4 days. Residues of 2-EHE were reduced to less than 0.05 ppm in less than 3 days, while the acid reached that level in less than 30 days. Grover (1975) reported a half-life of 3.5 days in a pH 5.3 soil.

Wilson *et al.* (1997) found that 2,4-D DMA degraded quickly with half-lives of 2.1 to 7.5 days for liquid formulations and 4.0 to 15.5 days for granular formulations. Barney reported a 2,4-D acid half-life of 39 days with disappearance in less than 180 days in an Oregon forest soil treated with DMA.

3.4.2 Degradation Products

In a laboratory aerobic metabolism study using ^{14}C radiolabeled 2,4-D acid applied to six soils, McCall *et al.* (1980) found no significant metabolites. The majority of the 2,4-D was rapidly converted to CO_2 and the remainder of the radioactivity was incorporated into "the high molecular weight organic fraction of the soil which is eventually converted to CO_2". Concha and Shepler (1994a) also found CO_2 to be the major metabolite in a 16-day laboratory aerobic metabolism study using a silty clay loam and 2,4-D acid. Besides the CO_2, which constituted 51.2% of the originally-applied ^{14}Carbon radioactivity (AR) at 16 days, they also found very small quantities of 2,4-dichlorophenol (0.4% AR at 16 days) and 2,4-dichloroanisole (1.5% at 16 days). Radioactivity was also found as fulvic acid (6.1% AR) and humic acid (11.1%). The remainder of the radioactivity was unextractable from the soil at the end of the study through irreversible binding of parent material or metabolites, or incorporation into other soil constituents.

Smith and Aubin (1991b) studied degradation of ^{14}C-labeled 2,4-D acid in three clays and a sandy loam from Saskatchewan. Soils were incubated aerobically in laboratory flasks at 85% FMC and 20°C for 24 days. One of the clays had a long-term history of treatment with 2,4-D products, while the other soils had no recent history of 2,4-D treatment. No 24-day data are reported for the long-term treated clay and complete sixteen-day data are only available for the long-term treated clay and the untreated clay and sandy loam. At 16 days into the study, the soils with no long-term treatment had released 14% to 17% AR as CO_2. 8% to 10% AR was present as 2,4-dichloroanisole, and 30% to 43% AR was unextractable from the soils. In the long-term treated clay soil, 51% AR was released as CO_2 by Day 16. Also present were 1% AR as 2,4-dichlorophenol, 2% as 2,4-dichloroanisole, and 28% to 45% as unextractable radioactivity.

Chakrabarty's (1982) proposed pathway for 2,4-D microbial metabolism is reproduced from Washington State, 1993 as Figure 3.2.4. Loos (1975) and Tiedje et al (1969)

identified several 2,4-D metabolites produced by *Arthrobacter*, an aerobic soil bacteria genus. The compounds were 2,4-dichlorophenol, 3,5-dichlorocatechol, 2,4-dichloromuconic acid, 2-chlor-4-carboxymethylene-but-2-enolide, chloromamaleyacetic acid, and succinate. Balajee and Mahadevan (1990) identified 2,4-D metabolites produced by isolated *Azotobacter chrococcoum*, a forest soil bacteria. They found 4-chlorophenoxyacetic acid, 4-chlorophenol, 4-chlorocatechol, and 3-chloromuconic acid. It should be noted that these metabolites were produced in bacterial cultures and many are probably intermediate products that were in the process of being further changed when sampled. Most do not appear in the soil matrix studies cited above.

Smith and Aubin (1991a) studied 2,4-dichlorophenol degradation in four soils at 85% FMC and 20°C for 14 days. In two clays, a clay loam and a sandy loam, 12%-17% AR was released as CO_2. Unchanged dichlorophenol was found to account for 5% to 23% AR, and 2,4-dichloroanisole constituted 4% to 8%. From 44% to 68% AR was unextractable from the soil and was associated with soil organic matter as humins fulvic acids, and humic acids. In all soils, 2,4-dichlorophenol remaining at 7 days accounted for 9% to 39% AR, indicating rapid breakdown and a very short half-life.

3.4.3 Physical and Chemical Factors

There are several physical and chemical factors influencing the rate of 2,4-D breakdown in soil. Among those investigated are temperature, soil moisture, soil microbial population, and prior treatment with 2,4-D. The last of these is discussed in more detail in Section 3.2.6.

- **Temperature**

  The rate of chemical reactions and most biological metabolic processes doubles for every 10°C increase in temperature. Johnson *et al.* (1995b) found that degradation of 2,4-D was more rapid at 30°C than at 15°C in two surface soils. Half-lives in dry soils were 14 and 18 days at 15°C and 4 and 8 days at 30°C. Similar increased degradation was observed in the same soils at 100% FMC. In soils from 24 inches in depth, however, the results indicated other factors also at work. Half-lives in 24-inch dry soils were 28 and 12 days at 15°C, and 39 and 23 days at 30°C. In saturated soils at 24 inches, the half-lives were 45 and 43 days at 15°C, and 44 and 9 days at 30°C. These results suggest that the primary effect of temperature was on soil microorganisms, and that soil moisture was a contributing factor. When the soil was dry, the organisms were probably not able to take full advantage of the more rapid biological processes occasioned by increased temperature. Veeh et al (1996) found that an initial lag phase after soil treatment with 2,4-D acid increased and maximum degradation rates decreased for two soils with decreasing temperature from 24°C through 17°C to 10°C. Willems *et al.* (1996), however, found degradation rates were quite similar at 10°C, 15°C, and 20°C in incubated sandy loam field soil, and that a major drop in degradation occurs at temperatures less than 7°C. Similarly, Smith (1989) reported in increase in soil half-lives from 5-10 days at 10-25°C to about 25 days at temperatures below 10°C.

- **Soil Moisture**

  Higher soil moisture can increase the rate of 2,4-D degradation in some soils. Johnson *et al.* (1995b) found that degradation in dry surface and deep (2 feet) soils

was two to three times faster than in saturated soils at 15°C. In one case, however, there was no moisture-dependent difference in degradation. Benoit *et al.* (1999) reported that increased moisture in soil organic matter can increase biodegradation of 2,4-D. Willems et al (1996) reported that in an aerobic sandy loam soil from a depth of 10 to 20 inches, degradation increased slowly when FMC increased from 15% to 30%, but was much higher at 40% FMC, because of the formation of a more favorable environment for soil bacteria, increased bacterial mobility, and greater solute concentration and availability of 2,4-D. Smith (1989) found that 2,4-D half-lives increased from 5-10 days at 65-100% of FMC to about 25 days at 50% FMC.

- **Soil microbes**

  Initial lag times, attributed to the growth time for 2,4-D-degrading microflora, have been reported (51, 83). Recent treatment of soils with 2,4-D has been shown to increase the degradation rate of 2,4-D, or to decrease initial degradation lag times (84, 85). Smith and Aubin (1991b) also correlated degradation with the number of 2,4-D-degrading organisms isolated from test soils. In a study of Saskatchewan clay soil field plots that had received annual applications of 2-EHE and 2,4-D DMA for 40 years, Smith *et al.* (1989) found 2,4-D residues were less than 0.02 ppm. The study illustrates the same effects as laboratory studies, namely the selection for and growth of a microbial population that can utilize 2,4-D as a carbon source.

  No data were found specifically addressing whether 2,4-D that adsorbed to soil was more or less available for microbial degradation. Adsorption may facilitate breakdown by concentrating the chemical on soil particle surfaces where microflora can utilize it more efficiently, or it may bind the chemical so tightly that microorganisms cannot use it.

Table 3.4: 2,4-D Persistence in Soil

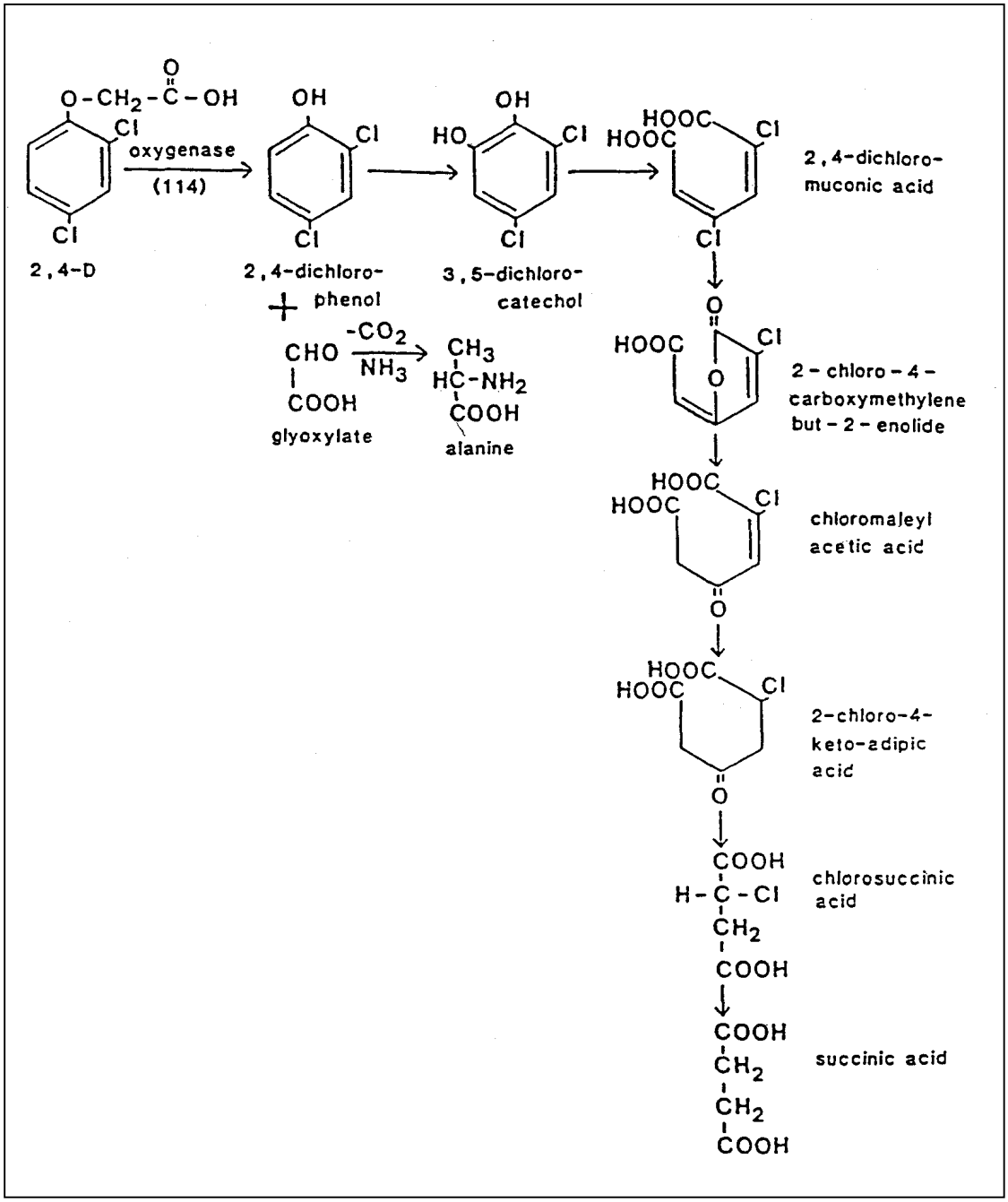
Experiment	Compound & application rate	Half-life (DT_{50})	Time to residues < 0.05 ppm	Reference
Lab aerobic clay soil microcosm*	2,4-D acid 5.1 ppm	1.7 D[1] at 25°C	n.r.[2]	(22) Concha and Shepler, 1994a
Lab aerobic soil microcosm (6 soils)	2,4-D acid 1 ppm	4 D mean, range 1.5-8.5 D, at 25°C	DT_{90}[3] mean = 11 D, range 5.9-25 D	(64) McCall *et al.*, 1980
3 clay soils	2,4-D acid	1 D[4] 3 D, 7 D at 20°C, 85% FMC[5]	n.r.	(83) Smith & Aubin, 1991b
clay soil - moisture comparison	2,4-D acid	At 20°C: 5-10 D at **65-100% FMC**, 45 D at **50% FMC**	n.r.	(81) Smith, 1989 in (99)
clay soil - temperature comparison	2,4-D acid	At 85% FMC: 5-10 D at **10-25°C** ca. 25 D **below 10°C**	n.r.	(81) Smith, 1989 in (99)
2 lab aerobic microcosms, **0-1 ft soils**, MT	2,4-D acid 0.75 lb/acre	**10°C** = 7 & 11 D **17°C** = 7 & 8 D **24°C** = 2 & 3 D	n.r.	(95) Veeh *et al.*, 1996
2 lab aerobic microcosms, **2-4 foot depth soils**, MT	2,4-D acid 0.75 lb/acre	**10°C** = 593 & 1691 D **17°C** = 25 & 31 D **24°C** = 12 & 10 D	n.r.	(95) Veeh *et al.*, 1996
Field dissipation, GA*	2-EHE 4 lb a.e.[6]/acre	2-EHE = 1.0 D acid = 4.0 D	2-EHE < 3 D acid < 30 D	(4) Barney, 1996
n.r.	2-EHE	3.5 D at pH 5.3	n.r.	(37) Grover, 1973 in (95)
Forest dissipation, OR*	DMA 4 lb a.e./acre	39 D (acid)	< 180 D (acid)	(3) Barney, 1995

Table 3.4: 2,4-D Persistence in Soil (continued)

Experiment	Compound & application rate	Half-life (DT_{50})	Time to residues < 0.05 ppm	Reference
30 soil dissipation studies	Acid, DMA, 2-EHE **liquid formulations**	Acid = 1.1-5.7 D DMA = 2.1-7.5 D 2-EHE = 1.0-12.8 D	n.r.	(102) Wilson *et al.*, 1997
30 soil dissipation studies	Acid, DMA, 2-EHE **granular formulations**	Acid = 3.6-11.6 D DMA = 4.0-15.5 D 2-EHE = 9.9-84 D	n.r.	(102) Wilson *et al.*, 1997
Silt loam, AR	n.r. 1 lb/acre 2,4-D	4 D	DT_{95} = 26 D	(46) Johnson *et al.*, 1995a
Silty clay, AR	n.r. 1 lb/acre 2,4-D	5 D	DT_{95} = 20 D	(46) Johnson *et al.*, 1995a
Volcanic ash with clay	n.r. 25 ppm	25 D	n.r.	(55) Kuwatsuka and Miwa, 1989

1 D/H = Days/Hours
2 n.r. = not reported
3 DT_{90} = time to 90% disappearance
4 Soil had recently been treated with 2,4-d
5 FMC = field moisture capacity; see text
6 a.e. = 2,4-D acid equivalent
* EPA guideline study

Figure 3.4: Proposed degradation pathway for 2,4-D acid
After Chakrabarty (1982)



3.5 DEGRADATION AND PERSISTENCE - AQUATIC SYSTEMS

Summary: 2,4-D degradation is primarily caused by the action of sediment microorganisms. 2,4-D acid in water is very stable in the absence of microorganisms that can break the molecule apart. Two laboratory studies in sterile water found no significant degradation in sterile water in a 30 day aerobic study, and a calculated half-life of 312 days in a one-year anaerobic study. In water plus sediment taken from ponds and lakes, the acid is broken down fairly rapidly. A half-life of 45 days plus an initial 30 day lag time where no degradation occurred was reported for soil plus sediment ("system") using a sediment that had not been previously treated with 2,4-D or related chemicals. When the experiment was repeated with a sample of the same sediment that had been inoculated with microorganisms cultured in the presence of 2,4-D, the initial lag time disappeared and a system half-life of about 6 days was calculated. The system half-life of additional 2,4-D added to the culture flasks was about 2 days. 2,4-D persistence in the unadapted sediment system was more than 50 days, but only about 16 days in the adapted system. In two soils/water mixtures, system half-lives were 16 and 26 days for microflora-rich surface soils, but 43 and 45 days for soils from 2 foot depths with lower microflora populations. In laboratory water/sediment systems having 2,4-D adapted microorganisms, system half-lives from 1.4 to 14.7 hours have been reported. In large cylinders containing water, sediment and plants, treated with 20 lb BEE per acre and maintained at a relatively cold temperature of 7-10°C, 2,4-D acid persisted in the water for less than 6 months. All of the studies summarized in this paragraph were conducted in laboratories in relatively small volumes, in the absence of water currents, daily temperature changes, and other dynamic processes to be found in natural waters.

Limited reporting of BEE persistence indicates that BEE breaks down to the acid very rapidly in aquatic systems. In laboratory flasks of water treated with BEE and inoculated with naturally-occurring microorganisms, the ester disappeared in time ranging from 15 minutes to 2.6 hours. In studies of lakes treated with BEE granular formulations, BEE half-lives in water were reported as 1.5 to 3 days.

Most outdoor studies reported just 2,4-D acid persistence rather than that of the ester used. In outdoor artificial ponds containing "virgin loam soil", water and watermilfoil and treated with 20 lb BEE granular formulation per acre, acid half-life in the sediment was 15 days, but the acid was detectable in water and sediment for an extended time, with disappearance in less than 182 days. Other studies reported half-lives for the acid in lakes treated with BEE granular of 3 days (water) to 7 days (sediment). Times to disappearance of the acid ranged from 8 to 59 days in water and 12 to 86 days in sediment except for one study where acid residues in the sediment persisted for more than 9 months. 2.4-D acid half-lives in an Ontario bog lake (pH 4.5) were 4.5 and 7.8 days in water for applications of 2-EHE at 0.9 and 2.2 lb/acre, with acid persisting for about 24 days in the water.

The overall pattern is that BEE is rapidly broken down in natural pond and lake systems in a few days, while the resulting acid is usually below detection levels (approximately 0.01 ppm) in treated area water within a month. In sediment, the acid may persist from a few weeks to as long as 3 months, with occasional instances of persistence to 6-9 months, though the latter is unusual. Longer sediment persistence is probably facilitated by the use of granular formulations that release BEE over a prolonged period. If the BEE or

acid is in contact with flocculent (light, fluffy) sediment, adsorption to the sediment particles and subsequent slow release may prolong the presence of residues near and in the sediment.

BEE breaks down to 2,4-D acid in aquatic systems. The major degradates of the acid are 2,4-dichlorophenol (immediate) and CO_2 (final). Humic and fulvic acids bound to the sediment are also important degradates. Small amounts of dichloroanisole, 4-chlorophenol, and related compounds have also been reported. Much of the carbon in the 2,4-D molecule is taken up by soil microorganisms and used to build cell tissues or used in their metabolic processes like carbon from any other source. As is the case for soil, the minor products are likely intermediate compounds caught in a "snapshot" of the metabolic process.

Numerous physical and chemical factors can effect the persistence and fate of 2,4-D in the aquatic environment. Temperature influences the rate of both chemical and biological processes. Hydrolysis and adsorption to soil will be slower at lower temperatures, and most importantly the degradation of 2,4-D by microflora will be inhibited at low water temperatures. Water pH plays a minor role in persistence. BEE hydrolyzes more rapidly in neutral and basic water than in acidic water. Therefore, BEE persistence is expected to be shorter in water of neutral to higher pH. 2,4-D acid persistence does not appear to be significantly affected by pH variations expected in natural waters. The amount of oxygen dissolved in a water body has a direct effect on the speed of 2,4-D metabolism since the microorganisms that break down the chemical are aerobes that must have oxygen to thrive. Warmer water, aerobic decay of organic materials on/in the sediment, and oxygen depletion resulting from decay of a large aquatic vegetation kill are examples of situations that can deplete dissolved oxygen. In many cases, eutrophic and even mesotrophic lakes are more likely to support large populations of microorganisms that can metabolize 2,4-D than lakes with lower nutrient levels. On the other hand, if carbon sources are not abundant, competition for the carbon in 2,4-D can favor the growth of the microbiota that can utilize 2,4-D exclusively. There is disagreement among researchers as to whether adsorption of 2,4-D to sediment increases the availability to microorganisms by concentrating it on the surfaces, or decreases the availability for metabolism due to strong binding.

Probably the most important physical process affecting 2,4-D persistence in larger water bodies is transport of treated water away from the treated area and replacement with untreated water through lateral circulation or vertical movement of water. In this regard, the larger the lake, the more wind blowing across the lake surface, and the more water exchange through inlet and outlet streams or rivers, the more likely it is that 2,4-D residues will be rapidly dispersed and diluted to below detection limits. In small lakes, detectable concentrations of 2,4-D may be carried a significant distance down an outlet stream if the flow is sufficient and degradation is slow. Vertical dispersion is the dominant mechanism of dilution in whole-treated lakes, while a combination of vertical and horizontal water movement contribute to dispersion and dilution in lakes treated over only a part of their surface.

Liquid formulations can be expected to result in higher initial water concentrations than granular formulations, since all of the 2,4-D is applied directly to the water. Granular formulations will generally yield higher sediment concentrations and longer persistence in or on sediments due to a prolonged release of 2,4-D from the granules. Granular

formulations can therefore result in lower water concentrations that may persist somewhat longer than if liquid formulations are used.

The disappearance of 2,4-D from a lake or other natural water body is influenced by a number of factors as discussed in earlier in Section 3.1.4.3. Various water chemistry conditions, physical conditions such as temperature, adsorption to the sediment, and the extent of water currents and dilution can all have very pronounced effects on 2,4-D persistence. This section reviews the disappearance times reported for natural water bodies and for artificial laboratory microcosm and mesocosm studies (small and medium scale simulations) and also looks at the reported factors that can influence such times.

3.5.1 Half-life and Disappearance Time

Table 3.5 summarizes the half-lives of various 2,4-D forms as reported in research papers, as well as the time to non-detection or very low levels as specified in the table. A half-life is the time required for an herbicide to reach half of its initial concentration immediately following application. Depending on the type of study and the data collected, a half-life may be mathematically calculated using several analyses over time, or may be interpolated from tabular data or figures given in a cited paper as was sometimes necessary in this review.

Time to disappearance is the time necessary for an herbicide concentration to drop below the lower limit of analytical detection. This value is usually 0.05 ppm for 2,4-D in sediment and 0.01 to 0.005 ppm in water. Because of the variety of analytical techniques used over time (chemical analysis, bioassay), the Limit of Detection (LOD, the lowest herbicide concentration that can be reliably quantified) has varied over time.

Half-life values are important for estimating persistence, but can be misleading if the herbicide remains in the environment at significant concentrations after the half-life time. Times to disappearance are a useful tool for predicting impacts on biota and wildlife, particularly when used with calculated or estimated half-lives. The persistence of 2,4-D varies widely depending on the conditions of the system being tested. Therefore it is not surprising that a wide range of half-life and disappearance times has been reported in the literature.

The majority of scientific literature describes laboratory microcosm/flask and aquarium studies as opposed to natural water body studies. Laboratory studies allow more control over water chemistry and temperature as well as the determination of degradates formed if desired. They are useful for isolating the effect of specific factors on 2,4-D persistence. On the other hand, studies in natural lakes and ponds subject the herbicide to 1) temperature and pH variations, 2) a greater variety of microorganisms, 3) a greater water:sediment surface ratio (thus affecting adsorption), and 4) to dilution and movement throughout the lake. There are few reported data concerning 2,4-D persistence in sediment; most of the water/sediment studies report half-lives in the water phase or for the two phases as a whole.

Two EPA guideline aquatic metabolism studies were conducted at 25°C using ^{14}C-labeled 2,4-D acid. In the 30-day aerobic study (30), no significant degradation was observed in flasks of Lake Mendota, Wisconsin sediment and water. In the other study, using pond sediment and water and conducted under anaerobic conditions at pH 7.6 to 9.6 (22), degradation occurred very slowly with a calculated half-life of 312 days.

However, other data cited by the authors suggest that this number is too high, and that the half-life is actually much shorter. In contrast with the very long persistence calculated in these studies, Bryant (1992) calculated a half-life for the acid of 45 days in a laboratory anaerobic study using pond sediment. In a parallel experiment, the same sediment was inoculated with sediment microorganisms adapted to degrading 2,4-D. The decline period of about 15 days followed an initial lag period of about 30 days, when little degradation occurred. Residues were still detected after 50 days. The acid half-life dropped to 6 days when the same sediment was conditioned with 2,4-D treatments prior to starting the study and the 2,4-D allowed to totally degrade. No residues were detected after about 16 days in this second experiment.

Two Arkansas soils, a silt loam and a silty clay, were mixed with water to a slurry and incubated in another laboratory flask study (Johnson *et al.*, 1995b). Soils were taken from the surface and from 24 inches in depth. Half-lives in the surface soils were 26 days for the silt loam and 16 days for the silty clay. For the deeper soils, the respective half-lives were 45 and 43 days.

Reinert and Rodgers (1987) reviewed several persistence studies. They reported a half-life of 14.7 hours in a laboratory test of 2,4-D acid in water plus a river sediment previously exposed to 2,4-D (92). Another 2,4-D acid laboratory test with sediment and water was inoculated with microflora selected for their ability to degrade 2,4-D acid. Half lives in the water were 1.4 to 2.8 hours.

Most of the studies of BEE persistence have already been reported in review articles or in Washington State (1993). They are briefly reviewed here, along with a several new studies, in order to bring relevant data together in one document.

Kolig (1985) used aquaria containing pond and river water and Teflon strips populated with typical aquatic micro- and macrobiota collectively called *aufwuchs*. The very short half-lives of 0.03 to 0.14 days were undoubtedly an effect of microbial action. Lim (1978) set up large cylinders of water, mud, plants, and "some organisms" in the laboratory, then treated them with BEE granular at a rate of 20 lb/acre. Temperatures were maintained at 7.5°C to 10°C. Initial concentrations of the acid in the water were 0.03 ppm. By 30 days, release of 2,4-D from the granules had caused residues to rise to 0.26 ppm where they remained for about 30 days then dropped to nondetectable levels at about 6 months.

Paris, et. al. (1975) inoculated flasks of water with a bacteria and a fungus from natural waters and applied BEE at a target concentration of 0.03 to 6.6 ppm and cultured the flasks at 28°C. BEE broke down very rapidly with a calculated half-life of 0.11 days (about 2.6 hours), although in some of their tests the BEE had disappeared in as little as 15 minutes.

Birmingham and Colman (1985) created outdoor artificial ponds containing water, a "virgin loam soil", and Eurasian watermilfoil. The ponds were treated with 20.5 lb/acre of a BEE granular formulation. Temperatures were 25°C at the start of the experiment but dropped to freezing within 60 days. According to a table of residues from this study presented in Washington State (1993), BEE concentrations in water were 0.16 ppm on Day 1 and dropped to 0.001ppm by Day 15. 2,4-D in the water increased from 1.8 ppm on Day 7 to a maximum of 3.0 ppm on Day 13, then declined to 0.2 ppm by Day 182. The initial slow rise of water concentration is characteristic of granular formulations,

which release the active ingredient slowly into the water. In sediments, the maximum BEE concentration occurred about 7 days after treatment, then declined to less than 0.1 ppm in the next 42 days. Acid residues in the sediment were 8.0 ppm on Day 1, declined to 0.4 ppm by Day 82, then remained at about the same level through Day 182. As noted above, temperature had dropped very low by two months into the study, which undoubtedly slowed the degradation of the chemical.

Reinert and Rodgers (1987) and Washington State (1993) reviewed a study reported by Hoeppel and Westerdahl (1983) on Seminole Lake, Georgia. Two plots, 3 to 6 feet in depth, were treated with BEE at 20 and 40 lb acid equivalent per acre. Using data reported, Reinert and Rodgers (1987) calculated a half-life in the water of 3.3 days for 2,4-D acid. The BEE form was not detectable in the water by 24 hours after treatment. The highest concentration in water was 0.68 ppm, observed on Day 1 after treatment in the 40 lb/acre plot. Concentrations of 0.51 ppm to 0.65 ppm were observed in the 20 lb/acre shallow plot. BEE was not detectable in the water by 24 hours after treatment, and 2,4-D acid had declined to less than 0.01 ppm by Day 13 in both plots. The maximum sediment concentration (8 ppm 2,4-D) occurred at Day 7 in the 20 lb/acre plot. The highest sediment 2,4-dichlorophenol concentration was found in the 40 lb/acre plot at an unspecified time.

Daly (1971) also reported on persistence of 2,4-D acid in Lake Seminole when treated with BEE at 100 lb granular/acre. Water residues increased from 2 ppm to 5 ppm acid from Day 1 to Day 7, after which water monitoring ceased. Sediments contained 0.1 ppm acid for 12 days, after which no 2,4-D was detected.

Reinert and Rodgers also reviewed a report of spot control of aquatic macrophytes in Fort Cobb Reservoir, Oklahoma using a BEE granular formulation. In that study (67), the overall aqueous BEE half-life was reported as 2.2 days.

Otto *et al.* (1983) reported the results of another Fort Cobb Reservoir study. Two deep coves in the 6.6 mile long reservoir were treated with granular BEE, one at 20 lb acid equivalent per acre and the other at 40 lb per acre. Water temperatures ranged from 25.6°C in late August when the application was made to 19.5°C 56 days later. Water pH was 7.5 to 8.1. Dissolved oxygen was 3.0 ppm (very low) at the start of the study, and had risen to 6.2 after 28 days.

Half-lives for the aicd were less than 4 days. In the treated plots, mean residues of 0.010 ppm (high rate) and 0.0006 ppm (low rate) were found at 14 days, the last water sampling point. The maximum residues found were 0.06 (surface) and 0.07 (bottom) on Day 1 in the high rate plot. In the low rate plot, maximum residues were 0.039 ppm (surface) and 0.065 ppm (bottom), also on Day 1. At three sampling stations 100-200 yards away at the mouths of the bays, residue profiles were similar, with maximum residues of 0.06 ppm (high rate) and 0.08 ppm (low rate) on Day 1. Residues similar in magnitude to those in the treated plot were still present at 14 days.

Mean sediment residues in the low rate plot were 1.11-1.16 ppm on Days 1 and 4, then declined slowly to a value of 0.124 ppm on Day 56. Maximum residue was 3.35 ppm on Day 1. In the high rate plot, the Day 1 mean sediment residue was 3.97 ppm (maximum 6.59 ppm). Residues then declined to a mean of 0.081 ppm on Day 56, except for Day 14 when high mean residues of 2.61 ppm (maximum 7.64 ppm) were found. The erratic nature of the residues found preclude calculation of a meaningful sediment half-life.

Frank and Comes (1967) applied BEE granular to a small pond near Denver, Colorado at a calculated rate of 1.33 ppm. The water concentration was only 0.024 ppm at Day 1, while concentration in the sediment was 5 ppm. The maximum concentration measured in the water was 0.067 ppm. Sediment concentrations decreased about 50% in one week and reached 0.10 ppm at 56 days, and were undetectable 85 days after treatment.

Smith and Isom (1967) treated plots in Watts Bar Reservoir and Guntersville Reservoir, Alabama, with 40 and 100 lb/acre of BEE granular. The maximum residue concentration found in the water was 0.157 ppm 2,4-D acid. In the Watts Bar Reservoir, limited sediment samples had concentrations of 0.95 to 56 ppm acid on Day 4 after treatment, 0.15 to 35 ppm on Day 24, and 0.24 to 58.8 ppm 10 months after treatment. Sediment residues in the Guntersville Reservoir, residues were in the "mid-ppb" (parts per billion) range over the 9 months following application, except for one sample with 33.6 ppm acid at Day 42.

Shearer and Halter (1980) attribute the "erratic" nature of sediment residues in this study and that of Frank and Comes to poorly-formulated granular products. However it is more likely that varying water temperatures (particularly in colder reservoir bottom water), sediment composition, and the presence or absence of water currents were much more influential in causing spatial differences in residues and extended persistence. In addition, sediment samples taken shortly after treatment, whether by bulk "grabber" type equipment or corers, have a high likelihood of including some of the granules lying on the sediments. Depending on the size of the sample, even 1 or 2 such granules can cause an inaccurate high apparent residue concentration.

Studies of 2,4-D persistence in Skaha and Okanagan Lakes, in the Okanagan Valley of British Columbia, were reviewed by Dynamac (1988) and Shearer and Halter (1980). Test plots were treated with BEE at rates of 9.8 to 40 lb/acre. BEE degraded rapidly with a half-life in water of less than 3 days. The review reports maximum water concentrations (presumably of 2,4-D acid) during the period of 0 to 7 days after treatment were 0.36 ppm, 0.07 ppm, and 3.25 ppm for the 22, 33, and 40 lb/acre treatments respectively. Maximum sediment concentrations were 17.6 ppm, 57.3 ppm, and 288 ppm for the same three application rates between Days 8 and 161.

One site in Lake Okanagan was treated with 10, 20.5, and 29.4 lb/acre BEE. Washington State (1993) reported that "the concentration of 2,4-D in the water reached a maximum of 4,000 mg/L 6 days after treatment, but was not detected after 59 days." Since 4000 mg/L is the same as 4,000 parts per million, or 4 parts per thousand, this is probably a typographical error and should be 4000 ug/L, or 4000 parts per billion, equivalent to 4 parts per million (ppm). Sediment concentrations were highest 2 days after treatment at 34 ppm, and were undetectable after 86 days.

Lim and Lozoway (1979) monitored 2,4-D residues in test plots in the north arm of Lake Okanagan following treatment with BEE granular at 20 lb/acre (100 lb granular/acre). 2,4-D acid residues were generally 0.002 to 0.25 ppm and peaked at 0.14 ppm. Residues were nondetectable in 3 days. Sediment residues were generally about 0.2 ppm and peaked at 0.5 ppm. No residues were detected in sediment by 17 days after treatment. The plot was known to have lateral underwater currents, which contributed to the short water residence time of the residues.

Otto *et al.* (1983) conducted tests in Banks Lake in eastern Washington State using BEE. Banks Lake is a 27 mile long reservoir fed by a canal from the Columbia River behind Grand Coulee Dam. There is a dam at the southern end. In July, 1981 two 40-acre shoreline plots approximately a mile apart and about 4 miles north of the dam were treated with granular BEE, one at 20 lb acid equivalent per acre and one at 40 lb per acre. Water pH was 7.1-7.8 during the study, temperatures were 15.7 to 20.4°C, and dissolved oxygen was 9.1 to 10 ppm. Water depth in the plots was 2-4 feet. No data are given regarding vegetation in the treated areas. Water samples were taken on the day after application and on Days 4, 7, and 14. Sediment samples were taken on the same days, and on Days 28 and 56.

Half-lives of the acid in both plots were approximately 4 days or less. Mean residues in the low rate plot were about 0.025 ppm on Day 1, when the maximum single-sample residue of 0.028 ppm was measured. The residues declined to 0.004-0.008 ppm by Day 14. At three sampling points 300-600 feet away from the plot (2-7 foot depths), the maximum residues of 0.012-0.013 ppm occurred on Day 1. By Day 14, residues at these distant points were 0.003 to 0.007 ppm.

In the high rate plot, acid half-lives were less than 4 days. Mean residues were 0.077 ppm at the surface and 0.022 ppm on the bottom on Day 1. The maximum residues measured were 0.138 ppm (Day 1 surface) and 0.200 ppm (Day 4, bottom). By Day 14, mean residues had declined to 0.003 ppm at the surface and 0.011 ppm on the bottom. At three distant sampling points 300-600 feet from the plot (3-10 foot depths), mean residues were 0.107 (surface) and 0.005 ppm (bottom) on Day 1, which declined to 0.003-0.004 ppm by Day 14. The maximum measured residues at these points were 0.195 ppm (surface) and 0.013 ppm (bottom) on Day 1.There were no consistent significant differences between surface and bottom water residues in either plot.

Sediment mean residues in the low rate plot were 4.60 ppm on Day 1. Mean residues increased to a maximum of 11.2 ppm on Day 7, then declined to 0.324 ppm by Day 56. The maximum single residue of 16.4 ppm occurred on Day 7. In the high rate plot, an initial mean residue of 24.7 ppm was found on Day 1. Single samples on that date ranged from 19.3 to 31.53 ppm. The Day 4 mean residue was 7.03 ppm. Day 7 and 14 mean residues were 25.6 and 23.7 ppm, with single samples ranging from 11.8 to 37.1 ppm (Day 7) and 10.4 to 35.7 ppm (Day 14). By Day 28, the mean residue had declined to 4.74 ppm, and were 0.0006 ppm by Day 56. The residue pattern suggests that the Day 1 high residues may have been caused by trapping some BEE granules along with sediment. The second peak at 7 and 14 days was probably caused by prolonged release of 2,4-D from the granules combined with little or no water movement to disperse the residues.

Water samples were also taken at the Banks Lake outlet dam, approximately 4 miles from the BEE-treated plots. Mean residues of 2,4-D acid were 0.0005 ppm on Day 1, reached a maximum of 0.007 on Day 4, then declined to 0.003 ppm on Day 14. It should be noted that two additional plots, about 6 miles from the dam, were treated with 2,4-DMA at the same time as the BEE experiments, and could be expected to contribute to residues measured at the dam.

DMA salt and 2-EHE formulations are not being considered for use in Washington State for aquatic weed control. However, a few studies using those forms of 2,4-D are briefly mentioned here as a matter of interest.

Concha and Shepler (1993b) added 2-EHE to water from a Michigan river. They found that breakdown of 2-EHE was very rapid and calculated a half-life of 6.2 hours. After 24 hours, only 7.2% remained as parent material. In another study, Solomon *et al.* (1988) set up polyethylene enclosures in an Ontario acid bog lake (pH 4.5) and treated at 0.9 and 2.2 pounds of 2-EHE per acre. Half-lives of 4.5 and 7.8 days in water were calculated for the low and high rates, respectively. Disappearance of 95% of the material had occurred by 24.2 and 23.5 days at the two rates.

Reynolds (1995) conducted a laboratory anaerobic aquatic study similar to the 2,4-D acid study by Concha and Shepler (1993b) reported above, but using 2,4-D DMA salt. He found a half-life of 1611 days (4.4 years).

Two pond aquatic dissipation studies using DMA salt were carried out in North Carolina and North Dakota by Hatfield (39, 40). Two applications were made 30 days apart and the ponds monitored for an additional 180 days. Half lives in the water were 19.7 and 13.9 days after the first application, and 2.7 and 6.5 days after the second application. DMA salt persisted in the water for more than 21 days and 60 days after the second applications in the two ponds. Sediment half-lives were 7.6 and 2.0 days after the first and second applications in North Carolina, and 29.5 days after the second application in North Dakota. The chemical was not detectable after 21 days following the second application in the North Carolina lake, but was still present at the end of the study, 180 days after the second application, in the North Dakota lake.

Johnson *et al.* (1995a) applied an unspecified 2,4-D liquid product at 0.98 pounds active ingredient per acre to a flooded rice paddy in Arkansas. They calculated a half-life of 4 days, with 95% degradation in 15 days and non-detection by 28 days. While not stated specifically in the reference, the information appears to apply only to the water. A half-life of 50 days in a flooded clay soil was reported by Kuwatsuka and Miwa (1989), but the formulation used was not specified.

Grover *et al.* (1997) monitored Saskatchewan farm ponds and dugout waters for herbicides from the fall of 1987 to spring 1989. They found residues of 2,4-D at 0.05 ppm or greater in 81% of the samples at mean concentrations from 0.07 ppm to 0.09 ppm. The residues were attributed to agricultural use of 2,4-D in the pond drainage areas.

Donald and Syrgannis (1995) sampled 19 prairie lakes in agricultural Saskatchewan for residues of several pesticides associated with agricultural activities in their watersheds. There were 10 permanent lakes with areas ranging from 0.8 to 40 square miles, and 9 semi-permanent lakes that dried partially or entirely during the summer having areas of 1.2 to 14.7 square miles, plus one lake 128 square miles in area. Detection limit in water was 0.03 ppm. 2,4-D was detected in the water of 64% of the permanent lakes and in 85% of the semipermanent lakes after they had refilled. The overall maximum and mean water concentrations were 0.43 ppm and 0.10 ppm, with a median of 0.05 ppm. The mean and median water values for permanent lakes were 0.071 ppm and 0.04 ppm. For semipermanent lakes mean and median were 0.117 ppm and 0.08 ppm. Less than 10% of the sediment samples from semipermanent lakes contained detectable residues of 2,4-D, but concentrations are not reported by the authors. Lakes were sampled during spring and early summer. Freezing winter air and water temperatures were probably responsible for the long persistence of pesticides, as well as a continuing input from spring agriculture.

Schultz and Harmon (1974) and Shultz and Gangstad (1976) treated three ponds each in Florida, Georgia and Missouri with 2, 4, and 8 lb/acre of 2,4-D DMA salt formulation. The ponds had from 5% to 10% aquatic vegetation present, with 50% in one Florida pond. They reported that residues in water were undetectable (less than 0.005 ppm) in 3 to 28 days in Florida, 14 to 28 days in Georgia, and between 28 and 56 days in the Missouri ponds. Maximum water residues were reached in the first three days after application and were similar in all 9 ponds. Maximum residues ranged from 0.087 to 0.692 ppm. Sediment residues in all three states were low, with maximums of 0.005 to 0.17 ppm, usually reached between Day 7 and Day 14. Sediment residues disappeared in 14 to 56 days.

3.5.2 Degradation Products

Unlike soil degradation studies, few of the reviewed aquatic degradation studies investigated the nature of metabolites formed. Information on degradates formed in moist soil should be similar to those in aerobic sediments.

In their anaerobic laboratory flask study with radiolabeled 2,4-D acid, Concha and Shepler (1994b) found the major metabolite to be 2,4-dichlorophenol (2,4-DCP), which reached a maximum at 30 days of 21.6% originally-applied ^{14}Carbon radioactivity (AR), then declined to 4.2% AR at 365 days. CO_2 was the other primary metabolite, reaching 22.1% AD after 365 days. Trapped volatile organic vapors contained small amounts of metabolites at one year. These included 4-chlorophenol (4-CP) at 1.9% AR, 2,4-dichloroanisole (2,4-DCA) at 0.7% AR, and 2,4-DCP at 0.7% AR. Humic and fulvic acids bound to the sediment were reported as 27.1% AR at 240 days.

Zhang and Weigel (1989) proposed a degradation pathway for 2,4-DCP in anaerobic sediments. 2,4-DCP is metabolized to 4-CP, then to phenol, to benzoate, and finally to methane and CO_2. They believe that at least five different organisms are involved sequentially in this process. This is predicated on one organism being responsible for each step, and does not address the possibility that a single organism can perform multiple steps in the breakdown.

Hatfield detected 2,4-DCP in sediment within one day of application of a DMA salt to a North Carolina pond (41). In both that study and one in North Dakota (42), 2,4-DCP and 2,4-DCA were detected at low concentrations in water samples immediately after application. Hoeppel and Westerdayl (1983) found 2,4-dimethylnitrosamine in Seminole Lake, Georgia, at concentrations less than 0.01 ppm after treatment with BEE granular at 20 and 40 lb acid equivalent/acre.

Otto *et al.* (1983) found traces of 2,4-DCP in some sediment samples in Banks Lake, particularly in DMA-treated plots, but 2,4-DCP was also found in some pre-treatment sediment samples. They attribute these residues to contamination from non-study sources. No 2,4-DCP was found in water samples.

3.5.3 Physical and Chemical Factors

Few studies were found that were designed to ascertain the effects of various water and sediment parameters on the persistence of 2,4-D in aquatic systems. Most were conducted under a controlled set of conditions, or were field studies under uncontrolled conditions. In most studies with variable conditions, it was not possible to separate out the effects of

the specific variables discussed below. Aside from hydrolysis, the major degradation mechanism for 2,4-D and its esters is microbial degradation. Some general principles expected to affect the degradation of most biologically degraded pesticides, including 2,4-D, in aquatic systems are discussed below. Several of the factors discussed earlier that affect soil persistence can also be expected to influence persistence in aquatic systems.

- **Temperature**

  Temperature has a pronounced effect on the rate of chemical reactions and metabolic processes. In the case of 2,4-D, where biological degradation predominates, temperatures outside the optimum range for 24,-D-degrading microflora will increase persistence. Johnson et al (1995b) added 2,4-D acid to slurries of a silt loam and a silty clay, then incubated them at 15°C and 30°C. 2,4-D degraded from about 1.3 to 4.8 times faster at the higher temperature. Their results indicate that temperatures as high as 30°C do not incapacitate 2-4-D degrading microorganisms in soil.

  Water temperatures high enough to inhibit 2,4-D metabolism in bacteria and fungi are unlikely to occur in Washington lakes. In this moderate climate, the most likely effect is that caused by cooler temperatures at night and at greater lake depths. Because of the high specific heat of water, it is a good thermal insulator, so the temperature of average size lakes does not vary much from night to day at the surface and even less at greater depths. Water temperatures of perhaps 50°F to 70°F may be expected in medium size lakes during the times when aquatic weed control is a concern. Smaller or shallow lakes may be expected to be warmer than larger lakes.

  In deeper lakes a thermocline can form during summer months wherein there is a sharp boundary between the warmer surface water and cold deeper water. Thermoclines can increase 2,4-D persistence in two ways. As there is little exchange of water across the thermocline, there is less water volume to dilute the herbicide, particularly in lakes treated over a large percentage of their surface. Any 2,4-D that penetrates the thermocline encounters a colder environment where degradation by microbes is slowed.

  Laboratory studies, typically conducted at 20°C to 25°C (68°F to 77°F), may yield half-lives that are somewhat shorter than studies in ponds or lakes. In addition, the latitude of the lake, with varying temperature regimes, make comparisons difficult.

- **pH**

  In the Arkansas soil slurry tests described earlier (Johnson *et al.*, 1995a), half-lives were shorter in pH 6.9 slurries using surface soil (26 and 16 days for the two soils) than in pH 5.0 slurries from 24 inches depth (45 and 43 days). However the 24-inch soils contained fewer 2,4-D degrading microflora than surface soils, so the pH effect can not be isolated, although the lower pH may have influenced the density of the microflora population to some extent. As reported above, an acid pH as low as 4.5 in an Ontario bog lake treated with 2-EHE did not appear to significantly affect 2,4-D degradation (90). Half-lives of 4.5 and 7.8 days were reported for two application rates, with disappearance times of 23 to 24 days.

While 2,4-D acid in water is chemically stable at typical environmental pH values, Table 3.2 illustrates that BEE degrades hydrolytically much more rapidly in neutral and basic waters (pH 7-9) than in acidic water (e.g. pH 5). The same is true for 2-EHE. Even at pH 6, BEE degrades much faster than it does at pH 5.

Most natural waters pH values are typically 6 to 9 (36), aside from unusual lakes such as bog lakes, alkaline lakes, or those subject to acid rain, few of which are found in Washington State. The higher values are generally found during spring and summer, when more vigorous algal growth use large amounts of dissolved CO_2, driving the pH toward alkalinity through the carbonate/bicarbonate cycle (103). The more eutrophic (nutrient rich) a lake is, the larger the chance of enhanced algal growth and a higher pH. BEE would therefore be expected to degrade more rapidly to 2,4-D acid in lakes with neutral or slightly alkaline pH and more eutrophic lakes than in more acidic or oligotrophic (nutrient deficient) lakes.

- **Aerobic state**

The amount of oxygen dissolved in the water can have an effect on 2,4-D persistence since degradation is largely the result of the action of aerobic microflora, which require oxygen, although a few anaerobic microbial 2,4-D degraders have been reported. Dissolved oxygen (DO) levels are typically 6 ppm to 10 ppm in well-mixed natural water bodies, though levels outside of that range are not unknown. The colder a water body, the higher the saturation value, or the maximum amount of DO that it can hold.

DO primarily enters the water from the atmosphere and from the photosynthesis of algae and submerged plants. Dissolved oxygen is consumed by fish and microflora in the water column on the sediments, and zooplankton and bottom-dwelling organisms such as aquatic insects. Plants also consume limited amounts of oxygen in their "dark cycle" metabolism at night. Decay of vegetation and other organic materials, primarily on the lake bottom, also consumes significant oxygen. If a thermocline forms, water circulation is impaired and the water below the thermocline will become anaerobic if all of the dissolved oxygen is consumed.

Some aquatic studies of 2,4-D degradation previously cited described an initial lag period caused by the inhibiting effect on aerobic 2,4-D-utilizing microflora of the depletion of dissolved oxygen in the water as the aquatic plants died and decayed. Little herbicide degradation occurred until natural restoration of adequate dissolved oxygen. After natural reestablishment of oxygenated conditions, the microflora reproduced rapidly using the newly-available carbon and began feeding on the endothall. No studies were found specifically addressing oxygen depletion resulting from macrophyte or algae kills for 2,4-D. Observed initial degradation lag periods were attributed to a low initial microflora population, rather than a depleted population. A degradation lag caused by heavy macrophyte kill may be expected in natural water bodies with a large macrophyte population treated with an herbicide over a significant portion of the lake where there is little water circulation to quickly restore dissolved oxygen.

The speed of restoration of oxygen in a natural lake would be dependent on water temperature, mixing throughout the water column, introduction of oxygenated water from elsewhere in the lake, and the contributions of algal photosynthesis. In the case

of a poorly mixed lake or of a treated shoreline area having a heavy macrophyte kill, reoxygenation might be delayed and 2,4-D persistence extended. The effect would be more pronounced in lakes with heavy macrophyte growth given a whole-lake treatment.

- **Trophic state**

  The trophic state of a natural water body exerts an indirect influence on 2,4-D persistence. Because eutrophic (high nutrient concentrations) and high-end mesotrophic lakes are likely to have a larger macrophyte population, they are more likely to be included in an aquatic weed control problem. In eutrophic lakes, with a high level of nutrients, microflora populations can be expected to be greater than in mesotrophic or oligotrophic lakes (medium to low nutrient concentrations). Therefore a larger population of microflora, many of which can degrade 2,4-D, can be expected to be present and persistence would be expected to be shorter. Conversely, when a large pool of carbon is available from decaying plant and animal matter, 2,4-D may not be utilized by microorganisms as readily as in lower-trophic state lakes. Mesotrophic and especially eutrophic water bodies usually have a higher population of algae that can substantially contribute to the restoration of DO following an aquatic plant kill from a 2,4-D application as discussed above, and can thus help speed degradation by aerobic microflora such as *Arthrobacter*.

  One possible negative effect of a eutrophic state on 2,4-D persistence should be mentioned. As stated above, the high nutrient levels usually give rise to a dense population of algae and various macrophytes as well as phytoplankton and benthic organisms. In any lake, there is a continuous process of decay of a large number of dead organisms occurring, particularly on the lake bottom. In a eutrophic lake a proportionately larger amount of decaying organisms can be expected. The first stages of this decay are generally aerobic, which uses dissolved oxygen. If conditions occur such as poor water circulation, the formation of a thermocline, or a population crash of a dense species population, the bottom of the lake (and possibly shallower depths) can become anaerobic. The inhibiting effects of low DO on 2,4-D-degrading microorganisms then becomes a significant factor in the persistence of the compound.

- **Adsorption to sediment**

  Adsorption and uptake of 2,4-D by aquatic macrophytes and algae is addressed in Section 4 of this document. The effect on persistence of adsorption to sediment particles was not addressed in any of the references found during this review. Any such effect would be expected to be manifested through increasing or decreasing the availability of the chemical to sediment microorganism. In the endothall papers reviewed in Section 3.1, there was some ambiguity among the authors as to whether adsorption to sediment increases the degradation rate by concentrating the herbicide so the microorganisms can utilize it more efficiently, or slows degradation by sequestering the chemical from the microorganisms. The nature of the effect probably depends on how tightly the chemical is bound to the sediment. It is reasonable to assume that the same thing applies to 2,4-D and its esters.

  In a turbid water body with significant amounts of particulate sediment suspended in the water, there is a greater solid surface area for 2,4-D adsorption and release to

lower-concentration water than in an essentially two-dimensional lake bottom. Since 2,4-D-degrading microflora can populate the suspended sediment as well as bottom hydrosoil, adsorption to suspended sediment can make the chemical more readily available for attack by those organisms. This can facilitate degradation in medium to large lakes without a large microbial population in the water column.

- **Transport and dilution**

  The most important and obvious physical processes affecting 2,4-D concentration in larger water bodies are most likely dispersion or transport from the treated site by water currents and dilution by untreated water. With its high water solubility, 2,4-D acid is easily transported within water currents in a lake. Obviously, the larger the area of a lake that is treated, the more water current will be needed to dilute and disperse the herbicide, with the extreme case occurring in whole-lake treatment.

  In lakes without significant inflow or outflow, most dilution of 2,4-D-treated water will occur through vertical movement in the water column. Solar heating is not as important to water movement in these lakes as the effects of wind. While sunlight can heat the surface waters, the warmer water tends to stay at the surface and little vertical circulation occurs. Wind can induce mixing between water depths even at low velocities. Surface water driven against a shoreline is driven downward and mixes with lower depth water, diluting the pesticide concentration of the surface water and may carry it into contact with sediment-dwelling microflora.

  In lakes treated over only a part of their surface, dilution is a very significant mechanism for reducing 2,4-D concentration in the treated areas. Dilution can occur from wind-driven water currents or water flow through the lake, both of which can give rise to both vertical and horizontal mixing and dilution. Movement of water through the lake can result from inlet streams and rivers, storm runoff outlets, submerged springs, or diffuse surface runoff into the lake from the surrounding basin. Operation of dams or weirs or other controls on lake outlets will impact the magnitude of water movement in lakes or reservoirs and consequently the dispersal of treated water.

  If a large portion of the lake is treated, 2,4-D can be carried out of a lake and into outlet streams if water movement is rapid or if there are insufficient microflora to break the herbicide down quickly. In view of the potential impacts on river biota, including fish, far from the treated lake, water mass movement and the specific water budget for a particular lake must be taken into consideration when applying 2,4-D. In western Washington, rainfall events, particularly in the months preceding July and after mid-September, can rapidly dilute 2,4-D residues in a treated lake due to stream inflow and surface runoff, and can also move treated water into outflow streams more rapidly than anticipated before degradation is completed.

  In contrast with endothall, few study reports were obtainable for this review that gave information on time and distance measurements of 2,4-D concentrations in lakes or ponds. Since the extremes of persistence times of endothall and 2,4-D overlap somewhat, the dispersion and dilution data presented for endothall in the Transport and Dilution subsection of Section 3.1.4 of this document can give a rough idea of the effects of dispersion and dilution on a biodegradable pesticide in general.

Monitoring of Guntersville Reservoir, at 68,000 acre waterbody on the Tennessee River, offers a good illustration of the effect of dilution and flushing on residue levels (74). A liquid formulation of 2,4-D DMA was applied to 60 acres in a shallow (2-3 feet depth) bay in the lower end of the lake at a rate of 18.7 pounds/acre (nominal 2.0 ppm). Water and sediment were monitored for residues for 14 days. It is not clear from the report exactly where sampling occurred, but it is believed that samples were taken from six sites at unknown locations within the treated area. No residues were found in sediments at any time. Maximum residues of 1.125 ppm were found in water 6 hours later (mean 0.499 ppm). By 48 hours after application, residues from 0.015, 0.026, and 0.097 ppm were found in three of the sites. On Day 7, only one site had 2,4-D residues (0.046 ppm), and by Day 14 the residues at the same site were 0.022 ppm.

As reported above, in a study of Banks Lake, Washington, Otto *et al.* (1983) found residues of 2,4-D acid from 20 lb a.e./acre and 40 lb a.e./acre BEE granular applications in water samples taken 300-600 feet outside the treated 40 acre shoreline plots on the day after application. Residues in these peripheral samples were from about 30% to 100% of the treated plot residues in both surface and bottom samples. Sediment samples were not taken outside of the treated areas. Since the treated plots were roughly square, judging from sketches in the report, a 40-acre treated plot would be about 1320 feet on a side. Therefore the peripheral samples were relatively close to the plots, given the treated areas. In the same study, water samples from the Banks Lake outlet dam, approximately 4 miles from the plots, showed residues of 2,4-D acid of 0.0005 ppm on Day 1, 0.007 ppm on Day 4, 0.006 ppm on Day 7, and 00.3 on Day 14. Pre-treatment samples were not taken. Two other experimental plots approximately 6.5 miles from the dam were treated with DMA at 20 and 40 lb a.e./acre at the same time as the BEE plots and undoubtedly contributed to the residues found at the dam.

In the same paper, Otto *et al.* reported on a residue study in Fort Cobb Reservoir Oklahoma, also reviewed above. Two narrow coves were treated with BEE at either 20 or 40 lb a.e./acre. The peripheral sampling stations were 300-600 feet from the treated plots, aligned across the mouths of the coves. Residues were found from Day 1 to final sampling on Day 14 at levels generally from about 20% to 200% of those in the treated plots. No inlet streams for the coves are indicated on maps of the area.

- **Type of formulation**

The use of liquid formulations usually results in higher initial water residues than with granular formulations since the entire application is present immediately in the water column. Sediment concentrations can be expected to be lower with liquid formulations since the chemical is injected in the upper water column, relatively far from the sediment surface, and must be carried to the sediment by water currents or dispersion.

In contrast, use of a granular formulation can be expected to give higher initial sediment concentrations and lower water concentrations. As 2,4-D (or most other pesticides) is released from the granules over time, sediment concentrations will likely persist, albeit at low levels, for a longer period than with a liquid formulation and water concentrations are likely to be very low or non-detectable. Since the bottom waters in deeper lakes and shoreline areas are frequently colder than surface

and mid-water depths, the higher sediment concentrations that granulars may produce are more likely to persist for a longer period in the colder water due to inhibition or slowing of microbial metabolism of the chemical.

Except in very shallow littoral areas, 2,4-D in liquid formulations can be expected to have less direct impact on deep-water or sediment-dwelling organisms than comparable granular formulations because of generally lower sediment concentrations and shorter persistence resulting from use of the liquid form.

Wilkinson (1964) tested the release of various 2,4-D derivatives from granulated formulations in 9-liter static flasks of distilled water. Initial concentrations of 10, 15, and 20 ppm were used, and the flasks were kept in a greenhouse (temperature not reported). For 2,4-D acid, steady-state concentrations in the water were achieved in 4 to 16 hours, with the lower concentration taking longer. From 72% to 87% of the acid was released at those times. BEE release at the initial 10 ppm concentration showed a maximum initial release plateau of 63% release at 4 hours; with a total release after 256 hours of 79%. In the 15 and 20 ppm initial concentration flasks, a plateau was reached at 1 hour (49% released), with only slow release thereafter until 256 hours when 71% and 68% of the initial material had been released in the 15 and 20 ppm tests, respectively.

These were static flask tests with no agitation and no sediment. Hence there were no potential biological degraders or potential sites for large-scale adsorption present. The pH of the initial distilled water is given as 5.6, but pH determinations of unbuffered distilled water are unreliable and easily influenced by the addition of compounds such as the granulated herbicides. No subsequent pH measurements were made of the test solutions. Nevertheless, since the flasks were kept in a greenhouse, it is likely that some hydrolysis and/or photolysis of the BEE and acid occurred, skewing the total release percentages downward. The author reported that for other tests conducted, release from attapulgite clay granules was generally faster at higher temperatures, as can be expected.

Lim (1978) used a large Plexiglas or similar acrylic cylinder 8 feet high and about 3.5 feet in diameter to test 2,4-D efficacy. Six inches of Lake Okanagan sediment was placed in the bottom, millfoil was planted, and the cylinder was filled with tap water. Amphipods and snails were also added. A total of 10.14 grams of Aqua-Kleen 20, a granulated BEE formulation, was added to the water. Assuming the cylinder was full, the expected concentration in the water from this treatment should be 5 ppm formulation, or 1 ppm BEE.

During the dosing, the author calculated the rate of fall of the granules to be 11.7 seconds per meter of depth (3.6 seconds per foot). She also observed that only one of the granules remained on the millfoil foliage, the rest coming to rest on the sediment. Residues were measured at top, middle and bottom of the cylinder. Water pH was 8.1-9.0, dissolved oxygen was 10.5-12.7 ppm, and temperature in the (apparently unheated) building was 7.2°C to 13.9°C.

The residue profile of 2,4-D acid measured in the water over 12 weeks of monitoring the cylinders is illustrative of the dynamics of 2,4-D in the undisturbed water. Mean residues on Day 0 (the date of application) were 0.50 ppm at 4 hours. Residues decreased to 0.025 ppm on Day 8, then slowly increased to a peak of 0.26 ppm on

Day 35. Thereafter, residues remained between 0.18 ppm and 0.24 ppm until the final reported sampling on Day 84, when mean residues were 0.084 ppm. No residues were found 24 weeks after treatment.

While adsorption by plants, hydrolysis of BEE, limited photolysis, and microbial degradation are expected to contribute to a decline in the residues of 2,4-D, the delayed occurring sustained residue peak was probably due to the time needed for growth of the sediment microbial populations (dissolved oxygen never dropped to levels expected to inhibit growth) as well as slow release of the BEE from the granular formulation and possible adsorption/desorption from the sediment.

Table 3.5: 2,4-D Persistence in Aquatic Systems

System	Formulation	Initial application rate	Half-life (DT_{50})	Time to disappearance[1]	Comments	Reference
Lab aerobic aquatic microcosm*	2,4-D acid	10 ppm	system: n.s.d.[2]	n.s.d	Lake Mendota, WI sediment and water. 30-day study.	(33) Fathulla, 1996b
Lab anaerobic aquatic microcosm*	2,4-D acid	4.0 ppm	system: 312 D[3] (?)	n.r.[4]	Pond sediment and water, Henry County, IL.	(23) Concha and Shepler, 1994b
Lab anaerobic microcosm, **non-adapted pond sediment**	2,4-D acid	n.r.	system: ca.[5] 45 D, including 30 day initial lag time	system: >50 D	1:1 water:sediment slurry	(11) Bryant, 1992
Lab anaerobic microcosm, **adapted pond sediment**	2,4-D acid	n.r.	system: ca. 6 D, with no lag time After 2d treatment with 2,4-D, DT_{50} ca. 2 D	system: DT_{95}[6] ca. 16 D After 2d treatment with 2,4-D, DT_{95} ca. 16 D	1:1 water:sediment slurry. System inoculated with 2,4-D metabolizing microorganisms. Second 2,4-D treatment made approximately 22 days after the first.	(11) Bryant, 1992
Lab microcosm, saturated silt loam AR	2,4-D (probably acid)	n.r.	system: 26D (from surface) 45 D (from 24 inch depth)	n.r.	Arkansas silt loam. 100% moisture capacity (slurry). 15°C.	(47) Johnson *et al.*, 1995b
Lab microcosm, saturated silty clay AR	2,4-D (probably acid)	n.r.	system: 16 D (from surface) 43D (from 24 inch depth)	n.r.	Arkansas silty clay. 100% moisture capacity (slurry).15°C.	(47) Johnson *et al.*, 1995b
Lab flasks, river sediment and water	2,4-D acid	n.r.	system: 14.7 H	n.r.	Microflora previously exposed to 2,4-D	(92) Spain and Van Veld , 1983 in (60)
Lab flasks, water/sediment	2,4-D acid	n.r.	system: 1.4-2.8 H	n.r.	Inoculated with microflora selected for ability to degrade 2,4-D acid	(66) Ogram *et al.*, 1985 in (72)

Table 3.5: 2,4-D Persistence in Aquatic Systems (continued)

System	Formulation	Initial application rate	Half-life (DT_{50})	Time to disappearance[1]	Comments	Reference
Lab aquaria: pond (Overlook Lake, GA) and river water	BEE	n.r.	water: 0.03 to 0.14 D (BEE)	n.r.	Short half-lives attributed to microbial/ algal communities on Teflon strips in tanks.	(54) Kolig, 1985 in (72)
Large indoor cylinders	BEE granular	20 lb a.i./acre	n.r.	water: 0.13 ppm at 12 weeks, <0.001 ppm at 24 weeks (acid)	8 ft tall x 3.5 ft dia cylinders with water, mud, plants, amphipods, and snails". 7.2 to 13.9°C. pH 8.1-9.0.	(58) Lim, 1978
Lab flasks, water	BEE	ca. 0.03 to 6.6 ppm	0.11 D (BEE)	water: "as little as 15 minutes" (BEE)	Water inoculated with a bacteria and a fungus from natural waters. 28°C.	(69) Paris, et.al., 1975 in (60 and (79)
Outdoor artificial ponds	BEE granular	20.5 lb/acre	water: n.r. sed: ca. 15 D (acid)	water & sed: <182 D (acid)	Ponds 3 feet deep with water and "virgin loam soil" and were planted with Eurasian watermilfoil. Temperature 25°C at treatment, dropped to below freezing within 60 days.	(9) Birmingham and Colman, 1985 in (99)
Lake Seminole, GA	BEE granular	20 and 40 lb a.e./acre	water: 3.3 D (acid)	water: < 24 H (BEE) < 13 D (acid)	Average depth ca. 3-6 feet. 27 acres treated. Water ca. 30°C.	(45) Hoeppel and Westerdahl, 1983 in (31) and (72)
Lake Seminole, GA	BEE granular	100 lb granular/acre	n.r.	sed: >12 D (acid)	Water residues 5 ppm acid at 7 days; no further water monitoring. 10-acre plot treated.	(28) Daly, 1971 in (79)
Skaha Lake and Lake Okanagan, British Columbia	BEE granular	22, 33, 40 lb/acre	water: <3 D (BEE)	n.r.	Max water concentrations at 0-11 D were 0.36 ppm, 0.07 ppm, 3.25 ppm for the 22, 33, 40 lb/acre applications. Max sediment concentrations were 17.6 ppm, 57.3 ppm, 288 ppm for the three applications.	(31) Dynamac, 1988 in (72)
Lake Okanagan, British Columbia	BEE granular	10, 20.5, and 29.4 lb/acre	n.r.	water: ≤59 D (acid) sed: ≤ 86 D (acid)	Single site. Max water concentration 4 ppm @ 6 days. Max sediment concentration 34 ppm @ 2 D.	(31) Dynamac, 1988 in (72)

Table 3.5: 2,4-D Persistence in Aquatic Systems (continued)

System	Formulation	Initial application rate	Half-life (DT_{50})	Time to disappearance[1]	Comments	Reference
Lake Okanagan, British Columbia	BEE granular	20 lb a.i./acre. 0.14 ppm max measured (acid)	n.r.	water: 8 D (acid) sed: <17 D (acid)	Lake "known to have lateral underwater currents". 13-16°C.	(59) Lim and Lozoway, 1978 in (79)
Banks Lake, WA	BEE granular	20 and 40 lb a.e.[7] /acre	water: ca. 4 D or less (acid)	water: >14 D (acid) sed: > 56 D (acid)	27 mile long lake (715,000 acre feet). Two shoreline areas treated, each 40 acres. 16-20°C. Max water residue 0.200 ppm at 1 D in treated plot, 0.195 ppm at 1 D 100 yards away; max sediment residues 36-37 ppm at 7 and 14 D in treated plot; both in high rate plot.	(68) Otto *et al.*, 1983
Small pond, Denver, CO	BEE granular	1.33 ppm	sed: ca. 7D (acid)	water: 0.02 ppm at 24 D, not detected at 36 D (acid) sed: <85 D (acid)	Maximum water 2,4-D acid concentration of 0.067 ppm	(34) Frank and Comes ,1967 in (79)
Watts Bar Reservoir, Guntersville Reservoir, AL	BEE granular	40 and 100 lb/acre	n.r.	sed: > 9 M (acid)	Very erratic pattern of sediment residues in the two reservoirs. Maximum water 2,4-D acid 0.157 ppm. Single 10-month sediment residue of 58.8 ppm.	(89) Smith and Isom, 1967 in (79)
Fort Cobb Reservoir, OK	BEE granular	n.r.	"overall aqueous": 2.2 D (BEE)	n.r.		(67) Oklahoma Water Res. Board, 1975 in (72)

Table 3.5: 2,4-D Persistence in Aquatic Systems (continued)

System	Formulation	Initial application rate	Half-life (DT_{50})	Time to disappearance[1]	Comments	Reference
Fort Cobb Reservoir, OK	BEE granular	20 and 40 lb a.e./acre	water: <4 D (acid)	water >14 D (acid) sed: >56 D (acid)	6.6 miles long lake (143,740 acre feet). Two deep coves treated, 32 acres (low rate) and 14 acres (high rate). 20-26°C. Max water residue 0.06 ppm at 1D in treated plot and 100 yards away; max sediment residue 6.6 ppm at 1 D and 7.6 ppm at 14 D.	(68) Otto *et al.*, 1983
Ontario "bog lake"	2-EHE	0.9 & 2.2 lb/acre	water: 4.5 & 7.8 D (low/high rate) (acid)	water: DT_{95} 24.2 & 23.5 D (low/high rate) (acid)	Average water depth 8 ft. Water pH 4.5	(90) Solomon *et al.*, 1988
Lab study using Tittabawasse River water MI	2-EHE	ca. 0.03 ppm	water: 6.2 H (2-EHE)	water: DT_{95} ca. 26 H (2-EHE)	24-hour study	(25) Concha and Shepler, 1993b
Lab anaerobic aquatic microcosm*	DMA salt	10 ppm	system: 1611 D (4.4 Y) (DMA)	n.r.	Water/sediment system.	(73) Reynolds, 1995
Aquatic dissipation NC	DMA salt	40 lb a.e./acre & 45 lb a.e./acre	water: 19.7 D & 2.7 D (acid) sed: 7.6 D & 2.0 D (acid)	water: < 21 D (after 2d application) (acid) sed: 21 D (after 2d application (acid)	Small pond. Two applications, 30 days apart. The 2 half-lives refer to time after the first application and second application.	(41) Hatfield, 1995a
Aquatic dissipation ND	DMA salt	41.8 lb a.e./acre & 41.8 lb a.e./acre	water: 13.9 D & 6.5 D (acid) sed: 29.5 D after 2d application (acid)	water: < 60 D after 2d application (acid) sed: >180 D after 2d application (acid)	Small pond. Two applications, 30 days apart. The 2 sediment half-lives refer to time after the first application and second application.	(42) Hatfield, 1995b

Table 3.5: 2,4-D Persistence in Aquatic Systems (continued)

System	Formulation	Initial application rate	Half-life (DT_{50})	Time to disappearance[1]	Comments	Reference
Three FL golf course ponds, four GA ponds, three MO ponds	DMA salt	2, 4, 8 lb a.e./acre	n.r.	FL water: 3-28 D GA water: 14 to 28 D MO water: 28+ D FL sed: 14 to 28+ D GA sed: 14 to 28 D MO sed: 14 to 28+ D	Data indicate water persistence dependent on initial rate. Ponds stocked with fish, water hyacinth - FL 10% of surface, GA < 5% of surface. Disappearance times of 28+ days mean residues were detected at 28 days but not at 56 days.	(78) Schultz and Harmon, 1974 and (77) Schultz and Gangstad, 1976
Flooded clay soil	n.r.	25 ppm	soil: 50 D (acid)	n.r.		(55) Kuwatsuka and Miwa, 1989 in (99)
Flooded rice paddy AR	n.r. (liquid formulation)	0.98 lb/acre	water: 4 D (acid)	system[8]: DT_{95}: 15 D (acid) water: not detected at 28D (acid)		(46) Johnson *et al.*, 1995a

1 Detection limit in water = 0.01 ppm, sediment = 0.05 ppm
2 n.s.d. = no significant degradation
3 D/H = days/hours
4 n.r. = not reported
5 ca. = approximately
6 DT_{95} = time to disappearance of 95% of initial residues
7 a.e. = acid equivalent
8 system = soil and sediment residues are not distinguished in the reference
* = EPA guideline registration study

3.6 MICROBIAL DEGRADATION

Summary: The primary mode of 2,4-D acid degradation is the action of microflora - bacteria and fungi that are found in soil, water and sediment. A number of genera of these microorganisms have been identified by researchers and appear to be widespread in the soil and sediment, though not necessarily at high enough population levels to play a part in initially reducing 2,4-D residues. Several experimenters have shown that the presence of such microorganisms in substantial populations is essential for 2,4-D breakdown to occur within a reasonably short time frame. When ponds and lakes without a large population of 2,4-D degrading microorganisms are treated with 2,4-D, there is usually an initial lag period while the microbes that are able to metabolize 2,4-D grow to sufficient levels to have an impact on the residues. Initial lag periods can also be caused by a large macrophyte or algal kill caused by treating heavy growths of these plants. Since most 2,4-D degraders are aerobes, decline in dissolved oxygen in the water as the dying plants decay will inhibit microorganism growth and metabolism of 2,4-D. Environmental conditions that are favorable to such microorganisms can be expected to decrease 2,4-D persistence.

Some microflora can utilize 2,4-D as their sole source of carbon, while others can metabolize 2,4-D, but still require other carbon sources. Genera identified as 2,4-D utilizers include *Achromobacter, Bordetella, Xanthobacter, Streptomyces, Aspergillus, Corymebacterium, Nocardia, Achrobacter, Alcaligenes, Arthrobacter, Flavobacterium, and Pseudomonas*. Members of the latter four genera have been shown to be able to utilize 2,4-D as a sole carbon source (1, 16, 88). Other as yet unidentified genera undoubtedly can also utilize 2,4-D as a carbon source. Han and New (1994) reported eleven bacterial and 72 actinomycete (fungus) isolates that degraded 2,4-D were found in a University of Sydney soil. Vijay *et al.* (1997) reported the fungi *Dichomitus squalens* and *Phanerochaete chrysosporium* can degrade chlorophenoxyacetic acids, such as 2,4-D.

Kamagata *et al.* (1997) sampled 668 pristine soil samples from six regions of the world. These soils had no human disturbance and no 2,4-D application. They found that 59% of the samples contained microorganisms that could degrade 2,4-D, but the degraders totaled only five species. They reported that 2,4-D degraders in pristine sites appear to be different from those from "disturbed" sites. Soil microbial DNA investigations by Ka *et al.* (1955) led to their contention that in soils treated with 2,4-D, a narrowing of the diversity of microbial strains took place, presumably those that degrade 2,4-D.

In the late 1980's, Smith and Mortensen (1991) isolated a *Pseudomonas* species from soil that had received annual 2,4-D applications since 1947. The organism could completely metabolize high concentrations of 2,4-D, but no other phenoxyalkanoic acids in the same class of compounds as 2,4-D.

A number of studies have presented evidence of the dependence of 2,4-D degradation on the presence of a microbial population, particularly a population adapted to 2,4-D metabolism.

In their laboratory microcosm studies of 2,4-D degradation in five soils, Voos and Groffman (1997) concluded that the degradation rate is most significant with microbial biomass (i.e. microorganism density), both measured as carbon and nitrogen, and with soil organic matter content.

As reported in Section 3.2.4.1,Veeh *et al.* (1996) investigated degradation of 2,4-D in laboratory flasks containing two Montana agricultural soils from depths down to 4 feet. They found that half-lives increased dramatically with increasing depth. They reported that bacterial populations decreased significantly with increasing soil depth, and were positively correlated to the rate of 2,4-D degradation.

Chen and Alexander (1989) added 2,4-D acid to a cultured suspension of lake microorganisms. After a lag phase of 12-18 days, during which little degradation occurred, 2,4-D started disappearing. When the acid was added to a suspension of lake microorganisms previously cultured in the presence of 2,4-D, the lag phase was less than one day and about 90% of the chemical disappeared within 2 days. In another experiment, the authors reported a 15 hour lag phase followed by a slow decline when 2,4-D was added to a bacterial culture grown with glucose as a carbon source. When 2,4-D was added to a culture of the same bacterial strain grown on glucose plus 2,4-D, there was no lag phase, and 2,4-D disappeared in about 3 hours.

Karelova *et al.* (1995) also described a 2,4-D degradation lag phase of about 2 weeks before degradation accelerated in a soil *Arthrobacter* culture grown in a medium containing 2,4-D. Smith and Aubin (1991b) found that 2,4-D added to two pH 7.3 Indian Head clays from the same region degraded with a half-life of 1 day in the clay with prior 2,4-D field applications, and in 3 days in the clay that had received no 2,4-D in the previous 18 months.

Smith *et al.* (1989) conducted laboratory tests on a Saskatchewan clay soil and found that 2,4-D breakdown was slightly faster using field soil that had received continuing applications of that chemical compared with soil from untreated control plots.

Soulas (1992) applied radiolabeled 2,4-D to flasks containing a sterilized silty clay at pH 7.8 and 72% FMC. No appreciable degradation occurred for at least 60 days. Fresh, unsterilized field soil was then added to the flasks. After an initial lag phase of a few days, 2,4-D degradation proceeded rapidly.

Cattaneo *et al.* (1997) found that higher soil moisture conditions resulted in higher rates of 2,4-D degradation, up to at least 50% FMC. He found that degradation did occur to some extent in dryer soils and suspected that some 2,4-D degraders that can better withstand dry conditions may be preferentially selected for in natural soils.

Most 2,4-D-degrading microorganisms are aerobic. That is, they require oxygen therefore they cannot function in anaerobic oxygen-starved water or sediments. However, the anaerobic decomposition of 2,4,5-T via 2,4-D has been seen in anaerobic methane-producing aquifer slurries (1). Bryant (1992) determined that 2,4-D was broken down by microflora in an anaerobic pond sediment/water slurry. He found that virtually no 2,4-D acid breakdown occurred in sterilized control slurries. In slurries without microbe addition, DT_{95} (the time required for disappearance of 95% of residues) was about 50 days. In a slurry containing microorganisms adapted to breaking down 2,4-D, DT_{95} was about 14 days. When two additional treatments with 2,4-D were made at about 22 day intervals, degradation was even more rapid as the adapted microorganisms flourished on that carbon source. Cheah *et al.* (1998) also found 2,4-D degradation occurring in acidic (pH 4.7) muck at 50% FMC. In muck maintained aerobically the half-life was 3.4 days, and 9.3 days in anaerobic muck.

It is clear from the above examples, and from numerous other 2,4-D fate studies that the primary mode of degradation of 2,4-D acid in natural systems is metabolism by microorganisms found widely distributed in soil, sediment, and to a lesser degree in water. In systems where 2,4-D has not been used recently, there is frequently an initial lag period following 2,4-D application wherein no appreciable degradation occurs. During this time, microbial populations able to metabolize 2,4-D increase in number to the point where they are able to significantly reduce 2,4-D concentrations. Previous exposure to 2,4-D shortens the lag phase. Environmental factors that influence the existence and growth of such microorganisms, such as dissolved oxygen levels, temperature, and soil moisture content, will therefore impact the persistence of 2,4-D residues. Repeated applications of 2,4-D to a pond or lake can be expected to produce a larger adapted population of 2,4-D degraders that can shorten the herbicide's persistence compared to the first application.

3.7 MOBILITY

Summary: Most of the data reviewed dealt with sorption to soil; there are very few published studies dealing with sorption to sediments. 2,4-D exhibits variable adsorption and desorption to soil depending on individual soil parameters. In most soils, adsorption is moderate to low, but the adsorbed material tends to stay bound to the soil particles once adsorbed. Adsorption is stronger in soils with higher organic carbon content, in soils with a more acidic pH, and in clay soils with higher levels of iron and aluminum oxides. 2,4-D has also been shown to bind to humic acid, a natural soil and sediment constituent.

These soil parameters interact so that it is difficult to pinpoint a single cause for high or low sorption in soil studies. Nevertheless, it is likely that 2,4-D will bind to a moderate degree to sediments with high organic matter concentrations, and to sediments in acidic lakes and perhaps to sediments with a high clay content. Many lake bottoms have fluffy, light (flocculent) sediments rather than a solid surface, particularly in more eutrophic lakes with a large amount of decaying organic material on the bottom. The much larger amount of particle surface in these flocculent sediments greatly increases the likelihood of 2,4-D adsorption compared with firm-surfaced sediment.

Results from a single adsorption study indicate that 2,4-dichlorophenol, the immediate degradation product of 2,4-D, exhibits adsorption properties similar to those of 2,4-D.

Overall, evidence indicates that 2,4-D does not bind strongly to most soils or sediments. This would normally raise concerns of potential groundwater contamination. However, the rapid degradation in soils and aquatic systems means that 2,4-D is likely to be destroyed before it has a chance to move very far through the soil or out of a lake or pond, and does therefore not pose a significant threat to groundwater.

When a chemical is applied to soil, a potential exists for the chemical to be carried down into the soil with water movement from rain and irrigation. Pesticides exhibit a wide range of leaching potential, from those that adsorb strongly to soil particles and are not released before they break down, to those that do not adsorb significantly (or adsorb, then desorb) and will travel considerable distances down through the soil, sometimes as far as the ground water table. The sorption of various chemicals to soil is affected in a number of ways by soil parameters such as organic matter, clay content and type, and pH.

Controlled laboratory "batch equilibrium" studies are designed to measure the adsorptive properties of pesticides to four representative soils. There are currently no comparable test guidelines specifically for sediment. The results for one of these soil tests conducted for the Industry Task Force II on 2,4-D Research Data (32) are presented in Table 3.7. The soil partition coefficients Kd_{ads} and Kd_{des} are measures of the potential for adsorption to soil and for desorption from that soil, respectively, and are calculated as the 2,4-D concentration in soil divided by the concentration in water at equilibrium in a soil/water system with a single 2,40D starting concentration in the water. Fathulla did not report Kd values, but several other researchers' Kd calculations are presented in the table. The Freundlich K_{ads} and K_{des} are another way of calculating leaching potentials, but use the results of a series of tests with different starting concentrations. The parameters are particular to the specific soil being tested, and soils are chosen to represent typical agricultural soil types. To calculate Kd_{ads} (and Freundlich K_{ads}), sterile soil plus sterile water containing radiolabeled 2,4-D acid are put in a sealed vial and shaken slowly for several hours until an adsorption equilibrium is reached (no more 2,4-D can be adsorbed by the soil). The amount of 2,4-D in the water and soil is determined by measuring the radioactivity in each. The water is then removed, replaced with fresh water, and the vial shaken again to allow the 2,4-D to desorb from the soil back into the water. From measurements then taken, the Kd_{des} is calculated in the same manner as Kd_{ads}. Taken together, the adsorption and desorption parameters indicate how well 2,4-D is adsorbed to and released from that typical soil and hence will give a measure of leaching potential.

Although there is some disagreement as to exact classification values, generally Kd_{ads} and Freundlich K_{ads} values greater than 5 are characteristic of compounds that are considered to be not appreciably mobile, values from about 1 to 5 indicate a potential for greater mobility, while values less than 1 denote considerable mobility potential. In a similar manner, high Kd_{des} and Freundlich K_{des} values indicate that an adsorbed compound will remain bound to soil and resist being carried downward.

Kd and Freundlich K values are composite values measuring adsorption caused by any of several soil characteristics such as clays, aluminum content, cation exchange capacity (CEC), and organic carbon. Koc values represent an attempt to separate out the role of organic carbon in soil adsorption from the other factors. Because organic carbon plays a significant role in the soil adsorption of many pesticides (57), Koc values are often used to predict pesticide mobility. But since Koc depends on two variables (Kd and carbon content), it must be used with caution.

Koc values are calculated by dividing Kd and K values by the decimal percent of organic matter or organic carbon in a soil (e.g. for a sandy loam soil (Cheah *et al.*, 1997), Freundlich Koc_{ads} is calculated as 0.57/0.013 = 43.8, or 43.9 when rounded by those authors). Koc values give an idea of the importance of organic carbon in a soil or sediment in adsorbing a chemical. Koc values generally are numerically higher than Kd or K values. A higher value indicates organic carbon is more influential in trapping a pesticide. For instance in Table 3.7, Baskaran *et al.* (1996b) reported that a Horotiu soil had surface and 24-inch depth organic carbon contents of 5.8% and 0.2% respectively. The Kd_{ads} values were 5.65 and 0.39, indicating a much lower adsorption of 2,4-D in the lower soil. However, Koc_{ads} values were 97 and 195 for the upper and lower depths. The similar values show that even the small amount of organic carbon at the greater depth played a very significant role in adsorbing 2,4-D.

It is emphasized that all of the "K" parameters discussed above are specific to a particular soil or sediment, and to the initial concentration of a chemical applied to the soil or to a sediment/water system. A Freundlich K for a particular soil is a single value calculated using the adsorption or desorption results from all of the initial concentrations used in an experiment, but a Kd is calculated from the result of each initial concentration separately. Unless specified otherwise, Kd and Freundlich K parameters reported in published literature are for adsorption; measurement of desorption values is rare. Where K values are given without the soil type and chemical concentration being specified, care should be exercised in using those values for evaluation of leaching potential.

3.7.1 Soil and Sediment

All of the adsorption constants (Kd, Freundlich K) located during this review deal with soil, as opposed to sediment. Very little information was found quantifying 2,4-D adsorption to lake sediments. Soil mobility data are directly relevant to the expected behavior of 2,4-D oversprayed on shoreline vegetation and to some extent indicates what may happen if a lake level drops, exposing shoreline sediment to drying, soon after treatment. Soil data can also be reasonably extrapolated to predict to some extent the adsorption of 2,4-D on pond and lake sediments. Sediment will usually have a higher organic material content than soils, except for muck soils, and therefore soil tests of higher organic content soils can be used as a guide to anticipate the potential for 2,4-D adsorption to higher-organic matter sediments.

Fathulla (1996a) conducted an EPA laboratory guideline study to determine the adsorption constants in four representative soils. Each of the four soils was mixed 1:1 with water to form a slurry, then ^{14}C-2,4-D acid was added to make four initial concentrations of 1, 2.5, 5, and 10 ppm. After agitation for 24 hours, Freundlich K values were calculated for each set of four concentrations for a given soil. Table 3.7 gives the results. In all of the soils, K_{ads} and K_{des} were very low indicating that 2,4-D was poorly adsorbed to the soils and that the small amount that was adsorbed, desorbed readily back into solution. This reviewer calculated percentages of organic carbon for each soil from Kd and Koc values presented in the study abstract, which was the only study document obtainable (see notes to Table 3.7). The soils used for the study all had a very low organic carbon content, which was probably largely responsible for the low 2,4-D adsorption. Pesticide sorption by soils has often been reported to be related to the organic matter content of soils (57). The Koc_{ads} figures are relatively low, while the Koc_{des} numbers are much higher. One may conclude that while soil organic carbon played relatively little part in adsorbing the 2,4-D, it clung tenaciously to the 2,4-D that was adsorbed.

Cheah *et al.* (1997) reported Freundlich K and Koc values for two soils from Malaysia, a sandy loam and an agricultural muck with 1.3% and 30.5% organic carbon, respectively. For the sandy loam, the Freundlich K_{ads} was 0.57 and the K_{des} was 43.9, while the Koc_{ads} and Koc_{des} were 43.9 and 198. These findings indicate that 2,4-D was weakly adsorbed, but strongly held by the soil. Organic carbon played a significant role in adsorption in this soil. In contrast, the K_{ads} and K_{des} values for the agricultural muck were 5.26 and 28.7, while the Koc_{ads} and Koc_{des} were 17.3 and 94.1. 2,4-D was adsorbed firmly by this soil and retained, though surprisingly the low Koc values suggest that carbon played less of a role in sorption than with the lower carbon content sandy loam. The difference may lie in the different pH values for the soils. The pH for the sandy loam was 6.7 (nearly neutral) while the muck pH was 4.7 (acidic). As discussed below, Johnson *et al.* (1995b) and Barriuso *et al.* (1992) also found greater adsorption to more acidic soils. Helling (1971)

Soil constituents such as clay minerals can cause increased adsorption in some soils (57), though adsorption of 2,4-D on pure clays and silts is negligible (99). Three soils, a sand, sandy loam, and a silt loam were reported to have Kd_{ads} of 0.291 to 1.18, while a clay loam had a Kd_{ads} of 12.7, which would make 2,4-D essentially immobile in that soil (31). The organic carbon content and pH values were not reported. Washington State (1993) points out the contrast with another study reported in Dynamac (1988). 2,4-D was leached with water through a column packed with a silt loam. All of the 2,4-D remained in the upper 2 inches of the 12-inch column and no pesticide was found in the leachate, indicating that 2,4-D was immobile in that particular silt loam.

Barriuso *et al.* (1992) calculated Kd values (Kd_{ads}) ranging from 0.7 to 17.7 for 20 ppm 2,4-D acid applied to samples of two Brazilian oxysoils. Oxysoils are highly weathered soils containing a large proportion of clay-sized particles dominated by hydrous iron and aluminum oxides. The soils had an organic carbon content of 1.28 to 4.9%. Adsorption was found to be highest in soils with a greater concentration of iron and aluminum oxides. An inverse proportionality was found between Kd_{ads} and pH. Kd_{ads} values were 15 at pH 4.1, 5 at pH 4.5, and <1 at pH 5.5, indicating greater adsorption and less leaching potential in more acid soils.

Table 3.7 summarizes investigations of the effects of pH on adsorption to Arkansas silt loam and silty clay soils from two depths by Johnson *et al.* (1995b). They found that 2,4-D was adsorbed more strongly at pH 5.0 than at pH 7, although adsorption even in acidic soils was low, possibly due to the low organic carbon content of 0.5% to 1.0%. Kd_{ads} values were 0.06 to 0.19 in pH 7 neutral soils and 0.37 to 0.59 in the more acid pH 5 soils.

Willems *et al.* (1996) aerobically incubated 2,4-D acid in sandy loam and loamy sand samples from several depths in a plot that had grown rye grass for several years. They found much higher rates of mineralization (formation and release of CO_2 as an end-product) in the soils from 4 to 5 feet depth than at the surface. They suggested that since the surface soils had relatively high organic matter content, microorganisms used that carbon in preference to 2,4-D while at greater depth the activity of the microorganisms was reduced by low amounts of available energy and carbon sources. The introduction of 2,4-D may have provided the resources necessary for a burst of growth and rapid metabolism of 2,4-D.

Adsorption of several pesticides to ten New Zealand soils was investigated by Baskaran et al (1996a, 1996b). As the Kd_{ads} values presented in Table 3.7 demonstrate, adsorption of 2,4-D was greater at the surface than at 28 inches (18 inches for two of the soils). However, Koc_{ads} numbers do not show as large a difference between the two depths. The authors interpret these results as demonstrating the significant role of soil organic carbon in adsorption. Overall, Kd_{ads} values demonstrate that 2,4-D would be essentially immobile in most of these high-carbon soils.

From their work with soils, Benoit *et al.* (1999) found that the effects of adsorption on the rate of 2,4-D, 2,4-DCP and 4-chlorophenol degradation depended on the nature and condition of the soil. In most of the experiments adsorption induced an increase in breakdown. They concluded that adsorption onto soil organic matter may provide a temporary protection from microbial attack, especially if desorption rates are slow. On the other hand, in soil with high organic content and high sorption coefficients, higher microbial activities can increase biodegradation. Increased humifaction, or decay into humic soil constituents of soil organic matter also increase the rate of degradation. Conditions of high organic content and soil humifaction are to be expected in many lake sediments, so Benoit's findings are relevant to many aquatic systems. Hesketh *et al.* (1996) found that there is a theoretical potential for 2,4-D to bind to aquatic fulvic and peat humic acids such as those found in some sediments, while Khan (1973) also reported that humic acid, a natural organic soil component resulting from humifaction, physically adsorbs 2,4-D.

Two Louisiana soils with pH values of 6.1 and 7.2, organic carbon content of 3.2% and 3.6%, and high clay content (56%) failed to adsorb significant amounts of 2,4-D applied as the isopropyl amine salt, and readily desorbed the small amount adsorbed (65).

Smith and Bridges (1996) constructed simulated golf greens with a potting mixture of 85:15 and 80:20 sand:peat moss both in a greenhouse and outdoors and seeded them with two species of grass, which were allowed to become established. Plots were treated with either 0.25 or 1.0 lb/acre of a 2,4-D DMA salt formulation and simulated rainfall was applied to all plots. At the end of 70 days, a total of only 0.4% of the applied 2,4-D was found to have reached six inches depth in the greenhouse plots. After two years, no 2,4-D had been detected in leachate captured at 20 inches depth in lysimeters in the outdoor plots.

In a Tennessee study, Stearman and Wells (1997) applied an unspecified 2,4-D ester to a silt loam soil at a rate of 4 lb/acre in the fall of 1992 and again in the spring of 1993. Soil pH ranged from 5.7 at the surface to 4.7 at lower depths. Organic carbon decreased from 0.85% at the surface to 0.4% at greatest depths. The plots were either planted with grass or clover or were bare. Following a 20-minute rainfall of 10mm (0.4 inches) 36 hours after the first application, samples taken 3 days after application had mean surface 2,4-D residues were approximately 590 ppm, while mean residues of about 60 ppm were found in soil cores from 3 feet depth. 2,4-D residues were near the detection limits (4.2 to 21 ppm depending on the soil) by 21 days after treatment at all depths except in the surface 0-4 inch stratum. After the second application, a rainfall of 0.2 inches was recorded. Mean surface residues 4 days after application were about 700 ppm, while mean residues from 3 feet were about 40 ppm 2,4-D. Interestingly, bromide tracer concentration soil profiles were very similar in shape to the 2,4-D profiles. While many of the studies reviewed here have shown 2,4-D to adsorb poorly to many low organic carbon soils, this reviewer feels from personal experience with soil studies that the elevated residues at lower depths may have been partially the result of sample contamination. When a single soil core is taken from the surface to say, 3 feet as seems to be the case in this study, extremely high surface residues usually transfer to the coring tube sides and contaminate the entire soil column.

In the only field aquatic mobility references found, Hatfield (41, 42) conducted aquatic dissipation studies using DMA salt in a North Carolina and a North Dakota pond. He found that residues of 2,4-D and 2,4-DCP were relatively immobile, remaining in the upper sediment profile, though minor detections of 2,4-D were made at all depths. The abstracts do not state the sediment sampling depths, but from standard practice in this type of study, the majority of residues were probably found in the upper 3 to 6 inches of sediment, with minor detections down to perhaps 12 to 18 inches.

Lovato *et al.* (1999) measured limited movement of 2,4-D from a treated pond sideways through a fine sand soil to a shallow sampling well 5 feet away from the pond. However a pumping well only 12 feet from the pond was pulling pond water through the soil past the sampling well at the time. Both wells were in a fine sand soil over a clay layer 10 feet down in the soil. The movement of the water through the soil was considerably faster that would be experienced under natural conditions, and there was little chance for the 2,4-D to be degraded or to adsorb to soil particles before being pulled past the sampling well. 2,4-D residues in the sampling well were relatively high (about 0.5 ppm) for about a month after treatment, then dropped abruptly and remained at about 0.05 ppm for two more months. Pumping well residues (0.25 to 0.3 ppm) slowly declined over a longer

period, reaching approximately 0.05 ppm after about 3 months. In the pond itself, residues were comparable to those in the sampling well, but became undetectable at Day 43. The continued detection in the pumping and sampling wells after that time points to a slow release of adsorbed 2,4-D from the soil.

It is possible that wells in a real-world situation drilled very close to a pond or lake shore could draw water directly from a water body so rapidly that any 2,4-D present in the lake would not have a chance to be diluted, broken down, or adsorbed to soil. Much would depend on the treatment rate of 2,4-D in the water body, the soil type and porosity, the depth of the ground water table, the frequency and volume of pumping, and other hydrological parameters.

Many lake bottoms have fluffy, light (flocculent) sediments rather than a solid surface, particularly in more eutrophic lakes with a large amount of decaying organic material on the bottom. The much larger amount of particle surface in these flocculent sediments greatly increases the likelihood of 2,4-D adsorption compared with firm-surfaced sediment, particularly in view of their typically higher organic content.

3.7.2 Groundwater

Over the many years of its use as a terrestrial herbicide, 2,4-D has been detected in wells and other groundwater samples. Washington State (1993) quotes Dynamac (1988) in reporting 2,4-D detection in about 100 of more than 1700 groundwater samples from nine states, but 2,4-D has not generally been found to contaminate groundwater. The most likely routes for contamination are spills during mixing of application solutions at wellheads, illegal dumping, surface water runoff from treated fields, and movement down through the soils from heavily treated agricultural land. With respect to groundwater movement, the difference between terrestrial uses of 2,4-D and aquatic weed control uses is that lakes provide, in essence, an isolated incubator in which 2,4-D degradation can take place without immediate impact on surrounding soil.

The data reviewed in this document indicate that 2,4-D is mobile in most soils to varying degrees. In general, the higher the organic carbon content of a soil/sediment and the more humifaction that has occurred, the more likely it is to adsorb to soil particles and hence the less mobile it will be. The lower the pH of a soil, and the more organic carbon that is present, the more likely it is to adsorb 2,4-D, although pH does not appear to influence mobility as strongly as the presence of organic matter. Helling (1971) concluded that soils with a high pH combined with low organic content are the least likely to adsorb 2,4-D.

2,4-D has a relatively short persistence in aquatic systems and any high concentrations at the sediment surface, as a result of application of granular formulations, decrease in a short time through dissipation into surrounding waters or microbial metabolism. Very low levels may persist in sediment for two or three months. At such low levels, very little 2,4-D would be available for movement either downward through the sediment or laterally into the soil surrounding the lake through subsurface water movement. Such movement would cause further dilution of the pesticide through continuing low-level adsorption to the soil that it moves through. A massive application to a small water body could result in movements of higher concentrations of 2,4-D into surrounding soil before it had time to degrade.

Almost no data were located relating to mobility of 2,4-DCP, the major initial metabolite of 2,4-D. As related earlier, Hatfield (41, 42b) found 2,4-DCP residues to be as immobile as 2,4-D in two pond studies. From the chemical structure, it is probably that 2,4-DCP may have similar mobility to 2,4-D, though more research is needed to determine this.

In spite of its mobility in various soil substrates, the leaching potential of 2,4-D, and its potential impact on groundwater when used for aquatic plant control is significantly reduced due to the its relatively rapid degradation rates in aquatic environments.

Table 3.7: 2,4-D Acid Adsorption/Desorption Constants

Soil/sediment type	% organic carbon	pH	Kd_{ads} [1]	Kd_{des} [1]	Koc_{ads}	Koc_{des}	Freundlich K_{ads}	Freundlich K_{des}	Freundlich Koc_{ads}	Freundlich Koc_{des}	Reference
Plainfield sand	0.47*	n.a.[2]					0.357	1.16	76	247	(32) Fathulla, 1996a
California sandy loam	0.24*	n.a.					0.167	0.811	70	338	(32) Fathulla, 1996a
Mississippi loam	0.24*	n.a.					0.281	1.48	117	617	(32) Fathulla, 1996a
Arizona silty clay loam	0.09*	n.a.					0.517	1.90	59	216	(32) Fathulla, 1996a
Sandy loam, Malaysia	1.3	6.7					0.57	2.57	43.9	198**	(19) Cheah *et al.*, 1997
Agricultural muck, Malaysia	30.5	4.7					5.26	28.7	17.3	94**	(19) Cheah *et al.*, 1997
Sand	n.r.[3]	n.r.	0.291								(31) Dynamac, 1988 in (99)
Sandy loam	n.r.	n.r.	0.363								(31) Dynamac, 1988 in (99)
Silt loam	n.r.	n.r.	1.18								(31) Dynamac, 1988 in (99)
Clay loam	n.r.	n.r.	12.7								(31) Dynamac, 1988 in (99)

Table 3.7: 2,4-D Acid Adsorption/Desorption Constants (continued)

Soil/sediment type	% organic carbon	pH	Kd_{ads}[1]	Kd_{des}[1]	Koc_{ads}	Koc_{des}	Freundlich K_{ads}	Freundlich K_{des}	Freundlich Koc_{ads}	Freundlich Koc_{des}	Reference
Tokomaru silt loam, New Zealand	3.2/0.6[4]	5.80	1.52/0.30[4]		45/50[4]		3.43/-		107**/-		(6) Baskaran *et al.*, 1996a, 1996b
Patua silt loam, NZ	8.2/5.2	5.56	9.60/9.36		117/180		17.42/-		212**/-		(6) Baskaran *et al.*, 1996a, 1996b
Egmont, NZ	8.7/1.9	n.r.	4.81/2.84		55/150		8.95/-		103**/-		(7) Baskaran *et al.*, 1996b
Papakauri, NZ	16.7/7.8	n.r.	11.50/6.60		69/84		18.84/-		113**/-		(7) Baskaran *et al.*, 1996b
Horotiu, NZ	5.8/0.2	n.r.	5.65/0.39		97/195		7.82/-		135**/-		(7) Baskaran *et al.*, 1996b
Warea, NZ	11.7/4.2	n.r.	13.28/4.64		114/110						(7) Baskaran *et al.*, 1996b
Hangatahua, NZ	6.9/0.8	n.r.	4.38/0.85		63/104						(7) Baskaran *et al.*, 1996b
Kerikeri, NZ	8.7/3.3	n.r.	5.45/4.22		63/129						(7) Baskaran *et al.*, 1996b
Hamilton, NZ	3.4/1.1	n.r.	3.78/2.21		111/197						(7) Baskaran *et al.*, 1996b
Himatangi, NZ	2.2/0.2	n.r.	0.94/0.08		43/35						(7) Baskaran *et al.*, 1996b

Table 3.7: 2,4-D Acid Adsorption/Desorption Constants (continued)

Soil/sediment type	% organic carbon	pH	Kd_{ads} [1]	Kd_{des} [1]	Koc_{ads}	Koc_{des}	Freundlich K_{ads}	Freundlich K_{des}	Freundlich Koc_{ads}	Freundlich Koc_{des}	Reference
Crowley silt loam, surface, AR	0.8%	6.9					0.43		53.8**		(47) Johnson *et al.*, 1995b
Crowley silt loam, **surface, buffered pH**	0.8%	**5.0**	0.59		73.8**						(47) Johnson *et al.*, 1995b
Crowley silt loam, **surface, buffered pH**	0.8%	**7.0**	0.19		23.8**						(47) Johnson *et al.*, 1995b
Crowley silt loam, 24 inch depth	0.7%	5.0					1.51		215**		(47) Johnson *et al.*, 1995b
Crowley silt loam, **24 inch depth, buffered pH**	0.7%	**5.0**	0.50		71.4**						(47) Johnson *et al.*, 1995b
Crowley silt loam, **24 inch depth, buffered pH**	0.7%	**7.0**	0.07		10.0**						(47) Johnson *et al.*, 1995b

Table 3.7: 2,4-D Acid Adsorption/Desorption Constants (continued)

Soil/sediment type	% organic carbon	pH	Kd_{ads} [1]	Kd_{des} [1]	Koc_{ads}	Koc_{des}	Freundlich K_{ads}	Freundlich K_{des}	Freundlich Koc_{ads}	Freundlich Koc_{des}	Reference
Perry silty clay, surface, AR	1.0%	6.9					0.57		57.0**		(47) Johnson *et al.*, 1995b
Perry silty clay, **surface, buffered pH**	1.0%	**5.0**	0.50		50.0**						(47) Johnson *et al.*, 1995b
Perry silty clay, **surface, buffered pH**	1.0%	**7.0**	0.12		12.0**						(47) Johnson *et al.*, 1995b
Perry silty clay, 24 inch depth	0.5%	5.3					0.65		130**		(47) Johnson *et al.*, 1995b
Perry silty clay, **24 inch depth, buffered pH**	0.5%	**5.0**	0.37		74.0**						(47) Johnson *et al.*, 1995b
Perry silty clay, **24 inch depth, buffered pH**	0.5%	**7.0**	0.06		12.0**						(47) Johnson *et al.*, 1995b

* % organic carbon calculated by this reviewer from Freundlich K and Koc values presented by Fathulla, 1996a using the equation % organic carbon = (K_d / K_{oc})x 100.

** K_{oc} values calculated by this reviewer using the equation K_{oc} = (K_d / % organic carbon) x 100. See text.

1 ads = adsorption, des = desorption

2 n.a. = data not available

3 n.r. = not reported

4 Organic carbon and sorption coefficients for surface/greatest depth. Greatest depth is 24-28 inches except for Kerikeri and Himatangi (16-18 inches

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2,4-D

Volume 3, Section 4

ENVIRONMENTAL EFFECTS

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4.0 ENVIRONMENTAL EFFECTS ASSESSMENT – 2,4-D

Executive Summary: The information contained in this report was compiled from studies submitted to EPA by the sponsor, data found on various EPA web-sites and the open literature on the toxicity of 2,4-D dimethylamine salt (2,4-D DMA), 2,4-D Butoxyethyl ester (2,4-D BEE), 2,4-D 2-Ethylhexyl ester (2,4-D 2-EHE) and 2,4-D acid and its inorganic sodium and potassium salts. Data collected included acute toxicity for the standard test species of bacteria, fungae, algae, plants, fish, free-swimming invertebrates and benthic (sediment-dwelling) invertebrates. Chronic toxicity data was for early life-stage studies for fish and life-cycle studies for free swimming invertebrates. No chronic toxicity data was collected for algae, plants or sediment organisms. Since chronic data was in short supply an estimate of the chronic no observed effects levels (NOEC) was made based on the acute/chronic toxicity ratio for animal species which have both acute and chronic data available. Additional data were collected on species other than the standard test species to supplement the data further. A risk assessment was conducted based on the procedures outlined in Urban and Cook (1986). Urban and Cook state that if acute risk quotients (RQs) are less than 0.1 and chronic risk quotients are less than 1.0, the biota should be safe from the toxic effects of the tested pesticide with assurance that 95% of the tested biota will be protected. These values are termed the acute and chronic level of concern, respectively. The acute RQ is defined as the acute LC50 divided by the short-term expected environmental concentration (EEC); and the chronic RQ is defined as the chronic NOEC divided by the long-term EEC.

Acute risk quotients are defined as the four day geometric mean of the Expected Environmental Effects Concentration (EEC) divided by the concentration of the herbicide that will cause mortality in 50% of animals exposed in a standardized acute toxicity test (EC50 or LC50). These values are calculated from the most typical initial concentration of 2,4-D DMA (1.36 mg a.i./L = 1.13 mg a.e./L); half-life is not considered for these acute exposures since the half-life of 6.4 days will not reduce the 4-day EEC significantly below 1.36 mg a.i./L. The most typical concentration (EEC) at zero time for 2,4-D BEE and 2,4-D acid is considered to be 3.25 mg/L at the bottom of the water column and 0.19 mg/L at the top of the water column. However, the short term EEC for a typical exposure is 0.100 mg/L after 2 to 6 days based on typical data from 15 open British Columbia waterways. These 2,4-D BEE values are further validated by recent work at Loon Lake, Washington. Chronic Risk Quotients are defined as the 28-day geometric mean for the Expected Environmental effects concentration (EEC) divided by the no observed effect concentration (NOEC) for animals after exposure in a standardized chronic toxicity test that can last up to several months. These values are calculated from typical day-1 concentrations of 2,4-D DMA and a typical half-life of 6.6-days (EEC = 0.091 mg a.i./L). The long-term EEC for 2,4-D BEE and 2,4-D acid is estimated to be 0.01 mg/L based on the geometric mean of the short term EEC (0.1mg/L) as described above and the five to 22-day concentration of 0.001 mg /L found in open British Columbia waterways. These values are conservative based on the 1999 Loon Lake data.

2,4-D acid, 2,4-D disodium salt and 2,4-D dipotassium salt were analyzed together for risk since EPA believes that the toxicity for these herbicides should be similar. Although 2,4-D DMA is similar in toxicity to 2,4-D acid, risk was analyzed separately for this product since a few species of benthic invertebrates appear to be extremely sensitive to 2,4-D DMA (possibly due to its DMA moiety). 2,4-D BEE is analyzed for risk separately from 2,4-D acid and its inorganic salts because its laboratory toxicity is much higher due to the presence of the BEE moiety. However, it is unlikely aquatic organisms will

encounter 2,4-D BEE due to its low solubility and rapid hydrolysis to 2,4-D acid. Summaries of the results of toxicity studies are presented in Table 2 for the herbicides specified above. Each subclass of plants and animals within the biota was evaluated separately by the risk assessment methods of Urban and Cook (1986). These separate subclasses included bacteria, fungi, algae and macrophytes, fish, free-swimming invertebrates and benthic (sediment) invertebrates. The risk analysis was conducted in this manner because the different classes of organisms had the potential to exhibit different toxic effects. Endangered species were evaluated under a separate acute risk assessment since the acute level of concern for endangered species is 0.05 rather the 0.1 value typically used for less sensitive members of the biota.

2,4-D products affect various species of bacteria and fungi. Various species of heterotrophic bacteria found in the water column have been stimulated to grow by treatments with 2,4-D sodium at 6.0 Kg a.i./ha (~2 mg a.i./L); such treatments have induced a 3-fold increase in bacterial counts shortly after treatment with 2,4-D sodium salt. Conversely, sediment bacteria (presumably obligate aerobes) have decreased in numbers shortly after treatment by about 3-fold due to a decrease in oxygen levels and then gradually increased in numbers during the eight weeks that aerobic conditions were restored. At a much higher concentrations of 400 mg /L 2,4-D, 50% inhibition of growth was observed in batch cultures of unspecified bacteria.

Fungi have also been observed to have an increased growth rate when exposed to low concentrations (3.0 mg/L) of 2,4-D 2-EHE while much higher concentrations of an unspecified formulation of 2,4-D ranging from ~100 to ~10,000 mg/L caused a reduced rate of growth in ectomycrrhizal and aeroquatic fungi. Ectomycrrhizal fungi form an important symbiotic relationship with many vascular plants and facilitate nutrient uptake and improve resistance to stress. However, a field study involving true fungi, yeast and mold found that 2,4-D DMA and 2,4-D BEE did not have a clear effect on fungal propagules in the water or sediment when the treatment rate was 1.0 mg /L. However, the total fungi numbers had a tendency to be depressed relative to the controls during the course of the study due to unspecified secondary effects.

*For the most part, 2,4-D products are not toxic to indicator species of algae, particularly 2,4-D DMA and 2,4-D acid. An exception may be freshwater and saltwater diatoms which can have EC50s that are quite low (~2.0 to ~5.0 mg a.i./L) for 2,4-D DMA, 2,4-D BEE and 2,4-D acid. The risk assessment scheme of Pedersen et al, (1994) (Table 21) indicates that risk (RQ = >0.1) is high to the diatom species. 2,4-D BEE also causes a very high risk to the indicator blue-green algae species (*Anabaena flos-aquae*). In the laboratory, low concentrations of 2,4-D (<10 mg/L) typically stimulate the growth of blue-green algae. However, 2,4-D products have a low toxicity to most blue-green algae at higher concentrations. There is some evidence that algal numbers increase when a water body is treated with 2,4-D DMA or 2,4-D acid for the control of Eurasian watermilfoil due to the release of nitrogen and phosphate. The phytoplankton cell count may double within a few days or weeks of treatment with 2,4-D at concentrations of 2 to 45 Kg a.i./ha. There may also be shifts in dominant species to those which find water temperatures and nutrient concentrations that occur after milfoil lysis ideal for growth (i.e.* Chlorphyta, Pyrrhophyta *and* Bacilariophyta*).*

2,4-D is toxic to the indicator species of aquatic macrophytes at low concentrations. The representative species in the laboratory is Lemna gibba *and the toxicities (EC50s) of 2,4-D DMA, 2,4-D BEE and 2,4-D acid are typically 0.58, 0.58 and 0.695 mg a.i./L,*

*respectively. Since use rates may be as high as 4.8 mg a.i./L (4.0 mg a.e./L), this macrophyte would be controlled under typical field situations. Results from field studies indicate that Eurasian watermilfoil (*Myriophyllum spicatum*,) variable-leaf watermilfoil* (M. heterophyllum*), crowfoot (*Ranunculus longirostris*), coontail (*Certophyllum demersum*), American waterweed (*Elodea canadensis*), pondweeds (*Potamogeton spp.*), water stargrass (*Heteranthera dubia*) and wild celery (*Vallisneria Americana*) declined substantially in impounded lagoons. While the Eurasian watermilfoil remained at low levels (<5% of areal cover) for up to two growing seasons, most of the native species regained 80 to 120% of their pretreatment standing crop by the end of the growing season. The Loon Lake model experiment reduced the levels of Eurasian water milfoil by 98% six weeks after treatment, but none of the other aquatic plant species appeared to be affected by the direct action of 2,4-D. Damage to plants appears to be controlled by both concentration and time of exposure. Chronic concentrations as low as 0.1 mg/L 2,4-D DMA may control northern watermilfoil (*Myriophyllum sibericum*) and sago pondweed (*Potamogeton pectinatus*) in Canadian prairie ponds. For list of species with which efficacy has been demonstrated please see Table 2 and Appendix 1 of Section 1).*

2,4-D DMA has a very low toxicity to green algae (EC50 = 66 to 185mg a.i./L) and blue-green algae (EC50 153 = mg a.i./L). 2,4-D BEE also has moderate to low toxicity to green algae (EC50 = 25 to 75 mg a.i./L) and high toxicity blue-green algae (EC50 = 6.37 mg a.i./L) while 2,4-D acid exhibits toxicity similar to that of 2,4-D DMA to green algae (EC50 = 26 to 98 mg a.e./L), and blue-green algae (EC50 = >2.02 to ~500 mg a.i./L). However, as described above, the toxicity of 2,4-D BEE and 2,4-D acid may be high enough to affect the diatom members of the algal biota (EC50 = ~2.0 mg a.i./L). The risk assessment scheme promoted by Petersen et al, (1994), indicates that diatoms may be at acute risk (RQ = >0.1) from the effects of 2,4-D BEE and 2,4-D acid. Furthermore, the mitigating factor that keeps 2,4-D BEE from adversely impacting blue-green algae is its fairly insoluble nature and its rapid hydrolysis to 2,4-D-acid, which is not significantly toxic to blue-green algae.

- ***Summary of 2,4-D DMA Effects on Aquatic Animals***

  Based on laboratory data, 2,4-D DMA has a low acute toxicity to fish (LC50 = >100 to 524 mg a.i./L for the rainbow trout and bluegill sunfish respectively). No Federally sensitive/threatened or endangered species were tested with 2,4-D DMA. However, it is likely that endangered salmonids would not exhibit higher toxic effects to 2,4-D DMA than those seen in rainbow trout. Since the maximum use rate of 2,4-D DMA would be no higher than the maximum labeled use rate (4.8 mg a.i./L) even the most sensitive fish species within the biota should not suffer adverse impacts from the effects of 2,4-D DMA. For example, the risk quotient is below the acute level of concern (0.1 for typical species and 0.05 for endangered species) for all species tested. RQ = 4.8 ppm a.i./ 100 ppm a.i. = 0.048 for rainbow trout.

  The use of maximum field rates of 2,4-D DMA has been shown to not adversely impact survival, condition, or movement within the treatment area of largemouth bass or the nesting behavior of bluegill and redear sunfish. Exposure of anadromous fish to sublethal concentrations of 2,4-D DMA that might typically be encountered in the environment will probably not interfere with the parr to smolt metamorphosis; when smolts are treated with 200 mg 2,4-D DMA/L for 24 hours, subsequent exposure to clean seawater resulted in no 96-hour mortality. Laboratory tests indicate that fish do not bioaccumulate 2,4-D DMA by up-take from the water or by

an oral exposure route. 2,4-D DMA was never found at concentrations that exceeded 0.94 mg/L in the tissue of multiple species of fish occupying water treated with concentrations up to 6 mg a.e./L. Field studies verified these observations for channel catfish, bluegill sunfish and redear sunfish. Rainbow trout have been reported to avoid 2,4-D DMA concentrations typically encountered in the field. However, it is not likely that fish exposed in the field would or could avoid 2,4-D DMA concentrations in the range of 1.36 to 4.8 mg a.i./L. Although much lower concentrations may produce more subtle behavioral or physiological changes, it is unclear whether or not these behavioral changes would have any effect on a species survival within the biota.

2,4-D DMA is practically non-toxic to free swimming-invertebrates like Daphnia magna *(LC50 =>135 mg a.i./L). However, the toxicity of 2,4-D DMA varies considerably for benthic invertebrates. 2,4-D DMA is practically non-toxic in chironomids, pink shrimp, glass worms, eastern oysters, aquatic sowbugs and fiddler crabs with acute LC50s above 100 mg a.i./L; but is highly toxic to glass shrimp (LC50 = 0.15 mg a.i./L) and is moderately toxic to seed shrimp (LC50 = 8.0 mg a.i./L). While formal risk assessment indicates that 2,4-D DMA poses low acute risk to free-swimming invertebrate biota (RQ = 1.36 ppm/>135 ppm = <0.010), the risk to sediment organisms is potentially high (RQ = 1.36 ppm/0.15 ppm = 9.0 in water and 0.21 ppm/0.15 ppm = 1.4 in sediment). However, field studies with mixtures of 2,4-D and dalapon or 2,4-Dulone showed no undesirable effects on the invertebrate population for up to six months after application.*

The chronic toxicity of 2,4-D-DMA has not been extensively evaluated in studies that would currently fulfil EPA study guidelines. However, the chronic toxicity of these test substances range from an NOEC of 17.1 mg a.i./L in a 31-day early life-stage test with the fathead minnow to 40 mg a.i./L in a 12 day sac-fry test with bluegill sunfish. At a projected use rate of 0.235 mg a.e./L (after 1-day), 2,4-D DMA will not chronically impact members of this segment of the biota. For example, the risk quotient is below the chronic level of concern (1.0 for typical species) for all species tested. RQ = 0.091 ppm a.e./17.1 ppm a.e. = 0.01. Predictions of a chronic NOEC can be as low as 5.56 mg a.e./L for early life-stage rainbow trout based on acute toxicity (LC50 = 100 mg a.i./L) divided by the acute to chronic toxicity ratio of 18. Using these predicted chronic NOECs produces a risk quotient that does not exceed the chronic level of concern (1.0) for protection of the biota; e.g., RQ = 0.091ppm a.i./5.56 ppm a.i. = 0.016. True chronic exposure probably does not exist in the field since treatment with 2,4-D DMA does not normally occur more often than once or twice per year in a typical water body. However, single exposures as described above in the field studies do not seem to significantly effect largemouth bass numbers, conditioning or movement within the treated area or nesting behavior in bluegill sunfish and redear sunfish. The general biological stimulation caused by 2,4-D DMA may cause adverse subacute effects such as early spawning activity, increased metabolic rate and decreases in bone collagen levels.

*2,4-D DMA has been tested for chronic toxicity on one species of free-swimming invertebrate (*Daphnia magna*). The experimental chronic toxicity (NOEC) to* Daphnia magna *is 27.5 mg a.i./L to 2,4-D DMA. At a typical use rate of 5.3 Kg a.e./ha (0.235 mg a.e./L = 0.283 mg a.i./L day-1 concentration), 2,4-D DMA will not chronically impact Daphnia magna (free-swimming invertebrate). The risk quotient*

is below the chronic level of concern (1.0 for typical species) for Daphnia magna, *RQ = 0.091 ppm a.i./27.5 ppm a.i. = 0.0033.*

For the benthic (sediment) invertebrates, predicted chronic NOECs for 2,4-D DMA are used to evaluate risk since no laboratory studies were conducted. The predicted chronic NOEC for the most sensitive environmental relevant species (glass shrimp) would be 0.0083 mg a.i./L which leads to a risk quotient that is much higher than the chronic level of concern of 1.0 (RQ = 0.091 ppm a.e./0.0083 ppm a.e. = ~11 for water and 0.21 ppm a.i./0.0083 ppm a.i. = ~25 for sediment). Therefore use of 2,4-D has the potential to chronically impact the benthic biota at typical use rates. However, it should be noted that these high chronic toxicity levels were expressed in only one of the ten benthic invertebrate species tested. Therefore, at least 90 % of the benthic species should be protected when typical use rates of 2,4-D DMA are used. True chronic exposure probably does not exist in the field since treatment with 2,4-D DMA does not generally occur more often than once or twice per year in a typical water body. Only one field study was conducted on benthic species with 2,4-D DMA; six months after exposure to a mixture of 2,4-D DMA and dalapon no undesirable effects were noted. There were no effects on numbers due to the direct effects of 2,4-D and diversity of species remained the same through out the study.

In conclusion, 2,4-D DMA will not effect fish or free-swimming invertebrate biota acutely or chronically when applied at typical use rates of 1.36 to 4.8 mg a.i./L. However, more sensitive species of benthic invertebrates like glass shrimp may be affected by 2,4-D DMA, but 80 and 90% of the benthic species should be safe when exposed to 2,4-D DMA acutely or chronically at rates recommended in the label. Field work indicates that 2,4-D has no significant adverse impacts on fish, free-swimming invertebrates and benthic invertebrates, but well designed field studies are in short supply. Furthermore, the concentrations listed on the label will control the aquatic macrophytes listed on the label including Eurasian watermilfoil (Myriophyllum spicatum) at 10.6 to 40.1 Kg a.e. /ha (12.7 to 48 Kg a.i./ha) and water hyacinth at 2.1 to 4.3 Kg a.e./ha (2.55 to 5.11 Kg a.i./ha). 2,4-D DMA should not be used in attempts to control species of weeds that are not specified on the label. 2,4-D DMA is not an algaecide and is generally ineffective in controlling algal species. Algal species may bloom after treatment with 2,4-D DMA if proper water quality conditions occur and released nutrients reach levels that can sustain algal growth.

- ***Summary of 2,4-D BEE Effects on Aquatic Animals***

2,4-D BEE, has a high laboratory acute toxicity to fish (LC50 = 0.3 to 5.6 mg a.i./L for rainbow trout fry and fathead minnow fingerlings, respectively). Formal risk assessment indicates that short term exposure to 2,4-D BEE should cause adverse impact to fish since the risk quotient is above the acute level of concern of 0.01 (RQ = 0.1 ppm/0.3 ppm = 0.33). However, the low solubility of 2,4-D BEE and its rapid hydrolysis to 2,4-D acid means fish are more likely to be exposed to the much less toxic 2,4-D acid. 2,4-D acid has a toxicity similar to 2,4-D DMA to fish (LC50 = 20 mg to 358 mg a.i./L for the common carp and rainbow trout, respectively). In contrast, formal risk assessment with 2,4-D acid indicates that short-term exposure to 2,4-D BEE should not cause adverse impact to fish since the risk quotient is below the federal level of concern of 0.01 (RQ = 0.1 ppm/20 ppm = 0.005).

Limited field data with sentinel organisms (caged fish) and net capture population surveys indicate that 2,4-D BEE lacks acute environmental toxicity to fish when applied at labeled rates. Exposure of smolts of several salmon species to 1 mg/L 2,4-D BEE for 24-hours did not affect the ability of these smolts to survive a subsequent 24- hour seawater challenge. This indicates that 2,4-D BEE probably does not interfere with the parr to smolt metamorphosis in andromonous fish species. Although bluegill sunfish and rainbow trout bioaccumulate 2,4-D BEE for the first 3 hours of exposure to 1 mg 2,4-D BEE/L, the material is rapidly metabolized to 2,4-D acid and eliminated from the tissues in the next 48 to 120 hours; the BCF reaches levels of 1.7 mg/L in trout and 46.6 mg/L in sunfish but rapidly falls to levels below 0.2. Several species of fish including sheepshead minnow and mosquito fish, are known to avoid 2,4-D BEE at concentrations typically found in the field. However, it is not likely that fish exposed in the field would or could avoid 2,4-D BEE concentrations in the range of 0.1 to 3.25 mg /L. 2,4-D BEE and 2,4-D acid produce a number of behavioral effects, paththological and metabolic effects at concentrations which are much higher than those typically encountered in the field. These effects are typical signs of stress in fish. Since these effects only occur at concentrations that greatly exceed concentrations likely to be encountered in the field, it is unclear whether a general decrease in vigor (ability to resist perdition, disease or chemical assault) would be important at dosages (1.0 to 2.0 mg a.i./L) typically encountered after treatment with 2,4-D BEE for aquatic weed control.

2,4-D BEE is moderately toxic to free-swimming daphnids (LC50 = 4.0 to 7.2 mg a.i./L) and highly toxic to moderately toxic to most benthic invertebrates (LC50 = 0.44 mg to 6.1 mg a.i./L). Formal assessment indicates a low risk to daphnids (RQ = 0.025). However, since the risk quotient is higher than the acute level of concern of 0.1 for benthic invertebrates (RQ = 0.1 ppm/0.44 ppm = 0.23) this segment of the biota is potentially at risk from the acute effects of 2,4-D BEE. However, the low solubility of 2,4-D BEE and rapid hydrolysis to 2,4-D acid would tend to limit exposure to the much less toxic 2,4-D acid. 2,4-D acid has a toxicity similar to the low toxicity of 2,4-D DMA to most species of invertebrates. For free-swimming invertebrates, the toxicity of 2,4-D acid and its sodium salt range from (LC50 = ~209 to >2000 mg a.i./L for Daphnia magna *and freshwater prawn, respectively) which leads to a toxicity evaluation of practically non-toxic for these species. The level of concern is also not exceeded for the most sensitive species of benthic invertebrate (lined scud) (LC50 = 3.2 mg a.i./L; RQ = 0.1 ppm/3.2 ppm = 0.031 for water exposure; or RQ = 0.15 ppm/3.2 ppm = 0.047 for sediment exposure). Short-term field studies indicate that zooplankton in water treated with 2,4-D sodium salt at 6.0 Kg a.i./ha are not adversely affected by 2,4-D and appear to increase in numbers due to the secondary effect of increases in the phytoplankton which occurs almost immediately and lasts up to 8 weeks. Also, while the 2,4-D BEE does not appear to have direct effects on benthic invertebrates, secondary effects such as a decrease of oxygen in the hypoliminion for several weeks after treatment may result in a shift of dominant species from those that require high oxygen like* Odonata *and* Ephemeroptera *to those that are tolerant of low dissolved oxygen content like oligochaete worms and Tendepedid midges.*

The chronic toxicity of 2,4-D BEE has not been extensively evaluated in studies that would currently fulfill EPA study guidelines. However, a couple of early life-stage studies and one life-cycle study have been conducted with this test substance. Studies indicate chronic NOECs of 0.040 and 0.081 mg a.i./L in an early life-stage test with

Chinook salmon and fathead minnow respectively. Furthermore, in a 10-month life-cycle study with the fathead minnow the NOEC was determined to be 0.3 mg a.i./L. There was no obvious correlation with exposure time and NOEC. Since only two species were tested, an estimate of the chronic NOEC was made from the acute LC50 for the most sensitive species (rainbow trout) and the acute to chronic toxicity ratio. At the projected maximum use rate, 2,4-D BEE will probably not chronically impact members of this segment of the biota. The risk quotient is below the chronic level of concern (1.0) for the most sensitive species tested (rainbow trout); RQ = 0.01 ppm/0.017 ppm = 0.59. True chronic exposure probably does not exist in the field since treatment with 2,4-D BEE does not normally occur more often than once or twice per year in a water body. 2,4-D BEE has not been extensively evaluated for chronic effects in the field. However, fish are unlikely to be exposed to 2,4-D BEE in the field due to low solubility and a rapid hydrolysis of 2,4-D BEE to 2,4-D acid. Since 2,4-D BEE is not chronically toxic to fish, one can assume that its hydrolysis product (2,4-D acid) will not be toxic to fish either; this assumption is borne out by the observation that 2,4-D acid is practically nontoxic to a variety of environmentally relevant species including common carp (most sensitive species with an LC50 of 20 mg a.e./L), white perch and cutthroat trout (LC50s = 40 mg a.e./l), lake trout (LC50 = 45 mg a.e./L) pumpkin seed sunfish (LC50 = 95 mg a.e./L, and rainbow trout and fathead minnow (LC50s >100 mg a.e.)/L. Further studies conducted with 2,4-D sodium salt indicate no direct adverse impact to fish exposed repeatedly for up to 1-year. There is an indication that secondary effects of exposure to 2,4-D acid may be improved survivorship and growth in Hamilton's carp and common carp that feed on benthic organisms. However, other carp species like rohu and catla that feed on plankton may have lowered survivorship and growth due to a decrease in the growth of phytoplankton in the water. Long-term effects seem minimal due no obvious reproductive effects on carp used as seed animals. In general the toxic potential of 2,4-D BEE as measured in the laboratory is apparently not realized under the 2,4-D BEE concentrations and environmental conditions present during actual field use.

The experimental chronic toxicity (NOEC) is 0.29mg a.i./L for Daphnia m*agna. At the projected maximum use rate of 112 Kg formulation/ha, 2,4-D BEE will not chronically impact this daphnid species (free-swimming invertebrate). The risk quotient is below the chronic level of concern (1.0 for typical species); RQ = 0.010 ppm/0.29 ppm a.e. = 0.034. Predictions of a chronic NOEC are not necessary since a predicted chronic NOEC cannot be more accurate than a value obtained empirically. True chronic exposure probably does not exist in the field since treatment with 2,4-D BEE does not normally occur more often than once or twice per year in a water body Since the hydrolysis product of 2,4-D BEE (2,4-D acid) is even less toxic than 2,4-D BEE to* Daphnia magna *and* Ceriodaphnia dubia*, no chronic effects are likely to be seen on these free-swimming invertebrates. The chronic NOECs are 19 and ~30 mg a.i./L for* Daphnia magna *and* Ceriodaphnia dubia*, respectively. No field studies were conducted to verify or deny the chronic risk associated with 2,4-D BEE® against this segment of the biota.*

Predicted chronic NOECs for 2,4-D BEE® are used to predict risk amongst the benthic (sediment) invertebrates since no laboratory studies were conducted. The predicted chronic NOEC for the most sensitive environmental relevant species (lined scud) would be 0.024 mg a.i./ L which leads to a risk quotient below the chronic level of concern of 1.0 (RQ = 0.01 ppm a.i./0.024 ppm a.e. = 0.42). Therefore, use of 2,4-D BEE at the maximum projected rate will not chronically impact the benthic biota

adversely if primary exposure is through the water column. However, if concentrations that may be found for 28 days in the sediment are considered (0.06 mg a.i./g) as representative of the EEC, the chronic level of concern of 1.0 would be exceeded (RQ = 0.06 ppm/0.024 ppm = 2.5) and the sediment biota would be judged to be at risk. However, benthic species may not be exposed chronically to 2,4-D BEE due to the low solubility of 2,4-D BEE and its rapid hydrolysis to 2,4-D acid. If this is the case, then benthic species should not be adversely impacted under chronic exposure since the risk quotient for 2,4-D acid is not above the chronic level of concern for exposure to the water column (RQ = 0.06 ppm/3.2 ppm = 0.018) or the sediment (RQ = 0.06 ppm/0.018 ppm = 0.33). However, treatment of a Pennsylvanian lake caused a change in population structure; e.g. while the numbers and diversity in the benthic biota did not change, the dominant species shifted from those that require high oxygen levels to those that can tolerate low oxygen levels. This was expected since the concentration of oxygen in the hypoliminion dropped to almost zero for approximately one week after treatment. True chronic exposure probably does not exist in the field since treatment with 2,4-D BEE does not normally occur more often than once or twice per year in a water body. However, exposure to a commercial product (presumably 2,4-D sodium salt) at 0.375 to 0.875 Kg/ha/month for 12 months caused an increase in the number of sediment microbes which subsequently lead to a 21% increase in the benthic biomass and an increase in survivorship and yield for carp species that feed on benthic organisms.

Conclusion: 2,4-D BEE will have no significant impact on the animal biota acutely or chronically when using applied rates recommended on the label. Furthermore, the concentrations listed on the label will control the aquatic macrophytes listed on the label including Eurasian *watermilfoil* (Myriophyllum spp.*), water stargrass (*Heteranthera dubia*) at 100 to 200 lbs. formulation/acre and bladderwort (*Utricularia spp*). Fragrant water lily (*Nymphaea spp.*) spatterdock (*Nuphar spp.*) water shield (*Brasenia spp.*) water chestnut (*Trapa natans*) and coontail (*Ceratophyllum demersum*) at 150 to 200 lbs./acre. Use of this product in* ***impounded waterways or waterways that lack significant lateral water exchange may result in significant injury to both Eurasian watermilfoil and native plant species. However, careful use of 2,4-D BEE at 100 lbs. formulation/ acre, as described for the Loon Lake, Washington project should result in control of Eurasian watermilfoil while sparing native species of pondweed and even those species typically controlled at higher use rates like water stargrass and bladderworts. 2,4-D BEE should not be used in attempts to control species of weeds that are not specified on the label. 2,4-D BEE is not an algaecide and should not be used for the control of algae. Although laboratory data indicates that 2,4-D BEE may be toxic to fish, free-swimming invertebrates and benthic invertebrates, data indicates that its toxic potential is not realized under typical concentrations and conditions found in the field. This lack of field toxicity is likely due to the low solubility of 2,4-D BEE and its rapid hydrolysis to the practically non-toxic 2,4-D acid.***

4.01 Objective

The purpose of Section 4 is to update the environmental toxicity data and to use this data to assess the potential risks to wildlife and the environment from using 2,4-D products including Aqua-Kleen® and Navigate®. When wildlife is discussed, the organisms

referred to include aquatic plants and animals, terrestrial plants and animals and microorganisms including algae, bacteria, and fungi.

4.0.2. Study Approach

4.0.2.1 Information Compilation

In order to collect appropriate information regarding wildlife toxicology, several sources of information were used. As a primary and definitive source of data, reports submitted to the EPA Environmental Effects Branch by the registrant (Dow AgroSciences) to support the registrations and re-registration of 2,4-D products were used. These submittals are considered to be definitive sources on the wildlife toxicology of 2,4-D because the tests are conducted in an agreed upon design with agreed upon organisms. These organisms are considered to be good representatives or good surrogates for plants and animals that are highly sensitive. Other sources of acute and chronic toxicity data include literature searches with the Dialog Online Database for referred journal articles and compilations of data in the form of literature reviews (Shearer & Halter, 1980; Ecology, 1982, 1989, 1991/1992; Ebasco, 1993; and JMPR 1997). Such literature reviews are a good source of information for older data that compares favorably with current data. Similar compilations of EPA data were also searched such as EPA's Brian Database (1999) and EPA's ECOTOX Database (1999). These are online databases for retrieval of data submitted to support registration (Brian Database, 1999) and data from refereed journals used as supplemental material to be used for risk assessment and evaluation (ECOTOX Database, 1999).

The US EPA and Washington's Department of the Ecology (Ecology) uses these data for the following evaluations:

- To establish acute toxicity levels of active ingredients to test organisms
- To compare toxicity information with measured or estimated pesticide residues in the environment in order to assess potential impacts to fish and wildlife
- To provide data which determine the need for precautionary label statements and permit requirements in order to minimize potential adverse effects to wildlife and aquatic organisms
- To indicate the need for further laboratory and field studies to support regulatory decisions

If an adverse impact is noted in the basic data, additional studies are conducted and evaluated to determine the effects of the product on sensitive species and sensitive stages of those species. These studies typically take the form of long term chronic, early life stage, reproductive effects and life-cycle effects. These studies take into account the toxicity of the product and compare that toxicity with expected environmental concentrations. If an adverse impact is noted at levels consistent with environmental concentrations, further "field" or laboratory work is necessary to evaluate the acute and chronic effects on different organisms.

4.0.2.2 Risk Assessment Methodology

Risk assessment is conducted in a manner similar to that described in EPA (1982), Brooks (1973 in Ebasco (1993), Ecology (1980,1989 and 1991/1992) and in Urban and Cook (1985). For assessment of acute risk, the LC50 is determined for a variety of organisms within a class (fish, aquatic invertebrates, algae, other aquatic and terrestrial plants, birds and mammals). The LC50 is the concentration at which 50% mortality is seen; the LD50 is the "oral" or "dermal" dose at which 50% mortality is seen. The relative toxicity of these values is determined in two ways: 1) The EPA has certain specific descriptive classifications for inter-chemical comparisons only and these classifications do not reflect actual environmental concentrations or hazards to the test species. For an example of these classifications please see Table 1; 2) The Acute LC50 or LD50 is compared with the Expected Environmental Concentration or Expected Environmental Dose (EEC or EED). The Acute Risk Quotient (ARQ) is determined by dividing the Expected Environmental, Concentration (4-day geometric mean or other appropriate evaluation of the EEC or EED) by the laboratory measured acute toxicity (4-day LC50, LD50). The ARQ is not based on values obtained for a single species but is based on the most sensitive environmentally relevant species in a specific segment of the biota; e.g. algae, other microbes, macrophytes, fish, free-swimming aquatic invertebrates, or benthic organisms. If the ARQ is <0.1, the evaluated pesticide is generally considered to be safe to that segment of the biota for exposures of short duration. A short duration is generally defined as 4 or 5 days.

Similar calculations are used for an assessment of chronic risk. However, chronic risk is based on an exposure period of 7 or more days. Seven days exposure is considered to be a short-term chronic risk. Typically 21 to 90 days exposure is considered to be a long-term chronic risk. Short-term chronic risk involves the exposure of sac- fry to the toxic substance and long-term chronic risk involves the exposure of newly fertilized egg through free swimming and actively growing fry. For invertebrates, the chronic life-cycle test involves exposure of newborns through 21 to 28 days when the maximum number of F1 newborns will have been deposited. The Chronic Risk Quotient is determined by dividing the 28-day EEC by No Observed Effect Concentration (NOEC). The CRQ is not based on the values obtained for a single individual but is based on the most sensitive environmentally relevant species in a specific segment of the biota; e.g. algae, other microbes, macrophytes, fish, free-swimming marine aquatic invertebrates, or benthic organisms. If the CRQ is <1.0, the evaluated pesticide is generally considered to be safe to that segment of the biota for exposures of chronic duration.

To determine how well acute toxicity can predict chronic toxicity, an acute (LC50)/ (chronic NOEC) was evaluated for species that had both values available. This ratio was taken regardless of the quality of the data and then the quality was evaluated. If an individual ratio was an extreme outlier, it was discarded for the purposes of assessing the acute/chronic toxicity ratio. If extensive chronic data was not available, the acute to chronic ratio was used to estimate the chronic toxicity for species where the test had not been conducted.

4.1 2,4-D

Summary: Two registered products containing 2,4-D BEE are used for controlling aquatic weeds and algae in the State of Washington. Aqua-Kleen® and Navigate® are used primarily for control primarily of Eurasian watermilfoil and certain other

macrophytes listed in the label. In order to write a label and determine if these products are safe to the biota, organisms with an extensive history of use in pesticide testing are evaluated for their response to acute and chronic exposure to these products. The most sensitive, easily culturable species are selected for testing. Also, the most sensitive stages are usually selected to determine the acute and chronic toxicity of 2,4-D products to algae, macrophytic plants, fish, free-swimming invertebrates and benthic (sediment dwelling) invertebrates. The most sensitive stages of any organism are usually those when rapid growth is occurring or the time of reproduction or shortly thereafter when eggs or newborn offspring are present. The most likely exposure route should also be selected to most closely mimic environmental reality. Products containing 2,4-D DMA may also be used for control of Eurasian watermilfoil, but currently 2,4-D DMA is only registered for this use in dams and reservoirs of the Tennessee Valley Authority (TVA) System.

There are currently over 30 registered formulations of 2,4-D in the United States. Washington State only registers one of these formulations for aquatic use. It is the 2,4-D butoxyethyl ester containing 19% 2,4-D acid equivalence (a.e.) [27.6% 2,4-D butoxyethyl ester (a.i.)]. This review directly addresses only those formulations registered for aquatic use by the Washington State Department of Ecology and Washington State Department of Agriculture as of 1999. The toxicity of the sodium 2,4-D formulation, potassium 2,4-D, sodium 2,4-D, 2,4-D acid and 2,4-D dimethylamine salts will also be addressed to support the risk assessment since under normal environmental conditions, 2,4-D BEE (butoxyethylester) hydrolyzes to 2,4-D acid within a few hours to a day of application (Shearer & Halter, 1980, Zepp, 1975 in JMPR, 1997 Hoeppel & Westerdahl, 1973 in JMPR, 1997). Salts of 2,4-D should have toxic effects similar to the acid because they disassociate to the conjugate base almost immediately when dissolved in water (Grover & Smith, 1975 in Ebasco, 1993). When 2,4-D BEE was originally registered for aquatic use it became quickly apparent that when 2,4-D BEE was adsorbed by fish it hydrolyzed rapidly to 2,4-D acid and was eliminated from tissue [Rogers and Stalling, 1972 (in Shearer & Halter, 1980)]. Since the 2,4-D esters are rapidly hydrolyzed by water with a relatively high pH, any risk assessment will be ultimately conducted using 2,4-D acid as the model compound despite 2,4-D BEE being known to have a high acute risk. This approach is suggested by the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group (JMPR), (1997). Subsequently, most of the acute and chronic toxicity, bioconcentration and metabolism studies have been conducted with 2,4-D acid.

The 2,4-D products currently registered for aquatic use in Washington State are as follows:

Aqua-Kleen® -- A granular slow release product containing 27.6% 2,4-D butoxyethyl ester (19% 2,4-D acid equivalence). Distributed by Nufarm.

Navigate® -- A granular slow release product containing 27.6% 2,4-D butoxyethyl ester (19% 2,4-D acid equivalence). Distributed by Applied Biochemists.

Aqua-Kleen® and Navigate® are both primarily used for control of *Myriophyllum spicatum*. (Eurasian watermilfoil) in Washington State. However, use of these products may incidentally control other species of weeds. These products are also registered for the control of *Heteranthera dubia* (water stargrass), *Utricularia spp*. (bladderwort),

Nymphaea spp. (fragrant water lily), *Nuphar spp*. (spatterdock), *Brasenia spp*. (water shield), *Trapa natans* (water chestnut) and *Ceratophyllum demersum* (coontail)

2,4-D Dimethylamine (DMA) has a federal aquatic registration and is distributed by several companies. All of the liquid formulations contain ~46.8% 2,4-D dimethylamine (~38.9% 2,4-D acid equivalence). The dry formulation (usually in a water-soluble bag) contains 95% 2,4-D dimethylamine (~78.9% 2,4-D acid equivalence). This product is used primarily for the control of *Eichornia crassipes* (waterhyacinth). It is also registered for control of *M. spicatum* (Eurasian watermilfoil) and has been used in programs conducted by the Tennessee Valley Authority (TVA) in dams and reservoirs of the TVA system. Rhone-Poulenc is currently seeking to remove this label restriction for use of 2,4-D DMA for TVA dams and reservoirs only.

2,4-D 2-ethylhexyl ester is generally not used to control submersed weeds in ponds and lakes. It can be used for control of weeds on drainage ditch banks but should not be used for direct treatment of water. This product is currently not supported for aquatic use by the industry task force.

Other formulations with current registrations are not supported by industry (Larry Hammond, personal communications, Dow AgroSciences, 1999).

4.1.1 Evaluated Organisms and Sensitive Stages (EPA, 1982)

In order to develop the most sensitive risk assessment possible, appropriate species and appropriate life stages must be chosen within each class of organisms. The classes of organisms of interest are microorganisms (bacteria, fungi and algae), macrophytes, fish, aquatic invertebrates, sediment organisms (includes several classes), terrestrial plants, birds, mammals and terrestrial invertebrates (includes several classes). The life stages that are tested are selected for high sensitivity and ease of manipulation. Each class of organism is broken down into appropriate species as indicated in Table 2.

- **Microbes --** Very little work has been conducted on microbes, particularly aquatic bacteria and fungi. Recent work primarily deals with the effects of bacteria on the environmental fate of 2,4-D. Most of the work dealing with the toxicity of 2,4-D to microbes (bacteria and fungi) was conducted before 1989.

- **Algae --** There are four standard species that are typically evaluated in algal toxicity tests. They are *Anabaena flos-aquae* (freshwater blue-green algae), *Selenastrum capricornutum* (freshwater green algae), *Navicula pelliculosa* (freshwater diatom) and *Skeletonema costatum* (marine diatom). These have been selected as the standard species because there is an extensive database on the effects of many pesticides on their growth rate. Additional algal species including *Dunaliella tertiolecta, Chloroccocum spp., Phaeodactylum tricornutum, Chlorella fusca, Nostoc spp., Anabaena dolium* and *Scenedesmus quadricauda* have also been tested with one or more of the 2,4-D products, particularly 2,4-D BEE or 2,4-D acid. The endpoint of interest in algal studies is a 50% reduction in log-phase growth after five days of exposure to a static solution (EC50). Field studies normally measure the amount of chlorophyll a or use cell counts at the site as an indicator of population size.

- **Aquatic macrophytes --** For macrophytes, one species (*Lemna gibba* or duckweed) is typically used in the laboratory. It is a standard species with an extensive database

on the effects of many pesticides on its growth rate. This was the only species of macrophyte tested with commercial 2,4-D BEE products for acute toxicity by the registrant (Dow AgroSciences) or others. However, 2,4-D acid was also tested against *Sinapsis alba*, a rooted aquatic macrophyte. The endpoint of interest in *Lemna* studies is a 50% reduction in growth after 7 to14 days of exposure to a static solution containing plants at a very sensitive period in the growth cycle**.** The field studies utilized whatever species were available in whatever growth stage they were in at the time and measured the percent reduction in lake or pond coverage as an endpoint. See Table 2 for a species listing and a summary of the available data**.**

- **Fish toxicity**

  - **Acute toxicity:** The standard species tested in the laboratory include *Oncorhynchus mykiss* (rainbow trout), *Lepomis macrochirus* (bluegill sunfish), *Pimephales promales* (fathead minnow), *Cyprinodon variegatus* (sheepshead minnow), and *Ictalurus punctatus* (channel catfish). Rainbow trout, bluegill sunfish, and fathead minnow were the only species tested on 2,4-D BEE, 2,4-D DMA and 2,4-D acid and these are representatives of a cold water species (salmonids), a warm water species (sunfish), and a standard sensitive test species (minnows). The standard acute LC50 test is run with juvenile fish of a uniform age-class or size. These acute toxicity tests are not typically run with smolts, eggs and sac-fry, but in some cases acute toxicity information is provided for these stages. The test is typically run for 96 hours although some of the LC50s may be based on 24, 48 or 120-hour data mortality. The measured endpoint is mortality. The species selected are considered to be representative of a broad sensitivity range and ecological, economic and aesthetic relevance. Other species may also be tested. Those of particular interest based on ecological relevance or sensitivity are *Oncorhynchus clarkii* (cutthroat trout) with 2,4-D BEE, 2,4-D 2-EHE (2-ethylhexyl ester) 2,4-D DMA; various salmon species with 2,4-D BEE, 2,4-D 2-EHE, and 2,4-D DMA. More species have been tested with 2,4-D acid than any other test substance. This no doubt reflects the fact that, the other test substances are rapidly converted to the free acid against which the standard acute risk assessment should be conducted.

  - **Chronic toxicity:** The standard species tested for chronic toxicity are fathead minnows, rainbow trout, and sheepshead minnow, which represent a warm freshwater species, a cold freshwater species and warm estuarine species. Consensus opinion is that rainbow trout are the most sensitive species in this group. Chronic toxicity tests can be run in the sac-fry stage for at least 7 days (standard time period, 28 days). In addition to mortality, the endpoints are growth and sub-lethal behavioral effects. Another study design is the early life-stage test where the endpoints are percent hatch, time to first and last (95%), swim-up or first-feed, growth and sub-lethal behavioral effects. The effective concentration is the lowest NOEC value obtained for the most sensitive endpoint. In summary reports that are obtained from agencies or registrants, the most sensitive endpoint is often not expressed. In some reporting formats, the effective concentration may be termed the No Observed Effect Concentration (NOEC), Lowest Observed Effect Concentration (LOEC) or the Maximum Allowable Toxic Concentration [(MATC), which is the geometric mean of the NOEC and the LOEC]. This is a very sensitive test and it often may yield an

unacceptably high CRQ when the ARQ indicates a high degree of safety for the more sensitive species in the biota.

- **Aquatic invertebrates**

  - **Acute aquatic invertebrate toxicity:** For aquatic invertebrates, the standard species tested for acute toxicity include *Daphnia magna* (daphnia), *Ceriodaphnia dubia* (daphnia), *Mysidopsis bahia* (mysid shrimp) and *Crassostrea virginica* (eastern oyster), which represent two warm freshwater species and two warm estuarine species. Consensus opinion is that the eastern oyster test is usually the most sensitive. Only three species have been tested on 2,4-D BEE, 2,4-D 2-EHE, 2,4-D DMA and 2,4-D acid; *Daphnia magna*, *Gammarus fasciatus* (scud) and the eastern oyster. The endpoints for these tests are immobility for the arthropod species and shell growth for the eastern oyster. The endpoint is expressed as the 48 or 96-hour EC50 or LC50 for the three arthropods, and EC50 (dosage causing 50% decrease in shell growth in 96 hours) for the oyster. A number of other non-standard species and non-standard methods were tested with certain of the 2,4-D products and are listed along with a summary of the data in Table 2. However, only a few of the standard species and the scud were tested on all of the 2,4-D products.

  - **Life-Cycle invertebrate toxicity:** Life-cycle invertebrate toxicity studies are typically done with *Daphnia magna* (daphnia), *Ceriodaphnia dubia* (daphnia) and *Mysidopsis bahia* (mysid shrimp). However, only a very limited database is available for 2,4-D products in their chronic effects on invertebrates. The only species of invertebrate that has been tested on 2,4-D BEE, 2,4-D 2-EHE, and 2,4-D DMA is *Daphnia magna*. Since these are the only life-cycle tests presented, the database may be insufficient to support life-cycle safety of 2,4-D on invertebrates. These tests are usually run for 21 days with *Daphnia magna*, 7 days with *Ceriodaphnia dubia* and 28 days with mysid shrimp. The parent generation is selected from a group of animals less than 24 hours old. The endpoints are immobility, reduction in number of live newborns produced per female, and growth of the parent daphnids or mysids during the test. The endpoint is expressed as the most sensitive EC50 in reference to immobility and reduction in neonate production and NOEC in reference to the most sensitive endpoint. The advantage of using the mysid shrimp as a test organism is that, since it shows sexual dimorphism, certain endocrine disruptive effects could be determined in the parental generation as it matures. However, these endocrine effects cannot be interpreted or correlated with similar effects on vertebrates since it is unlikely that the effects of steroid sex hormones like estrogen or testosterone determine sex in this species.

- **Sediment organisms**

  - **Sediment organism acute toxicity:** There are major disagreements among scientists as to how sediment organism studies should be conducted. The disagreements are so great that many researchers believe that daphnia studies make good surrogates for sediment organism studies. The main problem with sediment organism studies is that sediment organisms require sediment with a specific particle size in order to function properly in a physiological sense. However, in acute tests the sediment is often eliminated from the study because it

adsorbs the toxicant and interferes with analytical chemistry when the sediment phase must be extracted. Most short-term (acute) 96-hour sediment organism studies are conducted without sediment present. There is a need for these tests since there is no reason to assume that sediment organisms will respond in a manner similar to other aquatic invertebrates. These sediment organism acute toxicity studies are conducted in a similar manner as acute tests with other invertebrates except that the age at initial exposure and the exposure period is specific to each species. These specific characteristics are listed in Table 2.

4.1.2 Exposure Routes

Regardless of the organism, aquatic exposure to 2,4-D can take several routes. These include adsorption from the water column, consumption of water or organisms while eating, contact with plants or sediments that have been treated with the test substance, or eating the granules, in the case of 2,4-D BEE granular. More detail for exposure routes is given below:

- Aquatic Algae: Exposure is through adsorption from the water column.
- Fish and Aquatic Invertebrates: Fish and aquatic invertebrates can be exposed to 2,4-D by:
  - Adsorption through the "skin" or cuticle.
  - Adsorption through the gills.
  - Adsorption through the gut from the consumption of other animals or plant and algal material.
  - Adsorption through the gut after eating the formulated pesticide granules found at the bottom of the water body in the case of 2,4-D BEE granular.
- Detritovoirs can be exposed through eating detritus found in the sediment or catching the detritus from upper regions as it floats past.

For terrestrial organisms, exposure routes can be: 1) contact with treated water, 2) consumption of treated water, 3) organisms that have been in treated water, or 4) consumption of the pesticide granules if they have access to them.

4.2 ENVIRONMENTAL TOXICITY REVIEW: EFFECTS ON THE PHYSICAL AND CHEMICAL ENVIRONMENT ON HABITAT

Sites that have never been exposed to 2,4-D products may degrade 2,4-D DMA, 2,4-D BEE and 2,4-D acid more slowly than sites that have a previous exposure history. It may take several weeks for bacteria capable of using 2,4-D as their sole carbons source to develop out of the lag-phase and rapidly degrade applied 2,4-D DMA or 2,4-D BEE. Such rapid degradation leads to a half-life in ponds and rice paddies of 1.5 to 6.5 days. However, if degradation, sorption and dilution factors are interacting in open waterways, the field dissipation half-life may be even shorter. Typical half-lives in Northwest waters is less than one week. Therefore, long-term persistence of 2,4-D BEE at concentrations that will cause environmental damage is not likely. Furthermore, since 2,4-D BEE has a low solubility and is rapidly hydrolyzed to the generally less toxic 2,4-D acid, the likelihood of 2,4-D BEE coming into significant contact with sensitive members of the biota is much reduced**.**

Biomagnification in plants and animals is not likely for 2,4 D DMA, 2,4-D BEE or 2,4-D acid. Although benthic organisms and zooplankton have been observed to bioaccumulate

2,4-D BEE at extremely high levels (~10,000) and up to ~600, respectively, it is believed that this is a flawed observation carried out in an experiment not brought to equilibrium. However, these bioconcentration factors do not appear to be amplified as fish consume these species and no significant levels of 2,4-D have been found in predatory fish. Although concentrations of 2,4-D BEE accumulate in fish for the first three hours of exposure (up to 46.6-fold in bluegill) the test substance is degraded to 2,4-D acid and eliminated from the fish within 48 to 120 hours. Eurasian watermilfoil (*Myriophyllum spicatum*) appears to bioaccumulate 14C labeled 2,4-D at concentrations up to 94 times higher than the surrounding water. However, when the plant releases the 2,4-D upon death and decay, concentrations in the water column should not increase since the total amount of 2,4-D taken up by the plant will typically be less than 1% of the total 2,4-D found in the aquatic system.

4.2.1 Potential Soil and Sediment Interactions

4.2.1.1 Impact of Various Soils (Sediment/Substrate) Composition

Summary: Due to its high water solubility and low soil/water distribution coefficient, 2,4-D acid does not adsorb well to most soils. Therefore, in most cases the concentration of 2,4-D in hydrosoil is rarely higher than 0.46 mg/Kg and dissipation to below the detection limit occurred within 17 days. However, after heavy treatment with 2,4-D DMA, concentrations of 2,4-D in the hydrosoil ranged from 0.100 to 0.45 mg/L and persisted for up to three months. Furthermore, when 2,4-D BEE was either misapplied or the pellets were improperly formulated, concentrations of 2,4-D in the hydrosoil could be extremely high (up to 288 mg/Kg and persist at levels up to 57.3 mg/Kg for 52 days).

2,4-D residues will generally persist for longer periods of time at pristine sites than at 2,4-D contaminated sties. There may be a lag time of up to several weeks before bacteria capable of utilizing 2,4-D as a sole carbon source accumulate to levels where rapid degradation can occur. The most common species of bacteria capable of degrading 2,4-D to natural products and CO_2 is Alcaligenes eutrophus *but other species also perform this function. If treatment with 2,4-D occurs twice in a season, the first application often has a fairly long half-life (14 to 20 days). However, the second application usually has much reduced half-life (2.0 to 6.5 days). Nevertheless, a variety of conditions can effect the degradation rate of 2,4-D including the presence and number of appropriate bacteria, contact between the microbes and the substrate, pH, temperature, salinity, oxygen tension, redox potential, nutrient availability, presence of alternative carbon substrates, light quality and intensity, binding to surfaces, alternative electron acceptors and solubility of the formulation in water.*

2,4-D will not adsorb well to most soils. For most products, an accurate assessment of the soil adsorption characteristics are not possible because the test substance rapidly converts to the free acid when water and soil are slurried together (Racke, 1989 for 2,4-D BEE; Grover, 1973 in JMPR, 1997 for 2,4-D 2-EHE). However, the adsorption coefficients can be determined or at least estimated for the acid. Typical ranges of adsorption coefficients (Kd) on soils is 0.29 on sand, 0.363 on sandy loam, 1.18 on silt loam to 12.7 on clay loam (Dynamac Corporation, 1988 in Ebasco, 1993). In general, a compound with a Kd value of less than 5, and particularly with Kd values less than 1.0 are considered highly mobile in soil (U.S. EPA, 1986a in Ebasco, 1989). However, under normal conditions, the ability of 2,4-D to contaminate surface or ground water is limited by the rapid rate of degradation, binding to organic materials in the soil and by uptake in

the target plants (Shearer & Halter, 1980). More details on the nature of soil mobility and its ecological meaning are discussed in Section 3.

4.2.1.2 Potential for Increased Erosion and Re-suspension of Soils and Sediments from Plant Removal

Summary: Since these products are not generally applied terrestrially, classical erosion effects typically do not occur. However, removal of plants from irrigation canal situations may result in erosive processes occurring to a limited extent.

During aquatic weed control, 2,4-D products are applied directly to water and not to the terrestrial environment. Therefore, classic erosion, in a strict sense, generally does not occur from this use. That is soil and humic material is not dislodged by wind and water and washed into the waterway due to the removal of plants from the adjacent terrestrial environment. Removal of plants from non-flowing water systems may allow for the re-suspension of sediment from the bottom of a lake or pond due to wind mixing of the water, interactions with benthic organisms and direct interfering effects of human beings with the hydrosoil during periods of either work or recreation

.

The only likelihood of classical erosion occurring is if ponds treated early in the season evaporate or are drawn down. Under such conditions the previously submerged banks and possibly bottom of the lake will temporarily become terrestrial environment subject to classical wind and water erosion. Erosion in these areas would initially be high due to lack of plant cover. However, dead aquatic vegetation, if not yet broken down by natural decay processes, would function like a mulch to help reduce erosion until the area is re-vegetated with terrestrial plants or the area is re-flooded with water. A worst case scenario could occur if the area does not re-vegetate before the dead vegetation completely decomposes and exposes the underlying soil/sediment.

Without the presence of plant species providing soil stability, physical characteristics of the soil/sediment are the primary factors affecting soil erosivity. The two most important soil characteristics affecting water-influence are infiltration capacity and structural stability. Soil texture, organic content and clay content (i.e. swelling clays also influence infiltration capacity (Brady 1974 in Ebasco, 1993), structural stability depends on the ability of soil/sediment aggregates to withstand breakup caused by physical bombardment of water and wind. This depends on many factors, including both biological (mechanical, binding action of microorganisms, cementing action of the intermediate products of microbial synthesis and decay, and cementing action of the more resistant stable humus components) and the organic/inorganic component interaction that provides bridging between organic matter and soil clays (Brady, 1974 in Ebasco, 1993).

The Soil Conservation Service (USDA, 1978a in Ebasco, 1993) has developed simplified erodibility factors (K) based purely on soil texture of different topsoil and subsoil regimes. These K factors can be used as approximate erosivity estimates. The K values listed in Table 3 are used in predicting rainfall erosion losses with the universal soil loss equation (USDA, 1978b in Ebasco, 1993) and may be used as relative indicators of erosivity across different soil texture classifications.

The loss of soil by wind erosion involves detachment and transport mechanisms. Detachment results from abrasion by both wind and entrained particles. Transport may cause soil particles to travel along the land surface by saltation or to travel parallel to or

upward from the land surface by suspension. Soil moisture is the primary factor in determining erosion by wind. Other soil characteristics include mechanical stability of dry soil clods and aggregates, presence of a stable soil crust and bulk density and size of erodible soil fractions (Brady, 1974 in Ebasco, 1973 and Klik and Truman, 1997, Larney et al, 1999). Once detached, finer-grained particles are most likely to move in the wind and rain. Using similar reasoning, Donald and Syrgiannis (1995) hypothesized that wind erosion of dry lake sediments might carry off 2,4-D. However, 2,4-D acid was detected in 78% of the Saskatchewan prairie lakes and was found in the dried sediments of semi-permanent lakes less than 10% of the time. Unless the newly exposed area is barren or completely denuded, wind erosion is likely to be negligible. Water erosion has a tendency to have a greater effect on sandy loam than on sediments (particularly silt loam) since water effectively conglomerates and seals the finer soil/sediments. Greater amounts of 2,4-D or other pesticides are likely to wash out of sandy loam than silt loam sediments.

Re-vegetation of untreated areas with plantings can be used to mitigate the problem. It has been recommended that if noxious weed control is necessary in "forest ecosystems" that it should be conducted with either herbicides or burning. Mechanical removal of weeds from such an ecosystem increases the rate of erosion. Spot treating problem areas and over-sowing the "forest harvest" area with grasses or herbaceous species that can quickly colonize a site and stabilize soils can further decrease the rate of erosion. Although Neary and Michael (1996) were addressing the problem of harvested forest areas, the approach makes sense for any area where the soil/sediment is not stable.

Strictly aquatic herbicides like 2,4-D BEE and 2,4-D DMA are not commonly used to treat canals or ditch banks. However, 2,4-D DMA and 2,4-D 2-EHE may be used to control riparian weeds growing on the edges of ditch banks. In the case of canals treated with 2,4-D 2-EHE and 2,4-D DMA, erosion is unlikely to be a problem. Generally speaking, the major aquatic weed problems in irrigation canals are emergent or riparian weeds growing on the banks of drainage canals. There are times during the season when these riparian weeds may become a problem. These herbicides are used to control emergent macrophytes like purple loosestrife, black mustard, broad leaf plantain, pigweed, dandelion, Russian thistle or lambsquarters that are found growing on the banks of irrigation and drainage canals. Canals typically are constructed with 3:1 bank slopes and are designed to convey peak demand flows without eroding. Irrigation canals can be lined with a variety of materials including earth, blended earth (clay mix to reduce seepage loss), asphalt, concrete or geotextile. Although vegetation may invade the channel over time, vegetation-lined channels are typically not constructed because plant growth can reduce the canal's conveyance capacity. The main objective in canal design is to minimize losses from the canal and to maximize conveyance capacity. Therefore, the irrigation district actively removes nuisance plant growth. Plant removal operations are usually performed at the end of the irrigation season. The general procedure involves filling and sealing the canal after which the area is treated with a herbicide. The main purpose of 2,4 D 2-EHE and 2,4-D DMA applications would be to restore irrigation water flow by eliminating dense stands of purple loosestrife. Because irrigation canals are typically designed to operate at capacity under unvegetated conditions, removal of nuisance purple loosestrife is unlikely to result in destabilization of irrigation canals. However, depending on site-specific conditions, erosive processes and the amount of sediment trapped by loosestrife, removal of loosestrife may contribute to limited sediment erosion and transport.

- **Effects of removal of weeds on habitat**

  Removal of weeds from the newly formed terrestrial habitat may cause additional silt and nutrients to enter adjacent water bodies. Such an increase in nutrient load may lead to algal blooms and eutrification of the water body. Also, the removal of these terrestrial plants will decrease the amount of new terrestrial habitat that terrestrial animals may utilize. Removal of the newly established plants may increase the likelihood of flooding and return the water body to the previous aquatic condition (flooding). Flooding can increase the amount of habitat available for fish and amphibians to utilize for feeding and spawning (Goldman and Horn, 1983). Negative impacts from isolated flooded areas could be stranding or hydrological "jumping" of current flows to a new but not necessarily, superior channel.

4.2.1.3 Effects on Pristine Sites

If the treatment site has not experienced 2,4-D treatments before, it can take several weeks before the resident bacteria come out of the lag phase and start degrading 2,4-D at their maximum potential. Presumably, the lag phase represents that period of time during which the microorganisms capable of degrading 2,4-D develop (Cheah et al., 1998). The isolates of bacteria found at pristine sites appear to be genetically different than those found at disturbed or contaminated sites Kamagata (1997). Prior to this development, physical processes such as sorption may remove 2,4-D. See Section 3 and Section 4.2.1.1, "Impact of Various Soils Sediment/Substrate Composition," for an explanation of the process of physical removal of 2,4-D. Due to the combined effects of the degradation lag phase and the adsorption process, the concentration of 2,4-D in the aqueous phase decreases during the first couple of days while the concentration in the hydrosoil increases. After development, under aerobic conditions, *Acalogenes eutrophus* and other bacteria (*Arthrobacter, Bordetella, Flavobacterium, Pseudomonas* and *Xanthobacter*) that possess the appropriate plasmids should degrade 2,4-D 2,4-dichlorophenol and glyoxylate. Then through several steps dichlorophenol is metabolized to 2-chloromaleylacetate. Glyoxylate will eventually be converted to alanine. 2-Chloromaleylacetate will be converted to oxidoadipate, enter the oxidoadipate pathway and be converted to succinate and ultimately mineralized to humic acid and CO_2 (Whiting et al, 1997, Vollmer et al., 1993, Smith et al., 1994, and Shearer & Halter, 1980). Other pathways also exist which may be functional in degrading 2,4-D in absence of oxygen and these seem to depend on the reducing power of hydrogen and other electron donors Boyle, et al, 1999).

4.2.1.4 Effects on Contaminated Sites

If the site has previously experienced 2,4-D treatment, there will usually be no lag phase and the bacteria present will start degrading 2,4-D at a rapid rate almost immediately. For example, Chen and Alexander (1989) found that a laboratory culture previously inexperienced with 2,4-D acid had a lag phase of about 15 hours prior to the start of a slow degradation of 2,4-D. However, if this same strain was previously cultured with 2,4-D acid, introduction of additional 2,4-D involved no lag phase and 2,4-D persisted for only 3-hours before it was eliminated.

These effects have also been shown to occur in the field with applications of 2,4-D DMA. Hatfield (1995b in JMPR 1997) found that 2,4-D DMA applied at 46.81 Kg/ha (41.79 lbs/acre) to a North Carolina pond had a first treatment half-life in water of 19.7 days

while the second treatment half-life was only 2.7 days. The sediment at the same site exhibited a similar reduction if half-life after additional treatments. The half-life of 2,4-D DMA acid on sediment after the first treatment was 7.6 days while the second treatment half-life was only 2.0 days.

Similar observations were made by Hatfield in a North Dakota pond treated at the same rate with 2,4-D DMA. There was a half-life of 13.9 days in water after the first treatment and 6.5 days after the second treatment (Hatfield, 1995r in JMPR, 1997). Long periods of (about a year) between treatments may eliminate the advantage of contaminated sites in metabolizing 2,4-D in soil and presumable similar effects occur in water (Holben et al, 1992).

These half-lives were fairly long but half-lives as short as 1.1 days in water and 1.5 days in sediment have been noted in a Louisiana rice paddy treated with 2,4-D DMA at 1.68 Kg/ha (1.5 lbs/ha).

However, a variety of conditions can effect the rate of degradation of 2,4-D on soils and presumably in water as well. These may include the presence and number of appropriate microorganisms, contact between the microbes and the substrate, pH, temperature and salinity, oxygen tension and redox potential, nutrient availability, presence of alternate carbon substrates, light quality and intensity, binding to surfaces, alternative electron acceptors. The 2,4-D formulation used (BEE, 2-EHE, DMA, acid, K salt, sodium salt) and concentration and solubility of the herbicide in water also effect the degradation rate. Since, the esters and the salts are converted rapidly (typically within 1-day) to 2,4-D acid, chronic toxicity is due to the effects of 2,4-D acid. If anoxic conditions exist, bacteria like *Acalogenes spp*. and other species are not capable of metabolizing 2,4-D to the above mentioned natural products. This may be a significant consideration when treating heavily weed-infested ponds or lakes in total with 2,4-D where complete stripping of the dissolved oxygen content is likely to occur. 2,4-D acid will therefore remain in water and soil/sediment environment for longer periods of time and has a potential for toxicity due to protracted exposure to effect benthic organisms.

The maximum target concentration in water for a 2,4-D treatment is 2 to 4 mg a.e./L with the most typical application rate being 1.13 mg a.e./L (JMPR, 1977). If the typical adsorption coefficient is 1.0 and the sediment to water ratio is 1/200, the equilibrium value within soil would be expected to be between 1.1 and 4.0 a.e./L. The estimated and achieved concentrations were often very divergent. Therefore, the levels of 2,4-D found in sediments treated with reasonable rates of 2,4-D BEE (45 kg/ha = 40 lbs/acre) have been seen at concentrations ranging from 7.15 to 288 mg/Kg and persist at levels up to 57.3 mg/Kg for 52 days (Dynamac, 1988 in Ebasco, 1993). However, Halter (1980) believes that these high levels of 2,4-D in the sediment may have been primarily due to poorly formulated 2,4-D BEE granules. These sediment concentrations may effect benthic sediment species. The effect that sediment levels of 2,4-D have on the biota is discussed in Section 4.3.2.5.

Similar treatment with 2,4-D DMA yielded results that were much lower in concentration but had a moderately high persistence in sediment when this product was use to treat mesocosms in Florida and Georgia. When ponds were treated at levels of up to 8.96 Kg 2,4-D DMA/ha (8 lbs/acre), the highest levels of 2,4-D seen in the hydrosoil was 0.046 mg/Kg at three days after treatment (Gangstad, 1986). These concentrations may adversely effect the most sensitive sediment organisms (see Risk Assessment section).

4.2.2 Environmental Persistence

Summary: Treatment with 2,4-D DMA typically produces much lower concentrations of 2,4-D in the sediment than treatment with 2,4-D BEE. These concentrations are typically 0.005 to 0.046 mg/Kg for 2,4-D DMA and 4.3 to 8.0 mg/Kg for 2,4-D BEE. Due to the extremely high toxicity of 2,4-D BEE, there is limited potential for adverse impact to the biota based on the results of laboratory studies. Concentrations of 2,4-D BEE, although similar to those of 2,4-D acid, may be high enough to cause observable damage to the biota.

Bioconcentration in plants and animals is not likely for 2,4-D DMA, 2,4-D BEE or their hydrolysis/dissociation product (2,4-D acid). Although short term bioaccumulation of 2,4-D BEE can be fairly high in fish (BCF = 1.7 to 44.6 in rainbow trout and bluegills respectively) after three hours of exposure, 2,4-D BEE is converted to 2,4-D acid and excreted so that the BCF drops below 0.2 within 48 to 120 hours. If fish are "fed" 2,4-D acid, >90% is excreted within 24 hours. 2,4-D DMA also does not bioaccumulate in fish with the BCF value remaining below 0.2 for the entire 28-day exposure period. Work conducted in the field tends to corroborate this data since it was found that fish have little tendency to bioconcentrate 2,4-D in the field and when it does bioconcentrate it is rapidly eliminated.

Although benthic organisms and free-swimming invertebrates have been shown to bioaccumulate 2,4-D to very high levels (BCF = 8267 to 10,825 and 1 to 603, respectively) in the field, these results are probably artifacts since this experiment was not carried out to equilibrium. However, since these high levels are not found in fish, 2,4-D apparently does not bioconcentrate or biomagnify across trophic levels.

Eurasian watermilfoil apparently bioconcentrates 2,4-D to levels 33- to 94-fold higher than the levels found in water, but eliminates this material within 16-weeks after the watermilfoil mass has undergone extensive decay. The release of 2,4-D from decaying watermilfoil probably has little effect on the concentration of 2,4-D in water since the highest concentration in plants is only about one percent of the total 2,4-D in the aquatic system.

The environmental persistence of 2,4-D products in the field can be quite variable; the half-life in water varies from a few days to several months. Degradation in water usually depends on microbial degradation and generally occurs more rapidly at higher temperatures and under aerobic conditions. There may be a fairly long lag period that occurs (typically 5 to 7 days but can last as long as several weeks) before extensive 2,4-D degradation occurs (Kim & Maier, 1980 in Ebasco, 1993). The half-life in aquatic sediment is usually longer than on soil (terrestrial) and often longer than in water. Typically the half-life on clay sediments is from 20 to 50 days. While extremes of sediment persistence as described above in Section 4.2.1.4 can occur, this is not typical and the sediment concentrations for 2,4-D typically drop below the detection limit within 14 to 161 days. Typically the maximum concentration in sediment will be achieved within 3 to 6 days of initial application (Dynamac, 1988 and Schultz and Gangstad, 1990 in Ebasco, 1993). If the 2,4-D BEE pellets are formulated properly, the concentration in sediment is usually not extremely high. For example: 1) The maximum concentration in the sediment was 34 mg/Kg at Lake Okanogan, British Columbia 2 days following application of the pellets and disappeared after 59-days of dissipation; 2) The

concentrations are usually much lower in sediment than shown above; effects were found at Lake Seminole, Georgia where 0.100 mg/Kg 2,4-D was found shortly after application, which dissipated to non detectable levels within 12-days post treatment; 3) The maximum concentration of 2,4 –D in sediment at Currituck Sound, NC was 0.200 to 0.600 mg/Kg at application and during the subsequent three-week monitoring period (Shearer & Halter, 1980).

2,4-D is not expected to be found on soils when using the aquatic herbicides 2,4-D BEE or 2,4-D DMA unless an irrigation or flood incident occurs. However, the expected half-life on most soils ranges from 1.5 to 8.5 days (McCall et al, 1981 and Kawatsuka & Miwa, 1989 in Ebasco, 1993). Low accumulation and low leaching of 2,4-D are expected on soils receiving irrigation or floodwater due to effective degradation by microorganisms. Effects of 2,4-D on terrestrial crops after irrigation or flooding are discussed in Section 4.2.5.

4.2.2.1 In Water

A detailed review of the persistence of 2,4-D in water can be found in Section 3.1.3.3. The half-life for 2,4-D ranges from a few days to several months depending on the conditions found in the water at the time of treatment and during the breakdown of 2,4-D. The breakdown of 2,4-D BEE usually takes less than a day. In a typical treatment to control Eurasian watermilfoil, 100 lbs./acre of 2,4-D BEE was applied to impounded coves of Beulah Lake in southeastern Wisconsin. On May 21, 1993 (one day post-application), the concentration of 2,4-D in water ranged from 0.190 to 0.330 mg/L. At the end of the monitoring period on June 28th, the concentration ranged from ~0.030 to 0.090 mg/L Helsel et al. (1996). Since the monitoring period was 30 days, the half-life of 2,4-D at this site was approximately 13 to 17 days. If geometric mean is used, the estimated half-life, would be ~15 days. This is a reasonably good estimate for the half-life of the disassociated acid since the half-life has been determined for 2,4-D DMA to range in the time frame of 3.9 to 11 days by Reinert and Rogers (1987). Although the initial concentration values given here are reflective of the whole cove situations, 2,4-D BEE is designed as a slow release product where release into the root (benthic) zone should yield a higher concentration of 2,4-D than at the surface of the water body where the 2,4-D concentrations are governed by diffusion and mixing from the benthic zone. Gallagher (1992), reported that maximum Canadian 2,4-D concentrations in open water treatment areas should be 3.25 mg/L in bottom water samples and 0.190 mg/L for surface water samples. Water in treated water impounds have somewhat higher concentration of 2,4-D in bottom waters (4.0 mg/L) and surface waters (1.23 mg/L). In our water treatment areas the residue levels dropped to <0.100 mg/L in 2,4-D 6 days after application and <0.001 mg/L in 5 to 22 days after application. Similar results were obtained at Loon Lake, Washington where treatment of 2,4-D BEE at 100 lbs/acre (112 Kg/ha) resulted in maximum concentration of 1 to 2 mg/L within 1 to 3 days of application. The Loon Lake concentrations decreased to <0.005 mg/L within 3 to 7 days after application. These values were generated for the purposes of acute (0.100 mg/L) and chronic (0.001 mg/L) risk assessments as described in Section 4.0.2.2**.**

4.2.2.2 In Sediment

A detailed review of the persistence of 2,4-D in sediment can be found in Section 3.1.3.2. 2,4-D from application of the 2,4-D BEE product may have significant concentrations and half-lives of considerable length in the sediment. The stability of 2,4-D acid and 2,4-

D BEE in sediment is governed by pH, dissolved oxygen content and temperature. All of these physical parameters have a tendency to be less than optimal for the degradation of 2,4-D acid and 2,4-D BEE while it is in the sediment. In artificial pond experiments, Birmingham and Colman (1985, in Ebasco, 1993) found that typical sediment concentrations were approximately 4.4 higher than in the water phase on the first day after application. The concentration in water increased for the first two weeks after application while the concentration in sediment decreased for every observation time during the 180-day period. The half-life for 2,4-D in sediment for this example cannot be directly calculated since the regression is not linear. However, the 1st half-life can be estimated using the degradation data for the 1st 55 days as being ~35 days. Gangstad (1986) made similar observations in Florida and Georgia ponds where 2,4-D DMA had been used for the control of waterhyacinth. However, there were significant differences in how the two formulations behaved in the environment. Applications of 2,4-D BEE were approximately three times the 2,4-D DMA application rate, but the rates in acid equivalents were about the same (23 Kg/L vs. 8.96 Kg/L). Sediment concentrations of 2,4-D in the 2,4-D BEE experiment were 4.3 to 8.0 mg/Kg for the first two weeks, but concentrations were only 0.005 to 0.046 mg/Kg in the 2,4-D DMA experiment. This would indicate a much greater degree of sediment organism safety with 2,4-D DMA than with 2,4-D BEE when you consider that the most sensitive organism, lined scud (*Gammarus fasciatus*) has an acute LC50 of ~5.8 mg 2,4-D/L to 2,4-D BEE, >100 mg/L for 2,4-D DMA and 3.2 mg/L for 2,4-D acid.

Typical sediment concentrations for 2,4-D DMH and 2,4-D BEE are expected to range from 0.100 to 0.450 mg/L and 0.05 to 0.46 mg/L, respectively. Levels of 2,4-D DMA persist for up to three months after heavy treatment in a TVA reservoir while levels of 2,4-D BEE in Lake Okanogan typically persist for less than 17 days (Wojtalik, 1971 and Lim and Lozoway, 1978 in Shearer and Halter, 1990). These values were used in the calculation of the expected environmental concentrations (EEC) presented in Section 4.3.2.5.

4.2.2.3 In Soil

The presence of 2,4-D in soil is not anticipated from aquatic treatment unless flooding occurs or the water is used for irrigation. Use of water for irrigation is prohibited by the 2,4-D BEE label (current Aqua-Kleen® label as of January, 2000) and irrigation is prohibited with the 2,4-D DMA labels prior to analytical concentration not exceeding 0.1 mg/L (Terra 2,4-D amine label, 1999). According to Gangstad (1986), the top six inches of soil irrigated with water containing two to five mg/L of 2,4-D DMA (acid equivalents) contained not more than 10% of the concentration expected from the irrigation process. The half-life of 2,4-D DMA on soil is variable but is usually 1.5 to 8.5 days with a geometric mean of 3.6 days. The effects of 2,4-D in soil on plants after irrigation are discussed in Section 4.2.5.

4.2.2.4 Potential for Bioaccumulation or Bioconcentration in Fish, Aquatic Invertebrates, Phytoplankton, Zooplankton, Birds Mammals and Insects

The potential for bioaccumulation (BAF) and bioconcentration (BCF) is low to moderate for both 2,4-D BEE and 2,4-D DMA. In many cases 2,4-D acid will be adsorbed to a fairly high degree by plants and animals during the first few days of exposure. However, once converted to 2,4-D acid within the animal, the pesticide is rapidly eliminated [Birmingham and Colman (1985 in Ebasco, 1993)] and Reinert and Rogers and Stallings

(1972 in Ebasco, 1993). The expected bioconcentration factor for 2,4-D BEE is moderately high. Since the octanol/water partition coefficient K_{ow} is fairly high for all 2,4-D products (645 for 2,4-D acid and 2,4-D DMA and 14719 for 2,4-D BEE), it can be predicted that bioconcentration would be non-accumulative to slightly accumulative based on the classification scheme developed by Weber (1977 in Ebasco, 1993) (Table 4). The bioconcentration factor can be predicted throughout most of its range from the following equation: $BCF = K_{ow} \times 0.05$. Therefore the BCF is predicted to be ~32 for 2,4-D acid and 2,4-D DMA and ~740 for 2,4-D BEE. These values are similar to those predicted by Chou (1977 in Ebasco, 1993) and Veith et al. (1979 in Ebasco, 1993). Also since, the water solubility of 2,4-D acid and 2,4-D DMA products are fairly high (23,000 to 34,000 and 664,000 to 729,000mg/L, respectively), it would not be expected that these extremely hydrophilic compounds would bioconcentrate or bioaccumulate to a high degree. However, since the 2,4-D BEE product is insoluble in water, it would be expected that this compound would bioconcentrate at least to a moderate level.

- **Bioaccumulation and bioconcentration**

  When 2,4-D BEE is applied in the field at a rate of 23 Kg/ha (20.5 lbs./acre), 2,4-D appears to be rapidly adsorbed by Eurasian watermilfoil. (Birmingham and Colman (1985 in Ebasco, 1993). In an artificial pond, *Myriophyllum spicatum* was exposed to water containing 1.8 to 3.0 mg/L for the first three weeks. The plants accumulated the radioactivity at concentrations of 33 to 94-times the concentration detected in water. After three weeks the concentrations of 2,4-D in plant tissue decreased dramatically as the plants decayed and were not detectable after about 16-weeks (Table 6). Total plant collapse (death) can normally be expected after five days of exposure (Birmingham et al., 1983 in JMPR, 1997). Although little information is available on the breakdown products of 2,4-D in aquatic plants, it can probably be presumed that the breakdown products are similar to those found in terrestrial plants. Typical metabolites seen in plants were eight aglycones (glucose conjugates) of which 4-hydroxy-2,5-dicholorpheoxyacetic acid was the most abundant. Seven other polar metabolites including 2,4-D-glutamic acid and 2,4-D aspartic acid were seen. These amino acid conjugates appeared to be actively metabolized by plant tissue to free 2,4-D and water-soluble metabolites (Feung et al, 1972, 1973 and 1975 in JMPR, 1997). After the decay of the plant, the release of any remaining 2,4-D should have little effect on the concentration of 2,4-D in water since the highest concentration in plants is only about one percent of the total 2,4-D in the aquatic system.

  Sorption and metabolism has also been investigated with 2,4-D. There is little confirmed information that 2,4-D is adsorbed extensively by phytoplankton. Voight and Lynch, 1974 (in Shearer & Halter, 1980) found that 2,4-D acid was adsorbed weakly by *Coelastrum microporum*. Similar work by Boehm and Mueller, 1976 (in Shearer & Halter, 1980) showed a BCF in this species of only two-fold. However, Wojtalik et al. (1971 in Halter, 1980) found that when the Gunthersville Reservoir, Alabama was treated with 2,4-D DMA that algae removed nearly 100% of the 2,4-D in surface water samples at 24 hours post treatment. However, no other study has been evaluated that shows similar results. It is interesting to note that 21 species of algae have been observed to metabolize 2,4-D extensively when the applied active ingredient was 2,4-D BEE. 13% to 64% of the applied herbicide were degraded. How much depended on the algal species tested (Butler et al. in Halter, 1980).

The only extremely high BCF levels observed in the field were for benthic organisms and zooplankton. In Fort Cobb Reservoir, Oklahoma BCF values were observed to be 8,267 to 10,825 in benthic organisms and 1 to 603 for zooplankton in reservoirs treated with 2,4-D BEE. However, this is a somewhat flawed observation in that this experiment was not carried out to equilibrium. Although high levels of 2,4-D were seen in these fish food species, similar experiments at other sites did not detect 2,4-D BEE in fish 24 hours after treatment of Lake Seminole, Georgia (Reinert and Rogers, 1987). Although a complete study evaluating the BCF in both fish food stock and predatory fish was not conducted, this combined data indicates that 2,4-D does not appear to bioaccumulate across trophic levels. Trophic level magnification of bioconcentrated 2,4-D has not been seen. This biomagnification aspect of bioconcentration is discussed below.

Bioconcentration studies with both 2,4-D BEE and 2,4-DMA have been conducted in the laboratory on several animal species. 2,4-D DMA has been tested in the laboratory for bioaccumulation in bluegill sunfish, channel catfish, northern crayfish and freshwater clams. These species were exposed to 6 mg a.e./L of 2,4-D DMA for 28 days. For the first one to two weeks, all species gradually "accumulated" 2,4-D in their edible tissue. However, the maximum concentration never exceeded 0.94 mg a.e./L in any of the species tested. The bioconcentration factor for 2,4-D was therefore never higher than 0.16 in any of the species tested. By the end of the test (15 to 28 days of exposure), no concentrations in edible tissues from any species exceeded 0.56 mg a.e./Kg; the BCF therefore was only 0.09 (Biever, 1998 and Biever, 1996). Trophic level magnification of bioconcentrated 2,4-D has not been seen. The biomagnification aspect of bioconcentration is discussed below.

Similar tests with 2,4-D BEE were conducted with rainbow trout and bluegill sunfish. When these fish were exposed to 1 mg a.e./L under laboratory conditions, the edible tissue contained only 2,4-D acid and no 2,4-D BEE. Both species initially accumulated 2,4-D acid at concentrations higher than the concentration in water. After the first three hours of exposure the concentrations of 2,4-D acid were 1.2 to 4.4 mg a.e./L for rainbow trout and 1.7 to 46.6 mg a.e./L for bluegill sunfish, respectively. However, after 48 and 120 hours of exposure, the concentrations in rainbow trout and bluegill sunfish were below the detection limit (<0.05 mg a.e./L) and 0.1 to 0.2 mg a.e./L, respectively (Rogers and Stallings, 1972 in Ebasco, 1993). This indicates that 2,4-D in either commercial form does not bioaccumulate in aquatic animals.

Although 2,4-D is rapidly eliminated from their edible tissue, aquatic animals do not effectively metabolize 2,4-D. After four days of exposure to 2,4-D acid at 11 mg/L, almost all of the 2,4-D (80%) found in edible tissue was found as 2,4-D acid. The remainder was found as 2,4-Dichlorophenol (7.9%) and unknown materials (3.2%). In the viscera, some degradation of 2,4-D did occur; 30% of the recovered material was 2,4-D acid, 28% was 2,4-dichlorophenol and 40% was a mixture of chlorophenylacetate and chlorophenol, and 7.5% were unknown metabolites (Premkumar, 1994).

In most cases, animals did not accumulate 2,4-D in the field. For 2,4-D DMA, a variety of fish including channel catfish, bluegill sunfish and redear sunfish were monitored for 2,4-D concentrations. The concentration in fish tissue over a 28-day period was typically 0.009 to 0.0118 mg a.e./Kg while the concentration in water

varied with time and treatment rate (2.24, 4.48 and 8.96 Kg a.e./Ha) but was typically 0.02 to 1.18 mg a.e./L. Therefore, the bioconcentration factor in these fish was never higher than about ~9 and typically ranged from 0.9 to 3.00-fold [Schultz and Gangstad, (1975) and Gangstad, (1986)]. Some outliers that cannot be explained occurred on day 14 and day 28, but the total residues in these samples still remained very low (≤0.043 mg a.e./Kg) (Table 7). 2,4-D therefore has very little tendency to bioconcentrate in the field. However, in blue crab low levels of 2,4-D acid were seen in edible tissue some time after treatment for waterhyacinth control at St. Johns River, Florida. When concentrations in water were less than 0.01 mg a.e./L, the concentration in crabs was found to be 0.050 to 0.065 mg a.e./L. While the concentration is fairly low, the bioconcentration factor was at least 50 to 60-fold. Within a couple of months of the treatment date, the water concentration dropped to near detection limits and the concentration in edible crab tissue could not be detected. Therefore, 2,4-D has very little tendency to bioconcentrate in the field and when it does bioconcentrate, it appears to be rapidly eliminated.

2,4-D DMA or 2,4-D BEE was applied to 27 acre test plots in Lake Seminole, Georgia to control Eurasian watermilfoil (Hoeppel in Westerdahl, 1983 in Ebasco, 1993). The application rate was 22.5 to 45 Kg a.e./ha (20 to 40 lbs a.e/acre), for 2,4-D DMA and 2,4-D BEE respectively. Assuming an acre-foot of water, the estimated concentration of 2,4-D in water would be 7.3 to 15 mg a.e./L, respectively. Fish sampled included largemouth bass, bluegill sunfish, catfish and gizzard shad. None of the fish from the 2,4-D DMA treated area contained detectable concentrations of 2,4-D (1 mg a.e./Kg). However, in the 2,4-D BEE treated area, 18 of 20 gizzard shad contained detectable concentrations with the highest levels at 3.85 mg a.e./L. In game fish 4 of 24 fish contained detectable levels of 2,4-D with the highest edible tissue concentrations at 5.1 mg/L. These concentrations of 2,4-D gradually declined in gizzard shad to 1 mg a.e./Kg at 28 days and 0.13 mg a.e./Kg in game fish at 13 days. Again, it was found that 2,4-D has very little tendency to bioconcentrate in the field; and when it does bioconcentrate, it appears to be rapidly eliminated (Hoeppel and Westerdahl, 1983, in Ebasco, 1993).

- **Persistence within the organism**

Most organisms do not bioconcentrate 2,4-D and those that do rapidly eliminate the compound so that it is unlikely to be passed along trophic levels (Reinert and Rogers, 1987). Fish that adsorbed 2,4-D from the water eliminated the majority (more than 50%) of 2,4-D from their tissues within a few days despite continued exposure (Biever, 1996 and Biever, 1998).

One hypothesis is that 2,4-D was accumulated in gizzard shad because this benthic species “ate” the sediment and the pellets (Hoeppel in Westerdahl, 1985 in EBASCO, 1993). Data concerning the concentration of 2,4-D acid fed to channel catfish is presented below. Fish were “fed” 10 mg 2,4-D acid/Kg. The highest concentrations of 2,4-D detected in these fish was 1 to 4 hours after dosing when the concentration of 2,4-D in edible tissue was 0.6 mg/Kg. In other tissues, the concentration of 2,4- D at these times ranged from 4.4 mg/Kg in the head kidney to 12.4 mg/Kg in the trunk kidney. The concentration of 2,4-D in bile, spleen, plasma and liver was intermediate at 5.6, 6.0, 6.5 and 9.5 mg/Kg, respectively after four hours of adsorption. Within 24 hours over 90% of the administered dose had been eliminated with all tissues

containing less than 0.1 mg/ 2,4-D/Kg. The bile however still contained large quantities of 2,4-D (15.8 mg/Kg).

- **Potential impacts on the food chain**

  2,4-D BEE has a tendency to accumulate in sediment and plant from 1-7 days (Gangstad, 1986). This may be a reflection of plants and sediments "metabolizing" 2,4-D to products that can be incorporated into the plant structure or the sediment (as humus). Animals, however, rapidly hydrolyze adsorbed 2,4-D BEE to 2,4-D acid and excrete it unchanged back into the water. Higher animals (cows and chickens) did not bioaccumulate 2,4-D; approximately 81-114% of the administered dosage was eliminated by chickens in the excreta and less than 0.1% of the administered dose was collected from eggs and poultry tissue; in lactating goats and dairy cows, 98% of the administered dose was eliminated unchanged in the urine after dosing for three days, and less than 0.5% of the total test material was recovered from all tissues and milk (Puvanesarajah and Bliss, 1992, Krautter and Downs, 1996). In chickens, the main metabolites were not identifiable and in the goats and cows, only 2,4-dichloranisole was identified. Therefore, 2,4-D should not bioaccumulate; it should be rapidly eliminated from any organisms that ingest it; and it should not be bioaccumulated (biomagnified) as it is passed up the food chain.

4.2.3 Potential Impacts of Water Quality on Survival of Aquatic Organisms

4.2.3.1 Effects of Physiological Sustaining Water Chemistry

Summary: Exposure of living plant tissue to 2,4-D products or other herbicides usually results in secondary effects that may impact the biota. When plants start to die, there is often drop in the dissolved oxygen content associated with the decay of the dead and dying plant material. Reduction in dissolved oxygen concentration may result in aquatic animal mortality or a shift in dominant forms to those more tolerant of anaerobic conditions. There may also be changes in the levels of plant nutrients due to release of phosphate from the decaying plant tissue and anoxic hypolimnion. Also ammonia may be produced from the decay of dead and dying plant tissue which may reach levels toxic to the resident biota. Ammonia may be further oxidized to nitrite (which is also toxic to fish), and the almost nontoxic, nitrate. The presence of these nutrients may cause an algal bloom to occur. However, if significant living plant biomass persists after treatment, the released nutrients may be removed before an algal bloom can occur. Hardness and pH may have an effect on the toxicity of 2,4-D 2-EHE or 2,4-D acid, these parameters will probably not significantly impact the toxicity of 2,4-D amine salts since they are practically non-toxic at all environmentally relevant water hardness. The direct toxicity of 2,4-D BEE to salmonids will also probably not be affected by hardness since its toxicity does not appear to vary with varying degrees of hardness. However, increasing pH could cause 2,4-D BEE to degrade more rapidly to the practically non-toxic 2,4-D acid and thus decrease the potential for contact with 2,4-D BEE to fish and invertebrates.

- **Potential impacts of dissolved oxygen**

  Probably the key factor to survival and maintenance of the aquatic environment is adequate dissolved oxygen. The oxygen content of the water should ideally be as close to saturation as possible. For warm water environments (15 to 25°C) oxygen

saturation is 10 mg/L at 15 degrees and 8.2 mg/L at 25°C. For cold water environments (5 to 15°C), oxygen saturation is 12.2 mg/L at 5°C and 10mg/L at 15 degrees centigrade. Cold and warm water are somewhat arbitrary designations. Table 8 shows the sea level saturation concentration for oxygen at temperatures from 5 to 25°C.

Generally, speaking, warm water fish like sunfish, bass, catfish, carp and shiners can survive and reproduce at oxygen concentrations of about 5 mg/L (Litler, 1983, personal communications). However, while cold water fish are able to survive for short periods at dissolved oxygen concentrations of as low as 1 to 3 mg/L, concentrations needed for long term survival are much higher. It is unlikely that these cold water species could go through a life cycle at dissolved oxygen concentrations below 9.0 mg/L (Welch, 1992 in Shearer et al, 1996).

Treatment with 2,4-D products has been shown to decrease the dissolved oxygen content. Oxygen depletions are to be expected following application of 2,4-D due to the bacterial breakdown of the dead plants. This has been verified by a study at Stone Valley, Pennsylvania showing dissolved oxygen readings of 0.0 parts per million within six days of 2,4-D BEE treatment (Marshal & Rutschky, 1974). This dissolved oxygen slump occurred earlier in the season than usual due to treatment with 2,4-D. However, a natural oxygen slump has been observed in the hypolimnion during late summer due to a dissolved oxygen stratification, which closely follows the temperature stratification. High summer temperatures make this lake more suitable for warm-water species than cold water species. Rock bass, pumpkinseed sunfish, largemouth bass and bluegills make up the majority of fish in this lake. Also found in the lake are chain pickerel, bullhead catfish, white sucker, brown trout and rainbow trout.

Trout are stocked in this lake in spring and fall to correspond with the two trout seasons. These stocked fish have a poor survival rate but it is not known whether this is due to high temperatures, predation by pickerel and snapping turtles, or fishing. However, not many dead trout have been observed in the lake. It would be expected that the low oxygen content in the hypoliminion of this lake would effect the numbers and diversity of benthic organisms. But the while the absolute numbers did decrease significantly, the diversity index did not change. While the diversity index did not change, the dominant species shifted from odonates and mayflies to oligochaete worms and tendepedid midges. This shift in dominant species may be due to differences in tolerance of low dissolved oxygen concentrations in different species. Very few organisms can tolerate an anoxic environment for more than a brief period of time. Those species that can withstand low oxygen levels including oligochaete worms, tendepedid midges, chironomid midges and chaoborid midges are often associated with polluted environments (Marshal & Rutschky, 1974 and Goldman & Horne, 1983).

The above field study was conducted at rates that would be typically used to control Eurasian watermilfoil, American waterweed and pondweed. However, a recent study in a Kentucky lake indicates that applications of 2,4-D DMA at a target rate of 2 mg/L (11.25 to 45 Kg/ha = 10 to 40 lbs/acre) demonstrated oxygen levels that were lower than those found in control microcosms or microcosms treated with similar concentrations or concentrations up to 25-times the field rate (Kobraei and White (1997). Another study conducted with 2,4-D BEE and 2,4-D DMA to obtain

concentrations of 1 mg a.i./L, indicated that sediment respiration increased in each of the treated ponds during the period of milfoil collapse and decay (Scott, 1985 and Nagy et al., 1985 in Sherry, 1994). To what degree the oxygen levels slumped was not specified. However, it was noted that the treated ponds did not become anaerobic and that this would favor aerobic and microaerophilic organisms like fungi.

Effects of the presence of oxygen can influence the degradative pathway of 2,4-D. In the absence of oxygen, it is anticipated that 2,4-D would preferentially degrade to 2,4-chlorophenylphenol which is known to be toxic to fish (20 mg/L), *Lemna* (1.5 mg/L), to the marine crustacean (*Allorcheste compressa*) at 0.075 mg/L, the marine algae (*Phyllospora comosa)* ($<10^{-6}$ mg/L) and to certain unspecified soil fungi (Kamler et al, 1974, Ensley et al, 1994, Burridge et al, 1995 and Short et al, 1991). Until recently, the main concern regarding 2,4-dichlorphenol has been the toxicity of this chemical as a product contaminant and not as a metabolite of 2,4-D. However, since it is a primary product of anoxic marine sediment, it could potentially be of concern (Boyle et al, 1999).

- **Potential impacts of ammonia, nitrite and nitrate production**

It is very rare when nitrogen is the limiting factor for production within a freshwater body. The ability of several species of blue-green algae to fix nitrogen makes any additions of nitrogen to water bodies not a major issue. However, the toxicity of ammonia and nitrites to aquatic organisms is at times an issue. Experimental 2,4-D treatments in Lake Kentucky (Kobraei and White, 1996) produced a marked increase in the nitrate levels detected; from day 4 to day 8, the concentrations of nitrate in the field water samples increased from ~0.001 - 0.005 mg/L to over 0.060 mg/L. It was noted that concentrations of nitrate increased dramatically in the field samples containing 2 mg/L 2,4-D. There was also a brief and transient increase in field nitrate levels on the day after application of 2,4-D, when the concentrations of nitrate rose from <0.005 mg/L to ~0.020 mg/L. Increased nitrate production occurred during the periods of high community respiration in the field. The highest chlorophyll a concentrations occurred when nitrate concentrations exceeded 0.002 mg/L while the lowest chlorophyll a concentrations occurred shortly after the minimum nitrate concentrations occurred (~0.001 mg/L). That the highest chlorophyll a concentrations in the treated field plots were 50% higher than in the control is good evidence that nitrogen concentrations contributed to the algal "bloom". Again, increased levels of nutrients do not necessarily promote an algal bloom. Sherry (1994) noted that while there was an increase in nutrient level due to milfoil decay, phytoplankton and zooplankton did not increase in treated plots in any obvious manner (Sherry, 1994 cites Scott, 1985). Algal blooms could not be accounted for solely due to significant increases in ammonia, nitrate and nitrite. "Secondary" effects that include the increase in ammonia, phosphate levels and dissolved oxygen content were considered to be responsible for the increase in phytoplankton, zooplankton and bacteria in a carp pond in India treated with 2,4-D sodium salt at 6 Kg a.i./ha. Although the levels of ammonia were very high (up to 0.65 mg/L) shortly after treatment, no fish kill was recorded during the course of the study and after the treated vegetation had been eliminated, most water quality parameters improved over pretreatment levels including dissolved oxygen content, and free ammonia (Patnaik and Das, 1991) (Table 15).

In Washington waters, even a small release of ammonia can be a serious issue. The whole lake levels of ammonia-nitrogen in Lake Steilacoom during the 1995 season exceeded the criterion of 0.100 mg/L during the month of May and October. These levels of ammonia are toxic to fish and nearshore runoff containing fertilizers may have contributed to the October ammonia peak (Shearer et al., 1996). These levels of ammonia are higher than the maximum recommended levels for the culture of aquatic organisms and are higher than the EPA criterion (0.091 mg/L) for 4-day exposure of salmonids.

The toxicity of ammonia increases with both temperature and pH. As temperature and pH increase, the amount of unionized ammonia increases (Table 9). The unionized forms of ammonia (NH4OH + NH3) are toxic to aquatic animals. The ionized form of ammonia ($NH4^{+}$) is almost harmless (Goldman and Horne, 1983).

Adsorption of nitrogen containing nutrients by aquatic macrophytes and algae can influence the seasonal dynamics of nitrite and nitrate concentrations. The levels of nitrite/nitrate are often higher at the surface of a non-flowing water body than at the bottom because under anoxic conditions some bacteria utilize nitrate as a terminal hydrogen receptor when oxygen is not available.

Nitrite, although fairly toxic, is rarely a problem in well aerated waters because it is rapidly converted to nitrate and under anoxic conditions it is rapidly converted to ammonia. Nitrate is usually not toxic in the quantities found in lakes and rivers (up to 1 mg/L). The drinking water standard is set at about 10 mg/L). Polluted streams can contain up to 2 mg/L of nitrite and small areas near the thermocline may contain relatively large quantities of nitrite. Sewage, agricultural waste or other decaying matter including (aquatic plants killed by herbicides) can theoretically cause pollution (Goldman and Horne, 1983, Reid, 1961).

If nitrogen is the limiting nutrient, nitrate can participate in the next algal bloom. Nitrate and nitrite are formed from the oxidation of ammonia and may persist long after algae and plants have utilized the ammonia in their biological processes. The next algal bloom can be due to the presence of nitrate. However, it may take several days from the time ammonia becomes in short supply for the next bloom to occur because nitrate uptake is slow relative to ammonia uptake and induction of nitrate reductase in algae is also fairly slow. Nitrate must be reduced to ammonia in algae prior to the initiation of an algal bloom; algae cannot use nitrate directly and it must be converted to ammonia before it is utilized in their biological processes (Goldman and Horne, 1983).

- **Potential impacts of nutrient cycling and the release of phosphates and other plant nutrients**

Phosphate is usually the limiting nutrient in aquatic systems because it is tied up in growing plant and animal tissue as well as the sediment. The sediment typically retains phosphorus under aerobic conditions and releases it under anaerobic conditions. This released phosphate may result in growth of phytoplankton in the hypolimnion provided the depth is not so great that photosynthesis is precluded. When plants are treated with 2,4-D or other herbicides, they die and degradation of the plant tissue by microbes can cause phosphate and other nutrients to be released. Phosphorous in its organic form, cannot be utilized and must first be converted to

phosphate (PO4) by excretion and decay. Normally, phosphates will be at very low levels even in eutrophic lakes and rarely exceed 0.020 mg/L in the summer or 0.030 mg/L in the winter. Nitrate and ammonia levels are often many times higher than the phosphate levels and plants typically require a 7:1 nitrogen/phosphate ratio by weight for maximum growth rate. However, phosphorous depletion is likely in many freshwaters under normal circumstances. Therefore, the treatment of a water body with 2,4-D, which causes release of phosphates from the decaying tissue after the plants have died, has the potential to cause an algal bloom.

No change or a significant decrease in ammonia levels were noted at Kerr Lake, Oklahoma after treatment with 2,4-D BEE at 20 kg/ha (18 lbs./acre) (Morris and Jargon, 1981 in Ebasco, 1993). However, the total phosphorus concentration increased to 0.3 mg/L. Close monitoring of Kentucky Lake, Kentucky after treatment with 2,4-D DMA indicates that the soluble reactive phosphate increased dramatically from 0.010 mg/L on the treatment date to 0.040 mg/L 7 days after treatment. This increase in phosphate concentrations is likely due to the collapse and decay of aquatic weeds including Eurasian watermilfoil. The levels of phosphate and nitrate were similar in concentration over the course of this study and the concentration of chlorophyll a appeared to closely track the concentration of nitrate and phosphate. In this case chlorophyll a levels crashed when nitrate levels crashed and did not appear to be directly affected by phosphate levels. Therefore, unlike the case where phosphate is in short supply, nitrogen appears to be the limiting nutrient (Kobraei & White, 1996). However, the nitrogen and phosphate components can participate independently or simultaneously in producing an algal bloom.

Another nutrient, which frequently is in short supply, is iron. Ferric iron may either react with or be adsorbed with phosphate into the sediments under typical anoxic conditions and become biologically unavailable. Under aerobic conditions, ferrous iron is formed from ferric iron/phosphate complexes and is released into the hypolimnion where plants may utilize it for growth provided that the light is sufficient for photosynthesis to occur. Eh (oxidation/reduction potential), pH and DOC (dissolved oxygen content) govern this reaction. The heterogeneous nature of water/sediment phase reactions prevents easy extrapolation of laboratory results to real lake sediment systems. Iron availability may limit the growth of algae in lakes and streams especially when the production of ammonia (due to nitrogen fixation) is the limiting factor in algal growth (Goldman & Horne, 1983).

Nutrient cycling typically starts with a bloom of algae, which ends when one of the nutrients and/or other factors becomes in short supply. At that point the algae die and release phosphates, iron and ammonia through the degradative process. When enough of the nutrient in shortest supply becomes sufficient to sustain growth, algae will start growing again in the lag phase and will result in an algal bloom if conditions of temperature, pH, N: P ratio, light and iron and other essential nutrient concentration are adequate to sustain a log phase growth.

- **Potential impacts of pH changes**

The pH of most natural waters falls between 4 and 9. A pH of 7 is neutral, neither acid nor basic. One important way in which pH is controlled is by removing carbon-dioxide from the water. A pH of greater than 8 in a lake or pond is probably due largely to a high rate of photosynthesis, which increases pH by removing carbon

dioxide from the water. Anthropogenic sources of high pH may be due to enrichment of the water with fertilizers containing organophosphates. If the pH of a lake or pond is low (<6) it is likely due to leaching of organic acids from peat, and anthropogenic sources such as acid rain or leachate from mines. Bottom waters are typically lower in pH than surface waters because bacterial respiration and decomposition of organic matter produces carbon-dioxide and organic acids which lower pH (Shearer, 1996)

After aquatic macrophytes die due to natural process, treatment with an herbicide, or other control methods, the pH may drop. If an algal bloom occurs after the release of nutrients, the pH may rise due to the removal of carbon-dioxide from the water column by photosynthesis. A pH greater than 9 can be directly lethal to fish. Toxicity to high pH levels arises from the inhibition of ammonia secretion by gills and respiratory alkalosis (Heath, 1995 in Shearer, 1996). Sub-lethal alkaline or acidic conditions can indirectly harm fish and other aquatic animals by increasing their susceptibility to other stresses such a pollutants (like 2,4-D), ammonia, high temperatures and low dissolved oxygen.

Both pH and hardness can have an effect on the toxicity of 2,4-D. Although not directly connected to pH, hardness can have an effect on the toxicity of herbicides. Hard waters due to the presence of bicarbonate have a tendency to be alkaline (basic) while soft water due to the presence of low bicarbonate levels has a tendency to be acidic. This appears to be true for 2,4-D although the difference in toxicity of 2,4-D products in soft and hard water appears to be minimal. The two commercial esters of 2,4-D, 2.4-D BEE and 2,4-D 2-EHE appear to be affected by water hardness in relationship to toxicity. One of the non-commercial amine salts, 2,4-D diethanolamine (identified incorrectly in Ebasco, 1993 as 2,4-D DMA) also appears to be affected by water hardness (Table 10) (Wan, 1990 and Wan, 1991). 2,4-D BEE is more toxic by 3 to 4-fold to salmonids in soft water than in hard water (Soft water LC50 = 0.4 to 1.1 mg/L; hard water LC50 = 1.1 to 4.3 mg/L); 2,4-D 2-EHE appears to be more toxic in hard water than in soft water (hard water LC50 = 21 to 79 mg/L); soft water LC50 = 30 to 167 mg/L) and 2,4-D diethanolamine appears to be more toxic in soft water than in hard water (soft water LC50 = 291 to 472 mg/L; hard water LC50 = 438 to 744 mg/L). The 2,4-D BEE ester appears to be about 100-times more toxic to salmonids than the 2,4-D 2-EHE formulation and the 2,4-D-EHE formulation appears to be 3 to 20-times more toxic than the amine salt.

Also high pHs in water of intermediate hardness has a direct effect on the toxicity 2,4-D acid to rainbow trout fingerlings (Table 11) (Finlayson & Verrue, 1985 in JMPR, 1997). The toxicity of 2,4-D acid is >1000 mg/L at pH 8.48 and <100 mg/L at pH 4.54

In Washington State, hard waters with higher pH are generally found in Eastern Washington lakes (relative to Western Washington lakes). 2,4-D BEE formulations appear to be more toxic to juvenile coho, pink salmon and rainbow trout in soft water, and 2,4-D 2-EHE appears to be more toxic in hard water environments. Therefore, 2,4-D BEE has greater potential for adverse impact in Western Washington lakes while 2,4-D 2-EHE has a greater potential for adverse impact in Eastern Washington lakes. It is note worthy that 2,4-D 2-EHE is unlikely to be used directly on water bodies to control milfoil or other “strictly” aquatic weeds. Also due to the dilution of 2,4-D 2-EHE in ditch bank use, aquatic organisms are unlikely to

experience concentrations of higher than 0.1 mg/L for 2,4-D products applied for ditch bank weed control (Frank, 1972).

Although 2,4-D 2-EHE is likely to have significantly lower toxicity in Eastern Washington lakes than Western Washington lakes, the toxicity of 2,4-D BEE does not vary enough due to the effects of pH to affect the risk associated with slight changes in toxicity. E.g., LC50 of 2,4-D BEE in soft water ranges between 0.8 to 1.1 mg a.i./L and in hard water between 1.1 and 4.3 mg a.i./L depending on species. The toxicity of 2,4-D amine salts will be essentially unaffected since they are practically non-toxic to under both hard and soft water conditions.

One of the most likely reasons for low 2,4-D BEE toxicity is the low solubility in water limits potential contact of individuals within the biota to 2,4-D BEE. Furthermore, rapid degradation of esters to the free acid has a tendency to mitigate these factors although it is expected that this degradation to the free acid will occur more readily in hard/basic waters than in soft/acid waters JMPR, 1997). By contrast 2,4-D ethanolamine salt appears to be relatively non-toxic to juvenile salmonids and it is not anticipated that this product or other amine salts which have similar toxicities to fish (LC50>100 mg/L) will have adverse acute impacts in either Eastern or Western Washington lakes.

4.2.3.2 Effects of 2,4-D in Water

Summary: In the State of Washington, Pesticide residues that exceed the Federal drinking water standard (MCL) have not been found in public drinking water for many counties east of the Cascade Mountains. In some situations, 2,4-D has been seen in ground water where recharge areas have been treated with 2,4-D BEE. These recharge areas usually had porous bottoms (sand or gravel) with clay layers located below the bottom of the well shaft. Most down stream water treatment plants will not experience concentrations of 2,4-D higher than the Federal drinking water standard (0.07 mg/L) due to extensive dilution and lateral mixing. 2,4-D is not likely to be found in the water of sewage outfalls since waste water treatment plants only process water from household waste and water runoff from street level. Due to the short half-life, high levels of water exchange and dilution of 2,4-D in water bodies, additional procedures for removing 2,4-D from outfalls or potable water systems is not necessary. However, methods for treating relatively small wastewater streams include the use of biofilms impregnated with Flavobacteria spp.*, Fenton type UV/Ozonolyis reactors and dual-substrate chemostats containing bacteria capable of degrading 2,4-D.*

According to Scott Fink (2000, personal communications) of the Spokane Department of Health: Drinking Water Division, for all counties east of the Cascades, herbicides have never been detected in the surface water system at concentrations that exceed the Federal Drinking Water Standard. In public well water there has never been a herbicide detection that exceeds the EPA's Drinking Water Criterion. The current MCL for drinking water is less than 0.07 mg/L for 2,4-D products. However, there have been a few cases where herbicides were found in well water at concentrations that exceed Washington State's detection limits.

- **Potential impacts on recharge areas**

  In light of the above findings, it is unlikely that 2,4-D will have an adverse impact on sensitive well recharge areas. There has been one recent case of well contamination with 2,4-D due to the pumping of water upland from a treated lake that could have been considered a recharge area. 100 lbs./acre of 2,4-D BEE granular were applied over an 11.7 acre Michigan lake. This works out to a calculated rate of about 10.3 mg a.i./L (7.09 mg a.e./L) for the periphery of the lake assuming an average depth of one foot. This of course does not assume a timed-release product and assumes an instantaneous mixing. A pumping well was installed 12 feet from the edge of the lake and water was pumped from this well at a rate of 2.5 gal/min. Thirty samples were collected over 43 days from wells located 5 feet out in the lake, 5 feet inland from the lake edge and from the pumping well.

  After 21-days, the lake well exhibited a high 2,4-D concentration of 0.541 mg/L. At 11-days the lake edge well exhibited a high 2,4-D concentration of 0.595 mg/L and the pumping well exhibited a high 2,4-D concentration of 0.423 mg/L on day-16. The concentrations in these three wells exceeded the maximum permissible levels of a contaminant in water that is delivered to any user of a public water system (MCL) from day two through about day 30 at all three wells. After 43 days the concentration in the lake well fell below the detection limit (0.002 mg/L. However, the concentrations of 2,4-D did not drop below the detection limit for 257 days at the pumping well or the lake edge well. This is of concern since the high concentration in the pumping well is higher than the current U.S. EPA MCL level of 0.070 mg/L and higher than the current 10-day health advisory level (HAL) of 0.300 mg/L. Previous work by Regalbuto and Payne, 1988 (in Lovato et al, 1999) inferred that there is potential for migration of 2,4-D into the groundwater at this site. Furthermore, representative values for hydraulic conductivity in areas with a similar history indicate that it is reasonable to expect that hydraulic conductivity values for sand gravel aquifers to be an order of magnitude or greater than those at the study site.

  At first examination this data is alarming. However, some factors that made the observations unrealistic were that the wells were very shallow (not more than 12 feet deep) and the soil soil/sediment was very porous consisting of tailings from a gravel mining operation. The location of the wells were also close to the water (-5 feet, 5 feet and 12-feet from the water's edge) and the most upland well maintained a pumping rate of 2.5 gallons/min throughout the duration of this study.

  Each year numerous permits are issued by states other than Washington specifying a required "isolation distance" be maintained between the area of application and drinking water wells. Isolation distances are based upon several factors including herbicide mobility, environmental half-life and toxicity; 2,4-D is very mobile, has a short half-life ($t_{1/2}$ = <11 days) and a fairly low toxicity. Clearly, none of the procedures given in the above case are standard practice but it does indicate the extent of the problem if a state's guidelines are not followed. The Michigan State guidelines were not specified in this report (Lovato et al., 1999 Draft of article for "Scientific American" and Ragalbuto and Payne, 1988 in Lovato et al, 1999).
  In spite of this recent finding, 2,4-D has not been found to contaminate ground water. There is apparently due to degradation by soil microorganisms. 2,4-D has been detected in approximately 100 out of over 1,700 ground water samples collected from the United States (Dynamac, 1988 in Ebasco, 1993). Most contamination problems

were associated with point sources including spills from mixing and loading and application, and from back siphoning of spray solutions (Waite et al, 1992 a,b in JMPR, 1997 and Frank et al., 1987 in JMPR, 1997). The highest levels of 2,4-D reported in ground water outside of the recent experiments by Lovato et al (1999) were from Idaho (0.0365 mg/L) and from Ontario, Canada (0.029 mg/L).

- **Impact of pesticide application on downstream water treatment plants**

  Recent work on the effects of 2,4-D on downstream water treatment plants has not been conducted. Due to rapid degradation and dilution with untreated water, the effects of 2,4-D on downstream water treatment plants are expected to be minimal.

  A situation occurred in the Jagger Branch of the Gunthersville Reservoir (Alabama) which remotely pertains to this topic. After heavy treatment with 2,4-D for the control of watermilfoil, an excess of 5 mg/L was detected for the first five days following treatment and concentrations above 1 mg/L persisted for an additional 3 days. The concentration had dropped to about 0.6 mg/L after two weeks and was undetectable after four weeks. However, at two and three months post treatment, residues rose to 0.02 to 0.04 mg/L before returning to non-detectable levels four weeks later. The water entering drinking water treatment plants during herbicide application periods generally contained between 0.002 and 0.100 mg/L, 2,4 D. However, the North Marsh plant, pumped water containing in excess of 1 to 5 mg/L 2,4-D for over a one-week period. This was considered to be an extremely unusual situation since the Jagger Branch receives a very low water exchange.

  In the Northwest, in an area known to have underwater lateral current movements, similar treatments with 2,4-D BEE pellets exhibited much more acceptable concentrations of 2,4-D. Concentrations following these treatments did not exceed 0.14 mg/L and were typically in the range of 0.023 to 0.05 mg/L before declining to non-detectable levels three to eight days post treatment.

  By current standards, the Jagger Branch clearly violated the MCL levels for a protracted period. While Lake Okanogan, had levels that were higher than the MCL immediately after treatment, these levels did not persist and did not violate the 10 day health advisory level of 0.300 mg/L. (Shearer & Halter, 1980 citing Wojtalik et al, 1971 and Shearer & Halter, 1980 citing Lim and Lozoway, 1978).

- **Presence of pesticide in the out fall**

  Because concentrations of 2,4-D are so low at water intake pipes only three or four days post treatment, the amount of 2,4-D in the outfall of drinking water or waste water treatment plants is likely to be negligible. In Eastern Washington, there has never been any herbicide detected in surface water systems at concentrations that exceed the Federal Drinking Water Standard (Scott Fink, Spokane Department of Health: Drinking Water Division, 2000, personal communications). Since wastewater treatment plants only process water from household waste and water runoff from street level, 2,4-D from treatment of lakes, ponds, streams and irrigation canals will not be present in the outfall (Jim Milton, Ecology Central Regioal Office Manager of Sewage Treatment Plant Permits, 2000).

- **Need for additional procedures to remove pesticide from the out fall**

  Due to the short half-life, high levels of dilution, and low chronic toxicity to aquatic wildlife, additional procedures to remove pesticides from the out fall or potable water systems are not likely to be necessary. However, several methods have been proposed for the removal of 2,4-D from potable water systems. These included biofilms of *Flavobacterium sp*. (Hinteregger et al, 1995); a combination of Photocalysis and Ozonolyis (Muller (1998) the use of a Fe^{3+}/H202/UV: Fenton type system (Sun and Pignatello, 1993) and the degradation of 2,4-D by *Pseudomonas cepacia* in a dual-substrate chemostat (Daugherty and Karel, 1994). All of these methods seem to have promise in treating waste, water. However, at this point these methods are only designed for the treatment of relatively small wastewater streams. All of them appear to be a very long way from reduction to practice.

4.2.4 Mixtures with Other Pesticides and Incidental Presence of Other Pesticides

Summary: Tank mixes are not permitted in Washington State. However, when liquid 2,4-D products (2,4-D DMA) are used to control floating aquatic weeds, low levels of surfactants can improve the efficacy of liquid 2,4-D products. If surfactants are used, care should be taken to use surfactants that are registered for aquatic use since they have low toxicity to fish. Thickening agents like Polysar® or Nalquatic® (not currently registered for use in WA) have been used to control drift with liquid 2,4-D products that are applied to floating weeds and they may be of further use in allowing subsurface applications to sink more deeply into the water column where they can be most effective.

*There are some claims that combinations of 2,4-D and glyphosate are antagonistic to both terrestrial weeds and the green algae (*Chlorella fusca*). The fungicides, anilazine and prochloraz, and the insecticide, parathion, have also been reported to antagonize the effects of 2,4-D on* Chlorella fusca*. Since, 2,4-D appears to stimulate the growth of blue-green algae at concentrations below 10 mg a.i./L, there is a potential that 2,4-D may antagonize the action of algaecides.*

There have also been reports of 2,4-D and glyphosate exhibiting additive and possibly synergistic results for the control of weeds. 2,4-D has been show to have additive effects in control of Chlorella fusca *in combination with various herbicides, and 2,4-D has been shown to have slightly better than additive effects in combination with the insecticide, lindane.*

*Cumulative effects have been seen with 2,4-D against the southern house mosquito (*Culex pipiens fatigans*). Exposure to 2,4-D during each of three generations caused an increase in time between cellular divisions and a significant increase in the duration of the larval phase.*

Feldhaus et al (1998) has reported that low concentrations of 2,4-D in combination with low concentrations of malathion or carbaryl produces synergistic effects on insecticide induced behavioral effects in the brown planaria. However, at higher concentrations, these combinations were observed to be antagonistic on insecticide induced behavioral effects. In another case, although synergistic effects were not observed, parathion, carbaryl and the herbicide, dinoseb, have been noted to increase the persistence of 2,4-D in sediment, which could possibly lead to adverse impacts on the more sensitive sediment invertebrates.

Formulations of 2,4-D may act in combination with other pesticides under three scenarios: 1) Applied as a mixture; 2) Broadcast in separate applications (e.g., areas where pesticides are applied for mosquito and aquatic vegetation control), or 3) Accidentally combined as a result of over-spray in marginal areas or of run-off from neighboring areas treated with different products. Herbicide mixtures may result in antagonistic, synergistic, additive or cumulative effects (same herbicide applied more than once). It noted that tank mixes of pesticides are not permitted in Washington State for control of aquatic weeds.

Because very little work has been done on the effects of pesticide combinations it is unclear whether other pesticides applied for other purposes could substantially enhance the toxicity and persistence of 2,4-D.

In the State of Washington 2,4-D products are rarely mixed with other products. Since 2,4-D BEE is a granular product that is broadcast and then allowed to sink to the root zone for control of watermilfoil, adjutants would not be typically used with this product. 2,4-D DMA and 2,4-D 2-EHE may occasionally be used in conjugation with an oil or other carrier for the control of floating or emergent weeds. A number of surfactants are registered for use with water-soluble herbicides like 2,4-D DMA when they are applied to floating or emergent plants. The professional researcher (Getsinger, 2000) whom we consulted with believes that when a liquid pesticide is applied to floating or emergent vegetation that a surfactant and/or drift control agent should be used.

Not all formulations of 2,4-D have a similar toxicity on an a.e. basis. It has been shown that 2,4-D DMA is practically non-toxic to most species of fish and invertebrates except mullet (*Mugil cephalus*), bleak (*Alburnus alburnus*) and glass shrimp *(Palaemonetes kadiakensis*) while the ester compounds are more toxic. However, the esters are rapidly converted to 2,4-D acid in natural water systems and 2,4-D acid is relatively non-toxic to most species of fish and invertebrates except lake trout (*Salvelinus namaycush*), cutthroat trout (*Oncorhynchus clarkii*), white perch (*Roccus americanus*), striped bass *(Morone saxatilis*), (*Cyclops vernalis*) and lined scud. The conversion of the esters to 2,4-D acid is so rapid that JMPR (1997) recommends the use of the acid toxicity data to generate appropriate risk quotients if the values for the ester products are adverse. The "inert materials" and contaminants may interact with the pesticide to give antagonistic, additive, cumulative or synergistic effects against target (aquatic weeds and algae) and non-target fish and aquatic invertebrates (Kamler et al., 1975). For example, formerly commercial preparations of 2,4-D sodium salt containing 2% or 3% of 2,4-dichlorophenol are toxic within 24 hours to carp sac-fry at concentrations of 1600 mg/L 2,4-D sodium salt while preparations not containing the impurity have only a minimal toxicity (20% mortality in 48 hours). Current products used in the United States are not believed to contain these levels of 2,4-dichlorophenol. It is not necessary to use adjuvants with subsurface injections of 2,4-D (2,4-D DMA) or when using granular products of 2,4-D (2,4-D BEE). However, a thickener is often used with liquid products to allow the treatment to sink more deeply into the water column where it can be most effective.

Lemna gibba, marine plants and animals are more sensitive to 2,4-dichlorophenol than common carp. Other fish species were not evaluated for the effect of 2,4-dichlorophenol. Greater detail on the toxicity of 2,4-dichlorophenol can be found in Section 4.2.3.1.

- **Adjuvant effects**

When liquid 2,4-D products (2,4-D DMA) are used to control floating weeds by direct contact with a spray, the use of a surfactant and a thickening agent are recommended. The surfactant should be used to allow for better wetting of the floating weeds and the thickening agent should be used to prevent drift. There are a number of adjuvants registered for aquatic use by WSDA and approved for use by Ecology. Most surfactants should be mixed at 0.25% to 0.5% by weight of application solution when 2,4-D is being applied to floating (surface) aquatic macrophytes. The toxicity of these adjuvants to bluegill, rainbow trout and daphnia has been well documented. None of these aquatic adjuvants should be toxic to fish or aquatic invertebrates when applied at labeled rates. However, it has been noted by Watkins et al (1985) that some aquatic adjuvants have a potential to be toxic to aquatic organisms when applied in shallow water. For example: 1) If Spra-Mate® is applied at the labeled use rate to water with a depth of less than 1.5 meters, it can be toxic to bluegill sunfish. 2) If Cide-Kick®, X-77®, Formula 403®, or IVOD® are applied at the labeled use rate to water with a depth of less than 0.1 meters, they may be toxic to fish. Since the depths given are for concentrations of the adjuvant that will kill 50% of the treated animals, an additional safety factor of ~10-fold would need to be added to assure safety of the adjuvant to the biota. Details of the toxicity and depth considerations for a number of aquatically applied adjuvants can be found in Table 12. Although adjuvants are typically considered to be "nearly inert", they are not entirely inert. However, adjuvants labeled for aquatic use should not be subacutely, acutely or chronically toxic to fish or other aquatic animals. Adjuvants can either enhance, diminish, or have no effect on the activity of herbicides. Although acute aquatic testing has been done on a number of adjuvants, insufficient data exists on the toxic effects of adjuvants when mixed with herbicides and applied to the aquatic ecosystem.

One possible exception is the surfactant, Syndets® (Abdelghani et al, 1997); tests indicate that this surfactant is from 40 to 85 times more toxic than 2,4-D. Lethal concentrations of surfactant plus 2,4-D were found to be lower than the recommended field formulations. However, it was found that such hazardous amounts would rarely reach the target (roadside ditches) where the presence of large volumes of water is likely to provide dilutions to levels that pose no threat to aquatic life.

- **Antagonistic effects**

Antagonism is defined as a less than additive effect when using pesticides in combination with each other. There are a number of studies that show that combinations of 2,4-D and glyphosate are antagonistic when applied to terrestrial plants. O'Sullivan (1979 in Ebasco, 1993) reported that 2,4-D esters in combination with glyphosate are particularly antagonistic in effect when used on cereal grains, Yang (1978 in Ebasco, 1993) found glyphosate and 2,4-D mixtures to be antagonistic at higher application rates on mixed weeds in Indonesia. More recently, Faust et al, (1993) found that 20:40 mixtures of 2,4-D and glyphosate are antagonistic when applied to control the algae *Chlorella fusca* and that the decrease in effectiveness may be as high as 40% percent.

The fungicides, anilazine and prochloraz have also been reported to antagonize the activity of 2,4-D when applied in ratios with 2,4-D of 1.03:98.97 (Anilazine:2,4-D) or 0.0128:99.9872 (prochloraz:2,4-D). Both of these mixtures increase the observed concentrations over the predicted concentrations by nearly 100% or more in order to achieve control of Chlorella fusca (Faust et al., 1997). Even parathion has been reported to antagonize the effects of 2,4-D by increasing the observed LC50 over the predicted LC50 by nearly 100%.

However, since 2,4-D appears to stimulate growth of cyanophytes at concentrations below 10 mg a.i./L (Wang et al. 1991, Kobraei and White, 1996, Das and Singh, 1977, Wong and Chang, 1988, Mishra and Padney, 1998), there is the potential that 2,4-D could antagonize the effects of some good algaecides like Hydrothol® 191 that have not yet been tested with 2,4-D. Therefore, mixing 2,4-D and Hydrothol® 191 may antagonize the action of Hydrothol®. This has not been an issue when 2,4-D is present with endothall at low concentrations due to drift and/or run off from home lawn treatments.

- **Additive effects**

  There have also been reports of 2,4-D and glyphosate exhibiting additive and possibly synergistic results for the control of weeds (Ebasco, 1993 citing various authors including: Widyanto & Serjani, 1978 Ebasco, Tollervy et al, 1979 in Ebasco, Indian Tea Research Assn., 1979 and Proctor, 1975). In most cases 2,4-D in combinations with a variety of pesticides has been shown to have additive effects in control of *Chlorella fusca*. Some of the herbicides that have shown additive effects in combination with 2,4-D are bentazone, chlorotouluron, metazachlor, methabenthiazuron, simazine and triallate at various ratios of 2,4-D:additional herbicide (Faust et al, 1993). Even the insecticide lindane has been shown to have slightly better than additive effects when combined with 2,4-D for the control of *Chlorella fusca* (Faust et al, 1994).

  Shearer and Halter (1980) reviewed studies conducted on 2,4-D mixtures with dalpon, 2,4,5-trichloroacetic acid, and with multiple mixtures containing fenac and Banvel®. None of these mixtures were identified with supra-additive toxicities to aquatic organism.

- **Cumulative effects**

  2,4-D has cumulative effects on the southern house mosquito (*Culex pipens fatigans*) (Ahmed & Ali, 1994). When 2,4-D is applied at concentrations as low as 1 mg 2,4-D acid/L, there were effects on the mitotic index (percent cell division) and an increase in the duration of the larval phase from ~180 hours to ~225 hours after exposure for 4 hours to three generations. The effects of 2,4-D on the southern house mosquito are both dosage-related and cumulative from several generations of brief exposure (2–4 hrs. per generation). These effects could potentially alter the availability of insect food to both fish, benthic invertebrates and insectivorous birds by making the mosquito (larvae/adult) not available at the proper size and time for most effective utilization by the predator species.

- **Synergistic effects**

  There are no reports of true synergistic effects of 2,4-D in combination with the insecticides, parathion or carbaryl or with the herbicide (dinoseb) (Smith, 1989 in Ebasco, 1993). However these pesticides have been noted to increase the persistence of 2,4-D in sediment. This could have an adverse impact on the more sensitive sediment organisms like *Gammarus fasciatus* (lined scud) (LC50 = 3.2 mg 2,4-D acid/L) since the presence of 2,4-D under normal application rates can be quite prolonged (Smith and Isom, 1967 in Shearer & Halter, 1980); and the levels of 2,4-D in the sediment can be as high as 0.24 to 59 mg/L for 10 months post treatment (Smith and Isom, 1967, in Shearer & Halter, 1980).

  True synergistic effects with 2,4-D in combination with malathion and carbaryl have been noted in *Dugesia tigrina* (brown planeria). When very low concentrations of 2,4-D diethylamine salts and carbaryl or 2,4-D diethylamine salts and malathion were combined, heightened behavioral effects were observed. The effect of the combinations were approximately three to four times more than additive when 2,4-D and an insecticide were combined at very low concentrations. The concentrations of the insecticides were 0.0025 mg/L for malathion or 0.00025 mg/L for carbaryl in combination with 0.0025 mg/L 2,4-D. These concentrations were about 1/2000 of the LC50 for the insecticide. At higher concentrations (0.05/0.05 mg/L of the insecticide and 2,4-D) which was about 1/100 of the LC50, antagonistic effects between the insecticide and 2,4-D were observed. The effects of the combinations at these higher concentrations were approximately two-fold less than additive.

  Similar inconsistencies in the interactive effects due to differing dosage levels of pesticides have been noted with fish and fungi. Interactions could be antagonistic, additive or synergistic depending on the dosage of the tested pesticides. Interactions between stream pollutants and added reference standards have been reported (Marking and Mauk, 1975, Hill and Stratton, 1991 and Schaefer et al, 1991 all in Feldhaus et al, 1998). These studies indicate that this is a common phenomena when an organism is treated with a combination of pesticides and it is not confined to the effects of acetylcholinesterase inhibitors and 2,4-D.

4.2.5 Potential Impacts on Agriculture

Summary: At typical use rate concentrations, irrigation or flooding of crops with water that has been treated with 2,4-D DMA can cause damage to some crops, and non-target wild plants. Although early growth stage damage has been observed on many crops including sugar beets, soybeans, sweet corn, dwarf corn and cotton, no significant reductions in yield were seen at harvest for most crops. Residue levels that would interfere with the marketability of crops were not seen in various crops including potatoes, grain sorghum, Romaine lettuce, onions, sugar beets, soybeans, sweet corn or dwarf corn. 2,4-D will not bioaccumulate in crop plants, or fish at levels that will interfere with their marketability or consumption.

If water use restrictions are followed as described in Section 1 and the Federal Use labels, there should be no impact on agriculture. The Aqua-Kleen® and Navigate® labels (1999) does not permit the use of 2,4-D BEE to control weeds in water that is to be used for irrigation, agricultural sprays, watering dairy animals or domestic water supplies. The Washington Department of Agriculture has interpreted this label statement to mean that

people using treated water for the above purposes agree to suspend use until water in the treated area reaches the drinking water standard. The current drinking water standard is 0.07 mg/L for the MCL and 0.300 mg/L for the ten-day health advisory.

There are very strong indications that 2,4-D from granular treatments will not persist in the natural environment in Northwest waters (British Columbia's Skaha and Okanogan Lake). The persistence can be strongly affected by the application rate. The concentration reached 0.036 to 3.25 mg/L within 0 to 11 days of treatment with 44.8 Kg/ha (40 lbs./acre), 0.067 mg/L for the 33 kg/Ha (29 lbs./acre) and 0.099-0.36 mg/L for the 22 Kg/ha (20 lbs/acre) treatment. However, the highest concentrations, collected 800 feet from the treatment site, were 0.017 to 0.13 mg/L. Similar work with similar treatment rates yielded a maximum concentration of 4.0 mg/L 2,4-D 6-days after treatment with dissipation to non-detectable levels occurring within 59 days of treatment (Dynamac, 1988 in Ebasco, 1993). Indications are that treatment of coves in a Wisconsin pond at 112 Kg 2,4-D BEE/ha (100 lbs./acre) for the control of watermilfoil required at least 30 days for the 2,4-D concentration to drop below 0.100 mg/L. Concentrations appear to persist for some time in British Columbia and Wisconsin waters (Shearer and Halter, 1980 citing Wojtalik et al, 1971, Shearer and Halter, 1980 citing Lim and Lozoway, 1978 and Helsel et al, 1996). Therefore an analytical analysis to determine if the concentrations of 2,4-D has dropped below the drinking water criterion is necessary prior to the use of the treated waters for agricultural and household use.

Although a worst case scenario is given above, more typical persistence and concentration were correlated to water movement patterns. At Lake Okanogan addition of 2,4-D pellets resulted in typical residues of 0.02 to 0.5 mg/L declining to non-detectable levels 3-8 days following treatment.

In a demonstration project at Loon Lake, Washington, 2,4-D BEE pellets were applied at 100 lbs/acre (112 Kg/ha). This application lead to a maximum concentration of 2,4-D 1-2 days after application. The levels of 2,4-D dissipated to <0.005 mg/L in 3-7 days after application.

Work with 2,4-D DMA (Gangstad, 1986) conducted in Florida and Georgia indicate that concentrations of 2,4-D dropped to 0.025 to 0.395 mg/ha within seven days if the herbicide was applied at reasonable rates (2.4 kg a.e./ha = 2 lbs./acre to 8.95 kg a.e./ha = 8 lbs. a.e./h). See Section 4.2.3.2. The highest concentrations persist for at least seven days at levels that are higher than the federal MCL of 0.070 mg/L. These concentrations appear to persist for some time even in warm Florida and Georgia waters. Therefore, an analytical analysis to determine if the concentrations of 2,4-D has dropped below the drinking water criterion is necessary prior to the use of the treated waters for agricultural and household use. (Shearer and Halter, 1980 citing Wojtalik et al, 1971 and Shearer and Halter, 1980 citing Lim and Lozoway, 1978).

- **Potential impacts of water on irrigation**

  If water used for irrigation contains less 2,4-D than mandated by the MCL (0.070 mg/L or ten day health advisory (0.30 mg/L), 2,4-D (applied as 2,4-D DMA) should not have an adverse impact on crops irrigated with treated water. According to Scott Fink (Public Health Department: Drinking Water Division, 2000 personal communication), the levels of herbicides in public drinking water are always below the current MCL.

However, in general, broad leaf plants are susceptible to 2,4-D, while grasses and grains are resistant. 2,4-D is therefore toxic to many non-target broadleaf plants, including both crops and native vegetation. Adverse effects depend on rate of application, number of applications over a confined period and relative susceptibility of individual species. Plant susceptibility to 2,4-D has been qualitatively described for a number of weeds, crops and other terrestrial plants (Portman and Losey, 1979 in Ebasco, 1993) (Table 13).

The use of 2,4-D as a weed killer is described on individual product labels, with appropriate water use restriction specified for the specified application. If the water use restrictions are followed, minimal damage should occur to non-target native and crop species. However, because 2,4-D is a non-specific broadleaf herbicide, it may adversely affect some crops and other non-target species exposed to irrigation water containing the active ingredient.

Many species of plants may survive repeated exposure to 2,4-D, if key periods where they are more or less susceptible are considered. These periods include germination (more susceptible), seedling (more susceptible), dormancy (less susceptible) and senescence (less susceptible) periods. Other factors include plasticity, seed dispersal, hardiness and tolerance (Ebasco, 1993). For additional information please see Section 4.3.3.2.3. Indications are that when water containing up to 0.025 to 0.061 mg a.e./L was used to water various crops the maximum resides of 2,4-D in each crop were either non-detectable or lower than the FDA tolerances for these crops. These crops and there residue levels were potatoes (0.03-0.12 mg/Kg), grain sorghum (<0.05 to 0.12 mg/Kg, carrots (0.02 to 0.06 mg/Kg) Romaine lettuce (0.11 to 0.33 mg/Kg) and onions (<0.01).

Even concentrations of 2,4-D in irrigation water that were much higher than the experiment described, rarely caused crop damage. For example, grapes furrow irrigated with water containing 2,4-D at rates of 2.24 to 35.84 kg a.e./L exhibited damage that could be interpreted as likely to cause a significant yield reduction. For Further details, please see 4.2.2.4 (Bioconcentration). Toxicity studies were not extensively reviewed for crop plants but Table 4 provides information on the relative effects of 2,4-D on crop plants that may be adversely affected by irrigation with water containing 2,4-D.

Crop studies indicate that when water containing 2,4-D at concentrations as high as 2.21 to 5.51 mg/L was used for irrigation, many of the crops exhibited signs of phytotoxicity including abnormal curvature of the petioles, wilting, slumping, chlorosis and necrosis. However, crop yield reductions and unacceptable residue levels usually did not occur Table 4 (Gangstad, 1986).

No 2,4-D was lost from the top six inches of soil for the first 7 days after furrow irrigation. However, the concentrations in the soil were about 10% or less of the concentrations in the applied water. In the case of sprinkler irrigation, even at the higher treatment rates (2.21 mg a.e./L) less than 15% of the applied concentration in water was recovered from the soil. Such low levels of 2,4-D on soil certainly contributed to the safety of 2,4-D when applied in irrigation water.

- **Potential impacts of water used to water livestock**

  If water used for watering dairy animals contains less 2,4-D than mandated by the MCL (0.070 mg/L or ten day health advisory (0.30 mg/L), 2,4-D (applied as 2,4-D DMA) should not have an adverse impact on the animals or milk production. In general, 2,4-D BEE, 2,4-D 2- EHE and 2,4-D DMA will be rapidly converted to 2,4-D acid in natural waters. 2,4-D acid has an LC50 to rats of greater than 699 mg a.i./Kg and is therefore not significantly toxic to rats (Myer, 1981 in JMPR, 1997). The toxicity to birds for 2,4-D acid ranges between 200 and 400 mg/Kg for the most sensitive species (Hudson et al, 1984 in JMPR, 1997). After ingestion, 2,4-D is rapidly eliminated from the body of chickens and lactating goats (Puvanesarajahangesrajah and Bliss, 1992 and Guo and Stewart, 1993). Higher animals, chickens and goats, did not bioaccumulate 2,4-D. Approximately 81%-114% of the administered dosage was eliminated by chickens in the excreta and less than 0.1% of the administered dose was collected from eggs and poultry tissue; levels of 2,4-D in edible tissue and eggs was less than 0.030 mg/Kg and levels of <0.05 mg/Kg are considered to be negligible for most pesticides. The only compounds identified in hen tissue were 2,4-D and 2,4-dichlorophenol. In lactating goats, 98% of the administered dose was eliminated unchanged as 2,4-D in the urine after dosing for three days and less than 0.5% of the total test material was detected in the tissues and milk of goats. After seven days of depuration the levels of total residue in milk, liver, kidney muscle and fate were, 0.01, 0.39, <0.05, <0.05 and <0.05 mg/Kg, respectively. Only the liver maintained residues of potential concern and it must be pointed out that these residue levels were found in animals dosed with levels of 2,4-D that were three times the expected dietary maximum exposure rate. In chickens the main metabolites were not identifiable and in the goats only 2,4-dichloroanisole was identified. Therefore, 2,4-D should not bioaccumulate; it should be rapidly eliminated from any organisms that ingest it; and it should not be bioaccumulated (biomagnified) as it is passed up food chain from livestock to man.

- **Potential impacts of water used for agricultural sprays**

  Crops were irrigated with water that had been treated with 2,4-D DMA at rates much higher than would normally be expected to occur (0.070 mg/L = MCL). Typical concentrations of 2,4-D in sprinkler irrigation water were <2.21 mg/L significant phytotoxicity was noted in many cases but yields were not reduced. When crops were irrigated twice by an overhead sprinkler method with water containing 5.51 mg/L 2,4-D in the first irrigation followed by another irrigation with water containing 2.21 mg/L, significant 2,4-D phytotoxicity occurred in most crops (Table 4). The phytotoxicity to sprinkler irrigation was generally not as extensive as that resulting from furrow irrigation. Although phytotoxicity was a common effect, yields were not generally reduced, nor were residue levels detectable in the edible portion of the crop at harvest time. Use of overhead irrigation can probably be equated with the effects of using water for agricultural sprays. However, sprinkler irrigation does not take into account the potential antagonistic, additive or synergistic effects that might occur due to the presence of other pesticides, or adjuvants (surfactants, accelerator, thickeners, et cetera), and these effects have been known to occur. See Section 4.2.4.

- **Potential impacts on fishing and the consumption of fish**

  2,4-D DMA has not caused adverse impact on recreational or commercial fishing. One of the best recreational and commercial fishing seasons at the Gunthersville, Alabama Reservoir occurred after treatment with 2,4-D DMA in 1969. In addition, ponds treated with 2,4-D DMA at concentrations as high as 2.0mg/L did not exhibit increased fish mortality. After application, a successful reproduction of bluegill sunfish occurred. Bluegills in ponds treated at 2.0 mg/L 2,4-D DMA also showed no toxic effects and grew faster than fish in control ponds (Shearer and Halter, 1980).

  It has been previously shown that 2,4-D does not extensively bioaccumulate or bioconcentrate in fish (Section 4.2.2.4). Both 2,4-D BEE and 2,4-DMA bioconcentration studies have been conducted in the laboratory on several animal species. 2,4-D DMA has been tested in the laboratory for bioaccumulation in bluegill sunfish, channel catfish, northern crayfish, and freshwater clam. These species were exposed to 6 mg a.e./L of 2,4-D DMA for 28 days. For the first 1-2 weeks, all species gradually "accumulated" 2,4-D in their edible tissue. However, the maximum concentration never exceeded 0.94 mg a.e./L in any of the species tested. The bioconcentration factor for 2,4-D was therefore never higher than 0.16 in any of the species tested. By the end of the test (15 to 28 days of exposure), no concentrations in edible tissues from any species exceeded 0.56 mg a.e./Kg; the BCF therefore was only 0.09 (Biever, 1998 and Biever, 1996).

  Similar tests with 2,4-D BEE were conducted with rainbow trout and bluegill sunfish. When these fish were exposed to 1 mg a.e./L of under laboratory conditions, the edible tissue contained only 2,4-D acid and no 2,4-D BEE. Both species initially accumulated 2,4-D acid at concentrations higher than the concentration in water. After the first three hours of exposure, concentrations of 2,4-D acid were 1.2 to 4.4 mg a.e./L in rainbow trout and 1.7 to 46.6 mg a.e./L in bluegill sunfish, respectively. Predator salmonids like Chinook, Coho and Chum salmon should bioaccumulate 2,4-D in a manner similar to their congener, rainbow trout. However after 48 and 120 hours of exposure, the concentrations in rainbow trout and bluegill sunfish were below the detection limit (<0.05 mg a.e./L) and 0.1 to 0.2 mg a.e./L, respectively (Rogers and Stallings, 1972 in Ebasco, 1993). This indicates that 2,4-D in either commercial form does not bioaccumulate in fish.

  Additional field work indicates that similar effects occur under natural conditions. Hoeppel and Westerdahl (1983 in Ebasco, 1993) monitored the persistence of 2,4-D DMA and BEE residuals in fish in Lake Seminole, Georgia after four 27 acre test plots were treated to control Eurasian watermilfoil. Two plots were treated with 22.5 and 45 kg a.e./ha (20 and 40 lbs./acre) of 2,4-D DMA (Weedar 64®) and two were treated with similar amounts of 2,4-D BEE (granular Aqua-Kleen®) via aerial spray. Fish samples (largemouth bass, bluegill sunfish, catfish and gizzard shad were collected up to 69 days after treatment. None of the fish from the two 2,4-D DMA plots contained detectable concentrations (0.1 mg/L) of 2,4-D in muscle tissue. However, 2,4-D was detected in 18 of 20 gizzard shad and 4 of 24 game fish collected from the 2,4-D BEE treatment plots through day 13. The highest concentration (3.85 mg/Kg in shad muscle tissue) was observed on day 1 and declined to 1 mg/K by day 28. In game fish, the highest muscle tissue concentrations (5.1 mg/Kg was observed 4 days after treatment declining to 0.1 mg/Kg by day 13.

Gangstad (1986) and Schultz and Gangstad (1990 in Ebasco, 1993) studied fish uptake of 2,4-D in ponds located in Florida and Georgia that were treated with 2.24 to 8.96 Kg a.e./h 2,4-DMA. In Florida ponds, the highest residues (0.005 to 0.08 mg/Kg) in fish (largemouth bass, channel catfish, bluegill sunfish and redear sunfish) occurred 1 day after treatment. Within 3 to 7 days, there were no detectable residues (<0.005 mg/L) in any of the fish caught. However 14 days after treatment, one fish contained detectable residues of 2,4-D (0.075 mg/L) believed to have been caused by release of 2,4-D from decaying vegetation. In Georgia ponds, the highest residue of 2,4-D was observed in one of three bluegills collected 14 days after the pond was treated with 8 lbs a.e./ha 2,4-D DMA. No residues of 2,4-D were detected in fish caught from Georgia ponds 3 to7 days after treatment. However in none of the cases where 2,4-D was detected in fish flesh was the BCF higher than 8.6. Typical BCFs in this experiment were 0.5 to 3.00 (Table 7).

2,4-D is rapidly eliminated from the edible tissue of fish. Therefore, a more important factor effecting the consumption of fish is the tainting of flesh due to the presence of 2,4-dichlorophenol. 2,4-dichlorophenol has been reported to alter the taste of trout exposed to 2,4-D DMA for four hours at concentrations that ranged from 0.05 to 0.15 mg/L and then depurated in clean water for up to four days. Taste tests indicate that fish exposed to the highest dosages were inferior to control fish following all depuration periods. Other treated fish were deemed acceptable in taste but not as good as the control fish. Although 2,4-D and 2,4-D dichlorophenol residue concentrations were low to unmeasurable, tainting of flesh may have resulted from metabolic by-products.

Currently, the 2,4-D products are believed to contain very low levels of 2,4-dichlorophenol. However, in the absence of oxygen, 2,4-dichlorphenol and 4-chlorophenol have been generated by marine sediment organisms (Boyle et all, 1999) and in freshwaters (rice paddies and ponds (Hatfield, 1995r in JMPR, 1997). However, it is not known if the concentrations generated under these conditions would produce concentrations of 2,4-dichlorphenol that would cause tainting of fish flesh.

- **Potential impact of air quality on crop plants and livestock**

For 2,4-D BEE, the label states “Vapors from this product may injure susceptible plants in the immediate vicinity. Avoid drift of dust to susceptible plants” (Aqua-Kleen® and Navigate® labels, 1999). The 2,4-D DMA label warns “Do not apply when weather conditions favor drift from target areas, as this product may injure cotton, beans, other vegetables, certain legumes and ornamentals” (Terra 2,4-D amine 4 label, 1999). Table 13 gives examples to crops and native plants that are susceptible to foliar contact from 2,4-D. The main methods of using these products largely preclude the effects of drift. For granules, a cyclone seeder is used for spreading and the granules sink upon contact with water. The liquid products are either injected by subsurface methods (which precludes drift) or applied as large droplets at low pressure which mitigates the effects of drift. It is also recommended that a thickening agent be used to control drift when applying liquid herbicides to the water surface. However, even small amounts of drift can be an issue if many swaths are applied.

Due to a low vapor pressure of the commercial products of 2,4-D (2.4×10^{-6} mg/Hg at 25° C for the active ingredient 2,4-D BEE) applied aquatically and $<1 \times 10^{-7}$ mm Hg at 25°C for the active ingredient 2,4-D DMA), 2,4-D products should have very little tendency to affect air quality or cause crop damage. The mode of application is usually subsurface injection for liquid formulations, and the weight of the granular formulations makes drift unlikely. Drift outside the treatment area is therefore unlikely. For those cases where a liquid formulation is applied by boom sprayer, as much as 1% of the application may drift out of the treatment area. It has been estimated for general herbicides that this amount of drift could have an impact if 120 swaths were applied and 1% of the applied pesticide drifted out of the treatment area on each pass. In this case, dosage levels higher than that intended for the target could accumulate down wind of the treatment area. This could cause an effect on non-target plants that may damage habitat and decrease the amount of forage available for aquatic waterfowl and fish in non-target areas (Forsythe et al., 1997). In cases where aerial application might be necessary, as much as 17% of the treatment would not strike the target area. In aerial application, drift out of the treatment area could impinge on non-target organisms at a very great distance from the site of application. Depending on how much 2,4-D was deposited per unit area outside the site, there could be a significant impact on non-target wild plants or crops. In addition to effects on plants, non-target sensitive terrestrial wildlife may be adversely impacted.

Odor is unlikely to be noticed except for short periods of time following application of 2,4-D. Posting and communications requirements specified in the aquatic weed control permit should make the public aware of any potential odor problems and how long the odor problem will exist. Since there are is rarely more than one or two applications of 2,4-D per water body per year in the state of Washington, any adverse impact on quality of life due to problems with odor from 2,4-D applications should be weighed carefully with the impact on quality of life due to the effects of poor navigability, and effects on the recreational use of the water body. Typical odors associated with the use of 2,4-D are “phenolic” for the 2,4-D BEE product and “strong ammonia” for the 2,4-D DMA product.

- **Potential impact of flooding on agriculture**

Flooding of agricultural land with 2,4-D treated water should be a rare occurrence. When flooding occurs, the dilution effects should mitigate the effects of the concentration of 2,4-D. Flood irrigation is typically practiced with very few crops. Only cotton has been tested in respect to flood irrigation with a 2,4-D product, In 1953 to 1956, 2,4-D (triethanolamine or alkanolamine) was applied during the first irrigation after emergence to control weeds. The chem-irrigation was applied at 0.67 to 2.24 Kg a.e./ha (0.60 lbs./acre to 2.0 lbs/acre). When the cotton was 8 to 10 inches high. The 0.67 kg a.e./L application produced only slight injury on cotton and caused no decrease in yield. The 1.12 kg a.e./ha application caused some malformation of the foliage and abnormal development of early squares and bloom but caused no reduction in yield. In fact, total yields of cotton tended to be higher on the treated than on the untreated plots. Cotton however was noted to be an extremely tolerant or resistant crop to the effects of 2,4-D (Gangstad, 1986). It is not known, if other crops could withstand the effects of 2,4-D under flood application methods. However, similar levels of 2,4-D DMA applied by furrow or sprinkler irrigation were resisted by most crops, but grapes were affected (13% mortality) at treatment levels as low as 2.24 Kg a.e/ha Other signs of injury included destruction of the roots and

discoloration and tip enlargement on other portions of the grape plants. It is note worthy that grapes along with tomatoes, beans, lentils, peas, vetches red clover, young alfalfa, tree fruits, peppers, sweet clover, crimson clover, mint, sugar beets, hops and strawberries were observed to be easily killed or injured (Portman and Losey, 1979 in Ebasco, 1993). Therefore, it makes sense to avoid flood irrigation with water containing 2,4-D on these susceptible crops or on any crops (other than cotton) in which sever injury cannot be tolerated. The data from the irrigated crop studies indicate that "flooding" (furrow or sprinkler irrigation) will not adversely impact most crops if the "flood" water contains less than 2.21 to 5.51 mg/L mg a.e./L 2,4-D DMA.

- **Potential impacts on aquaculture**

  Under most conditions, it is not anticipated that the use 2,4-D BEE or 2,4-D DMA should have acute effects on aquaculture when the concentration of 2,4-D acid is below 10 mg a.e./L. The target concentration for 2,4-D in aquatic use is usually ~2-4 mg a.e./L but the most typical use rate in the United States is ~1.13 mg a.e./L (JMPR, 1997). Measured concentrations in open waters in British Columbia indicate that when used at maximum rates, 2,4-D BEE should have a concentration in surface waters of 0.190 mg a.e./L and in bottom waters of 3.25 mg a.e./L (Gallagher, 1992). A real application to a Wisconsin pond yielded effective concentrations on the first day after application of 0.19 to 0.330 mg/L (Helsel et al, 1996). Since this real case treatment of 112 Kg product/ha (100 lbs. product/acre) yields very similar surface water levels to that proposed by Gallagher (1992), these values are used to assess the impact of 2,4-D on aquaculture under worst case situations. Water is not generally used for agricultural purposes until the concentration in the treated water body falls below the MCL (0.07 mg/L for 2,4-D) If water used for aquaculture is taken in through surface intake pipes, the concentration will be in the range of 0.30 to 0.19 mg/L. Presumably, almost all of that dosage will be in the form of 2,4-D acid since 2,4-D BEE is converted to 2,4-D very rapidly within one day in natural environments (see Section 3). The levels that aquaculture organisms will be exposed to is somewhere between the applied formulation (2,4-D BEE) and 2,4-D acid. JMPR encourages a risk approach that takes the two extremes into account: 1) Evaluate risk based on the applied formulation; 2) If risk is too high based on this scenario, re-evaluate risk assuming that the 2,4-D has been converted to the acid. In order to assure safety, we would generally assume that long-term toxicity (NOEC) needs to be at least 10x lower than the acute toxicity.

  Because the concentrations of 2,4-D BEE that acutely effect the most sensitive commercial species run from very low to very high (0.3 mg a.i./L for estuarine crab zoels and rainbow trout to approximately 3000 mg/L for juvenile and adult estuarine crabs), 2,4-D BEE is likely to adversely impact cultured aquatic organisms since the exposure concentration would be at least at the Federal MCL 0.07 mg/L and under less controlled situations at 0.19 to 0.330 mg/L. In order to protect these commercial species from the effects of acute toxicity, you would expect the maximum concentration would have to be five to ten-fold lower than the lowest LC50 of 0.3 mg/L. However, the maximum exposure would expose the cultured organisms to dosages that are only four-fold lower than the lowest LC50. If one assumes that the low solubility of 2,4-D BEE and its rapid conversion to 2,4-D acid decreases contact of the biota to 2,4-D BEE, a substantial safety margin is present. The typical acute LC50 for culturable aquatic animals ranges from 25 mg a.e./L for cutthroat trout to

212 mg a.e./L for bay mussel. Even if the worst case scenario of 0.330 mg/L is assumed, a safety factor of 76-fold is provided for the most sensitive species (Table 14).

Even in chronic sac-fry tests where the 8 day LC1 (~NOEC) for largemouth bass is equal to 3.2 mg a.e./L, a safety factor of 9.7-fold occurs. A greater safety factor can probably be determined since 2,4-D BEE will have been converted entirely to 2,4-D acid in the period of a chronic study. Taking into account the lower molecular weight of 2,4-D acid, the chronic exposure concentration would be 0.227 mg a.e./L rather than 0.330 mg 2,4-D BEE/L. Furthermore, even if the half-life of 2,4-D acid is assumed to be fairly long (15 days), the geometric mean for the concentration of 2,4-D acid over 28 days would be 0.127-mg a.e./L. This would lead to a safety factor of between 14 and 25-fold for the most sensitive culturable species. Please see Appendices 1 to 5 for toxicity data used in this evaluation.

Since 2,4-D DMA, is not likely to be used in Washington State for the control of aquatic weeds, its potential effects on aquaculture are not discussed in great detail. However due to the extremely low toxicity of this product to culturable species [acute LC50 = >100 mg a.i./L (83 mg a.e./L)] and very low bioconcentration potential, it is unlikely to have any significant impact on aquaculture. In addition, the predicted chronic toxicity levels are also extremely low [NOEC = 4.5 mg a.i./L = 3.7 mg a.e./L (Table 13)] which should also provide significant safety since 2,4-D concentrations in natural waters are typically not higher than 0.281 mg a.e./L three days after treatment when concentrations are expected to be highest (Table 7 and Gangstad, 1986). Note that chronic toxicity is predicted based on the geometric mean of acute/chronic toxicity ratios; in this case this mean ratio was ~18.5 which gives a predicted chronic toxicity (Table 14) for 2,4-D DMA of 5.5 (100 mg a.i./L/18.5)

4.3 ENVIRONMENTAL TOXICITY REVIEW – 2,4-D TOXICITY TO THE BIOTA AND RISK ASSESSMENT

2,4-D products are not chronically toxic to most aquatic life except for their direct contact effect on plant foliage, and algae. However, the 2,4-D BEE product is apparently acutely toxic to some species of algae (EC50 1.66 to 1.86 mg a.i./L for *Skeletonema costatum* and *Navicula pelliculosa*) and aquatic macrophytes (EC50 = 0.58 mg a.i./L for *Lemna gibba*) and all species of aquatic animal (0.3 mg a.i./L for rainbow trout fry to 7.2 mg a.i./L for *Daphnia magna*) that are usually tested (Hughes, 1990; EPA, 1986 in Brian, 1999; Martens, 1980 in Ecology, 1989 and Alexander, 1983 in JMPR, 1997). This may be of short-term concern since the concentrations of 2,4-D BEE are often higher than the EC50 in surface waters than 0.20 to 0.3 mg a.i./L (Gallagher, 1992 and Helsel, 1996). However, 2,4-D BEE has a very short half-life (see Section 3 and Zepp, 1975 in JMPR, 1997; Racke, 1989; Wojtalik et al, 1971 in Shearer and Halter, 1980, Leonard, 1982 in Ecology, 1992,). The usual half-life for 2,4-D BEE is approximately one day, and it is rapidly converted to 2,4-D acid which has as very low acute toxicity. E.g., the acute toxicity (LC50) on plants and algae is 0.695 mg a.i./L for *Lemna gibba* and ~500 or more for many blue-green algae (Table 2; Hughes, 1994; Mishra and Pandley, 1989) and the toxicity for most aquatic animals is generally low (Usually >40 mg a.i./L with at least one important exception amongst sediment organisms).

The toxicity of 2,4-D DMA is rarely a serious issue since typical concentrations found in treated water bodies are below the LC50. The highest residue concentrations are usually around 0.281 mg a.e. /L. Concentrations this low will not generally affect aquatic plants. In standard toxicity tests, 2,4-D DMA is only toxic to aquatic macrophytes (LC50 =0.58 mg a.i./L = 0.48 mg a.e./L) with virtually no toxicity to the standard species of algae tested (LC50 = >60 mg a.i./L = 50 mg a.e./L) with at least one important exception amongst the freshwater diatoms (Hughes, et al, 1990). However, 2,4-D DMA has virtually no acute toxicity to aquatic animals with an LC50 typically >100 mg a.i./L (83 mg a.e. /L); important exceptions are a few species of estuarine shrimp with LC50s of approximated ~0.15 to 8.0 mg a.i./L (EVS, 1991 in Brian, 1999, Johnson and Finley, 1980.

The activity of 2,4-D BEE does not require the use of any adjuvants since it is a granular product. However 2,4-D DMA's effectiveness may be improved on emergent weeds by adding surfactants and accelerators so that 2,4-D DMA is more readily adsorbed. 2,4-D products are primarily applied from boats using a spray boom or subsurface injection for liquids, or the use of an electrical hopper spreader for the granular formulations. 2,4-D liquid products may occasionally be applied from a shore vehicle using a spray boom. It is very unusual for 2,4-D to be applied by aircraft except for application to remote sites. Aerial application is usually avoided due to public perception that drift problems may have an adverse impact on the human habitat (Getsinger, 1999, personal communications). The activity of liquid formulations of 2,4-D DMA) may be improved by adding a thickening agent to assure that subsurface applications drop lower in the water column where they can do the most good or by decreasing drift when the formulation of 2,4-DMA are applied by a spray boom. On the rare occasion that 2,4-D 2-EHE is used it can be formulated with diesel oil, Syndets® or another surfactant. However, these adjuvants should be used with great care since even those approved for aquatic application may have some toxicity to fish when applied in shallow water or for control of weeds on ditch banks with little or no flow within the canal (Wan et al, 1990, Abdelghani et al, 1997, Watkins et al, 1985). One commercial product (Weedone®) which is a mixture of 2,4-D BEE and 2,4-Dichloropropionic acid appears to have greater toxicity when mixed with diesel oil and applied to soft acidic water than the herbicide alone.

The acute effects of 2,4-D are not of major concern. However, possible problems could occur with food chain issues, disruption of habitat (Frank, 1972, Marshal & Rutschky, 1974, Wright and Bourne, 1990,), potential disruption of nesting (breeding behavior) in fish (Bettoli and Clark, 1992), disruption of behavior by causing avoidance of treated areas (Folmar, 1976, Hansen, 1973), sub-acute effects which disrupt biochemistry and cause pathogenic conditions (Neskovic, 1994, Elezovic, 1994) and changes in numbers, diversity and quality of aquatic macrophytes and animals associated with them (Helsel, et al, 1996, Monteiro and Moreira, 1990, Marshall & Rutschky, 1974, Bain and Boltz, 1992, Sarkar, 1991) .

Due to the mode of action, 2,4-D can take a very long time for control of aquatic weeds to occur particularly if low rate technology is an issue. It has been suggested that much lower concentrations than are typically used for the control of milfoil may be effective while preserving native species of plants. Control of milfoil is a function of exposure concentration and exposure time. Laboratory experiments have shown that control of Eurasian watermilfoil biomass is greater for an exposure to 2,4-D at 0.5 mg.a.e./L for 72 hours than for exposure to 2.0 mg a.e./L for 12 or 24 hours (Green and Westerdahl,

1988). Further laboratory work by Sprecher et al, (1998), indicates that while concentrations of 2,4-D at 1, 1.5 or 2.0 mg/L will control *Myriophyllum spicatum* (Eurasian watermilfoil), *Potamogeton pectinatus* (Sago pondweed) will not be affected. The selective effect of this growth regulator will provide suitable control for Eurasian watermilfoil in habitats where native pondweed is to be maintained. Sago pondweed and other narrow-leaf monocots should not be seriously affected by concentrations of 2,4-D that are used to control Eurasian watermilfoil. It has been suggested by Forsythe et al, (1997) the *Myriophyllum sibericum* (watermilfoil) and Sago pondweed could be eliminated from the waters of prairie wetlands with concentrations of 2,4-DMA as low as 0.1 mg/L if exposure was maintained for the entire season.

Biochemical degradation of 2,4-D is extensive. Bacteria metabolize 2,4-D rapidly initially converting it to 2,4-dichlorophenol and eventually to oxidoadipate and succinate (Volmer et al, 1993 and Short et al. 1991), which can eventually be converted to cellular products which enter the Krebbs' cycle to be ultimately metabolized to various cellular products, humic acid and carbon dioxide (Shearer and Halter, 1980). The toxicity of 2,4-D to microorganisms that can utilize 2,4-D as a sole carbon source appears to be very low. Concentrations of 2,4-D higher than 100 mg/L often are not toxic to these organisms but instead stimulate their growth in the water and soil environment. Current experiments to determine the toxicity of 2,4-D to microorganisms are primarily conducted to determine which genes in the *tdfA* through *tdfF* cluster are responsible for the transformation of 2,4-D and its metabolites to various metabolic products. The soil/sediment microorganism species most commonly used for these experiments are *Alcaligenes eutrophus* and *Pseudomonas putida*.

In general, there have been few studies done to ascertain the toxicity of 2,4-D to microorganisms. Using batch cultures of unspecified bacteria, Orhon et al (1989 in Ebasco, 1993) found that growth inhibition of 50 percent of the test organisms occurred at concentrations of 400 mg/L 2,4-D. In a recent field study, treatment with 2,4-D sodium salt at 6 Kg a.i./ha to control *Euryale ferox* (thorny lily), increased heterotrophic bacterial counts increased from 360 bacteria/L before treatment to 942 bacteria/L immediately after treatment when Eurasian watermilfoil started to die. When the treated plants had completely decayed, the levels of heterotrophic bacteria returned to 298 bacteria/L. Conversely bacteria counts (presumably aerobes) in the sediment decreased from 158,700 before treatment to 46,799 shortly after treatment and regained some of this loss (92100) eight weeks after treatment. These changes in heterotrophic and sediment bacteria were tied to secondary effects including the increase of free ammonia and phosphate levels. It was interesting that the levels of phytoplankton and zooplankton also appeared to be affected by these secondary effects attributable to plant decay accompanied by an oxygen slump and increase in inorganic nutrients (Table15) (Patnaik and Das, 1991).

In an experiment using three species of ectomycrrhizal fungi, which form important symbiotic relationships with many vascular plants, facilitating nutrient uptake and improving resistance to stress, Harley and Smith, 1983 (in Ebasco, 1993), and Estock et al (1989 in Ebasco, 1993) reported significantly reduced growth rates with various concentrations of 2,4-D. They observed reduced growth rates in all three species of ectomycorrhizal fungi (*Censcoccum geophilum*, *Pisolithus tictorius* and *Hebeloma lonicaudum*) at concentrations >1000 mg/L 2,4-D. Reduced growth rates occurred in two more sensitive species (*P. tictorius* and *H. lonicaudum*) at concentrations <100

mg/Kg 2,4-D, but the authors concluded that growth conditions in laboratory media tended to predispose the bioassay fungi to herbicide toxicity.

However, recent work by Premdas and Kendrick (1991) indicates, that 2,4-D 2-EHE formulated in an emulsifiable oil carrier (EOC) can stimulate the germination of fungal propagules in aero-aquatic fungus (*Pseudoaegerita matsushimae*) at concentrations of ~3.0 mg/L. However, higher concentrations inhibited the germination of these fungal propagules and at 100,000 mg/L, propagule germination was entirely inhibited (EC50 = ~10,000 mg/L). The growth of blue-green algae is also stimulated by low concentrations of 2,4-D in various formulations. This is discussed in Section 4.3.1. Field work at 1.0 mg/L indicated that 2,4-D DMA did not have a clear effect on fungal propagules in either the water column or sediment, but that the mean levels of molds and total fungi in ponds treated with 2,4-D DMA or 2,4-D BEE tended to be depressed, relative to the controls, for up to 114 days after treatment. However, these differences were erratic and a thorough statistical analysis of the data was not possible; the author concluded that the observed effects were probably due to unidentified secondary effects from the pond treatments (Sherry, 1994) and not the treatments themselves. Concentrations of 2,4-D that are stimulatory to blue-green algae growth are usually less than 10 mg/L. However, exact concentrations (0.05 to 100 mg/L) that stimulate growth vary with species and condition.

Animals do not appear to metabolize 2,4-D. 2,4-D BEE is rapidly converted to 2,4-D acid (Rogers and Stallings (1972 in Ebasco, 1993). Then the 2,4-D is rapidly eliminated unchanged from the animal's body in the urine and feces. This short residual time within an animal's body occurs for chickens (Puvanesarajah and Bliss, 1992), lactating goats and lactating dairy cows (Guo and Stewart, 1993 and Krautter and Downs, 1996) and a variety of fish and aquatic invertebrate species (Plakas et al, 1992, Premkumar 1994, Biever, 1996 and Biever, 1998).

4.3.1 Effects and Selectivity on Aquatic Plants

Summary: 2,4-D DMA and 2,4-D BEE are not generally toxic to aquatic algae. However, 2,4-D BEE and 2,4-D DMA may be toxic to some species of diatoms (EC50 = ~2 to ~ 5mg a.i./L) and 2,4-D BEE may also be toxic to blue-green algae (6.37 mg a.i./L). At low concentrations (<10 mg a.i./L), some products of 2,4-D have been observed to stimulate the growth of green and particularly blue-green algae. In laboratory tests, 2,4-D BEE and 2,4-D DMA are highly toxic (EC50 = 0.58 mg a.i./L) to aquatic macrophytes like Lemna gibba. In field tests, 2,4-D DMA and 2,4-D sodium salt may cause algal blooms due to the release of nutrients from decaying aquatic macrophytes. However, many species to algae are unaffected by 2,4-D DMA or 2,4-D BEE (EC50 = 25 to 150 mg a.i./L).

These laboratory results are similar to the effects observed in field studies where algal blooms occurred after treatment with 2,4-D DMA at rates up to 45 Kg a.e./L and with 2,4-D sodium salt at 6.0 to 8.0 Kg a.i./L. Treatment with 2,4-D DMA caused nearly complete clearance of Eurasian watermilfoil and native weeds in 44 days while 2,4-D sodium salt caused an eighty percent clearance of thorny lily within 8-weeks. Treatment with 2,4-D BEE at 19 kg a.e./ha almost completely eliminated Eurasian watermilfoil for two growing seasons and caused a temporary but significant decline in native plant species as well. Field studies indicate that removal of aquatic vegetation with 2,4-D does

not affect the numbers, size, condition or movement of largemouth bass. However this may be a reflection of the small size of the treated areas (5 to 10 acres).

Diversity of algae and aquatic macrophytes appear to be affected by the use of 2,4-D DMA and 2,4-D BEE. Before treatment with 2,4-D BEE Eurasian watermilfoil was the dominant species in Beulah Lake, Wisconsin. However, after treatment, the native species regained all of their pretreatment standing crop by the end of the season. At Loon Lake, Washington, treatment with 2,4-D BEE reduced Eurasian watermilfoil biomass by 98%, but the native pondweeds, naiads, American water weed, water celery, bladderwort, water stargrass and Chara spp. *were largely unaffected. After treatment with 2,4-D DMA, algae in the orders* Chlorophyta, Phyrrhophta *and* Bacilariophyta *dominated the treated lake system.*

2,4-D can be extremely selective or non-selective depending on conditions in the water body. However, the labeled used for 2,4-D BEE and 2,4-D DMA in aquatic ecosystems is limited. 2,4-D is used primarily for the control of *Myriophyllum spicatum* (Eurasian watermilfoil). However, it also has utility in the control of other species, i.e. *Myriophyllum spp., Heteranthera dubia* (water stargrass) at 100 to 200 Kg product/ha and *Utricularia spp*. (bladderwort), *Nymphaea spp*. (fragrant water lily), *Nuphar spp*. (yellow water lily), *Brasenia spp*. (watershield), *Trapa natans* (water chestnut) and *Ceratophyllum demersum* (coontail) at 150 to 200 Kg product/Ha. The use of 2,4-D BEE at 100 Kg product/ha can eliminate Eurasian watermilfoil within 3 to 6 weeks after application. Native (Wisconsin) plant species like *Ceratophyllum spp*. (coontail*), Elodea canadensis* (American waterweed) and, *Potamogeton crispus* (curly-leaf pondweed*), P. zosteriformis* (flat-stem pondweed), muskgrass, *Najas spp*. (naiads), *M. sibericum* (northern watermilfoil), *M. heterophyllum* (variable leaf milfoil), *Rununculus spp*. (water crowfoot), *H. dubia* (water stargrass) white-stem pondweed and water celery also declined within the first five weeks after treatment in early spring. However, 80 to 120 percent of the pretreatment standing crop returned by late August. Eurasian watermilfoil remained at low levels of dominance (3%-5%) of the areal cover for two years after treatment (Table 16 (Helsel, 1996)). The amine salt of 2,4-D was used in a manner similar to 2,4-D DMA to control *Eichornia crassipes* (waterhyacinth) and *Myriophyllum aquaticum* (parrotsfeather) in Portugal. The application rate was 6.48 Kg a.i./ha (1.6 mg/L 2,4-D at zero hour). Control of parrotsfeather often contributed to the spread of other undesirable species like *Sparangium erectum, Typha spp*. and *Paspalum pasapalodes*. The aquatic macrophytes, currently of greatest concern in the Northern Tier of States (including Washington), are *Myriophyllum spicatum* (Eurasian watermilfoil), *Potamogeton crispus* (curly-leaf pondweed), *Egeria densa spp*. (Brazilian elodea), *Monoesius hydrilla, Spartina alterniflora* (smooth cordgrass), *Lythrum salicaria* (purple loosestrife), *Phragmites australis* (common reed), *Nuphar spp*. and *Nymphaea spp*. (water lilies) and *Trapa natans* (water chestnut). Of these, only Eurasian watermilfoil, purple loosestrife, water lilies and water chestnut are effectively controlled with a 2,4-D product. 2,4 D BEE and 2,4-D DMA are effective against Eurasian watermilfoil, and water chestnut (Robinette, 1998-1999 and Westerdahl et al., 1988 and Getsinger, 2000 personal communications).

Treatment of a demonstration plot at Loon Lake, Washington resulted in the effective suppression (87%) of Eurasian watermilfoil for one year after treatment at 100 lbs/acre. However, other indigenous plant species were not reduced in biomass or frequency due to the affects of 2,4-D treatment. The plants that appeared to be unaffected by treatment with 2,4-D BEE included: American waterweed, several species of pondweed

(*Potamogeton spp*.), naiads, water stargrass, and *Chara spp*. Although *Megalodonta beckii* and *Vallisneria americana* appeared to slightly stimulated in growth by 2,4-D, these effects were considered by the authors to be seasonal and unrelated to the use of 2,4-D BEE (Parsons et al, 1999 in press).

The differences in the scenarios for these results were as follows: 1) The Beulah Lake, Wisconsin applications were to coves which had been isolated from the main body of the lake by polyvinylchloride curtains. This allowed for little water exchange and resulted in increased exposure times. 2) The Loon Lake applications were made to an open water body which allowed for extensive mixing and dissipation leading to decreased exposure times. Getsinger and Westerdahl (1986) and Sprecher et al (1998) previously found that both exposure time and treatment rate have a strong influence on the degree of damage due to treatment with 2,4-D.

4.3.1.1 Acute Effects on Aquatic Plants

The indicator species for aquatic toxicity in aquatic plants and algae are *Lemna gibba* (duckweed, aquatic macrophyte), *Anabaena flos-aquae* (blue-green algae), *Selenastrum capricornutum* (green algae), *Navicula pelliculosa* (fresh water diatom) *and Skeletonema costatum* (marine diatom) (Table 2 and Table 17). However, in the case of 2,4-D acid, a wide variety of surrogate species were also tested including *Chlorococcum spp*. (green algae), *Chlorella fusca*, (green algae), *Dunaliella tertiolecta* (green algae), *Scenedesmus quadricauda* (green algae), *Phaodactylum tricornutum* (marine diatom), *Isochrysis galbana* (marine hapatophyte), *Anabaena dolium* (blue-green algae), *Chlamydomonas reinhardtii* (blue-green algae), and *Nostoc spp*. (blue-green algae). Some of these species were also tested with 2,4-D BEE, 2,4-D 2-EHE and 2,4-D DMA. It is expected that the esters would be more toxic based on their greater lipophilicity (Frank, 1972). However, due to the poor solubility of the esters, accurate LC50 values are often difficult to achieve. Since many of these toxicity values are reported in terms of nominal concentrations, values are often suspect as to accuracy. Also, aquatic algae and plant species are often not tested at concentrations that exceed the expected environmental concentration (EEC). The maximum EEC is only 2.9 mg/L for the acid (Peterson et al, 1994) according to Canadian guidelines; and in terms of initial acid equivalents, this is the target concentration at time of application for most of the 2,4-D products. For those species tested at concentrations that exceeded the EEC, most blue-green algae, diatoms, green algae and macrophytes could withstand 2,4-D acid at concentrations in excess of 50 mg/L for the esters. Green algae and marine hapatophytes were able to tolerate 2,4-D BEE at concentrations in excess of 25 mg/L. However, two standard species of diatom were acutely affected at concentrations of 2,4-D BEE one might expect to see in the environment.

The other main 2,4-D product used aquatically in the United States (2,4-D DMA) has a fairly low toxicity to blue-green algae, green algae and marine diatoms (LC50 = 37 to 362 mg a.i./L = 34 to 338 mg a.e./L). The only species of macrophyte, tested on the three commercial products and 2,4-D acid was *Lemna gibba* (duckweed) and in all cases, the toxicity was fairly high (LC50 = 0.50 to 0.695 mg a.i./L). Since the effective concentration of 2,4-D acid (equivalence) for the three commercial products are all about equal (LC50 = 0.50 mg a.e./L) it is likely that the active ingredient in these cases is converted to 2,4-D acid before it becomes toxic to this plant. It is apparent that all esters and salts are converted rapidly to 2,4-D acid in the field. For the *Lemna* study, the field case and the laboratory case are similar since the tests are run statically for 14-days.

Because plants are the intended targets of aquatic herbicides containing 2,4-D, a risk assessment would not usually be conducted to determine the safety of 2,4-D products on plants. A realistic level of concern may be used for aquatic plants since even a reduction in growth of 50 percent will still leave a significant amount of forage and habitat (refuge). However, freshwater and marine diatoms can be adversely affected by the two 2,4-D ester products since the concentrations that they would be exposed to could be expected to reduce growth by more than 50 percent (RQ = 2.9 ppm a.e./2.72 ppm a.e = 1.1; 2.9 ppm a.e/0.15 ppm a.e. = 19). Since diatoms can be an important element of the food chain (Goldman and Horne, 1983), the high risk quotient leads to a level of concern for those species and animals that depend on them for food. Aquatic macrophytes are also very susceptible to the effects of 2,4-D esters which means that the risk quotient would be higher than unity; and it is (RQ = 2.9 ppm a.e./~0.5 ppm a.e. = 5.8) for the most sensitive species (*Lemna gibba*). Aquatic (emergent) macrophytes are of importance in providing both food and habitat to fish, amphibians, aquatic invertebrates, wild birds and mammals (Frank, 1972); therefore, this high RQ exceeds the level of concern for aquatic macrophytes. However, the 2,4-D DMA product appears to provide a high degree of safety to all aquatic algae [RQ = 2.9 ppm a.e./4.38 ppm a.e. = 0.66 for the most sensitive species (*Navicula*)]; but not to aquatic macrophytes (RQ = 2.9 ppm a.e/0.48 ppm a.e. = 6.04) However, concern has been expressed by Peterson et, al (1994), that RQs of less than 1.0 that are used to evaluate pesticides on aquatic plants by Environment Canada and U.S. EPA do not provide a significant safety factor. Peterson et. al. (1994) propose that RQs of less than 0.1 could provide a significant and more meaningful safety factor; and recommended the guidelines expressed in Table 18. If the products are rapidly converted to 2,4-D acid before they have an effect, only the standard diatoms and the standard macrophyte are affected at levels above the most liberal level of concern (2.9 ppm a.e./ 2.0 ppm a.e. = 1.45 for diatoms and (2.9 ppm a.e./ 0.695 ppm a.e. = 4.17 for *Lemna gibba*). Confirming this, Peterson et al (1994) found that 2,4-D acid could be considered to have a high hazard rating (RQ = > 1.0) in only one species in eleven. That one species was an aquatic macrophyte (*Lemna minor*). The other species with a potentially low hazard rating (RA = < 0.1) were various species of green and blue-green algae. While the WHO/FAO considers that this RQ of greater than 0.1 poses a significant risk to aquatic plants, they discount the level of concern since, aquatic macrophytes are the targets of the 2,4-D products (JMPR, 1997).

4.3.1.2 Chronic Effects on Aquatic Plants

Laboratory work to determine the chronic effects of herbicides on algae and aquatic plants is currently not conducted for the purposes of registration.

4.3.1.3 Potential Impacts of Single Versus Multiple Applications

Studies performed in both the field and laboratory indicate that algal response to a variety of 2,4-D formulations varies from no effect (<1 mg/L) to stimulation of growth at low concentrations (5-200 mg/L) to growth inhibition (100 to 1000 mg/L) to a temporary loss of species (400 to 1200 mg/L) (Shearer and Halter, 1980, Okay and Gaines, 1996, Wong and Chang, 1988, Fargasova, 1994a, Fargasova, 1994b, Mishra and Pandley, 1989, Das and Sing, 1977, Wang et al, 1991, Swain and Adhikary, 1991, Swain & Adhikary, 1994 Kobraei and White, 1996, Patnaik and Das, 1991, Torres, 1976).

Many species of algae, (particularly cyanophytes) appear to be stimulated to growth by low concentrations of 2,4-D. In the laboratory, the reason for this stimulation is not fully

understood. Some suggestions have been the presence of ammonia from the disassociation of the amine salt (Okay and Gaines, 1996) for a green algae and marine diatom; loosely defined hormonal effects from 2,4-D acid (Wong and Chang, 1988 and Fargasova, 1994) for a green algae (*Chlamydomonas reinhardtii*); stimulation of nitrate uptake and atmospheric nitrogen fixations by 2,4-D acid (Mishra and Pandey, 1989) and Das and Sing, 1977) in blue-green algae commonly associated with plants requiring microbe assisted nitrogen fixation for proper growth. The highest levels of growth and nitrogen fixation occurred in cultivated *Anabaena* species where a yield increase could be as high as 914% in the presence of very small amounts of 2,4-D (0.05 mg/L). Growth stimulation due to a relationship between cell physiology and photorespiration has been hypothesized as a likely cause for this increase in production of *Anabaena* biomass. Other older studies (1969 to 1975) have also shown 2,4-D BEE, 2,4-D sodium salt, and 2,4-D acid to stimulate growth of algae (Table 19).

Laboratory results indicate that algal survival, growth and productivity are not adversely affected at moderately high concentrations (>200 mg/L) of a variety of 2,4-D formulations including 2,4-D acid, 2,4-D sodium salt and 2,4- D DMA. However, field observations indicate that low concentrations of 2,4-D appear to stimulate algal growth at concentrations as low as low as 2 mg/L (Kobraei and White, 1996 and Pierce, 1991 in Ebasco, 1993). It is generally agreed that 2,4-D exposure can possibly lead to algal blooms, but there is disagreement whether this is due to direct stimulatory impact of 2,4-D or increases in nutrient levels due to the decay of dead and dying vegetation (Kobraei and White, 1996, Sherry, 1994 and Patnaik & Das, 1991). With the exception of Fargasova (1994) most of the cited authors believe that use of 2,4-D products at the labeled use rate (2 to 4 mg a.e./L) will not have a significant impact on phytoplankton growth and therefore adverse impacts on the food chain as a result of aquatic weed control are unlikely.

Sorption and metabolism of 2,4-D has also been investigated. There is little confirmed information that 2,4-D is adsorbed extensively by phytoplankton. Voight and Lynch (1974 in Shearer and Halter, 1980) found that 2,4-D acid was adsorbed weakly by *Coelastrum microporum*. Similar work by Boehm and Mueller (1976 in Shearer and Halter, 1980) showed a BCF in this species of two-fold. However, Wojtalik et al. (1971 in Shearer and Halter, 1980) found that when the Gunthersville Reservoir was treated with 2,4-D DMA that algae had removed nearly 100% of the 2,4-D in surface water samples at 24 hours post treatment. However, no other study has been evaluated that shows similar results. 2,4-D products do not appear bioaccumulate in algal tissue. A BCF value of 6.8 was measured for 2,4-D acid in the green algae (*Chlorella fusca*) (Reinert and Rogers, 1987) which according to Weber's (1977) system for evaluating pesticides would indicate that 2,4-D will not bioaccumulate. Twenty-one species of algae have been observed to metabolize 2,4-D extensively when the applied active ingredient was 2,4-D BEE. 13% to 64% of the applied herbicide was degraded. The amount of degradation depended on the algae species tested (Butler et al, 1975 in Halter, 1980).

Aggressive aquatic herbicide treatment may create more open water for fish habitat. However, aggressive treatment may eliminate areas containing milfoil and other macrophytes that are used by juvenile fish as a refuge from predators and as general habitat (Killgore et al 1987 in Ecology, 1980, 1989). Invertebrates are more abundant on macrophytes other than milfoil, so a community shift to other plant species may result in greater abundance of invertebrates, which would provide more food for the grazing planktovoric fish. Therefore, in most cases where an adverse effect has occurred on fish

food organisms, it has been as a result of anaerobiosis rather than loss of habitat. (Frank, 1971).

Ecology (1992) suggests retaining 20% to 25% of native vegetation as fish rearing habitat in treated areas. This also creates more open water with fewer macrophytes and increases habitat for post- and non-breeding adult fish while at the same time allowing for increased invertebrate habitat, which increases the food source for fish. This intermediate approach provides improved habitat and food source for both the juvenile, sub-adult and adult fish and should decrease the impact of extreme approaches such as no treatment or complete removal of aquatic weeds.

Although these approaches have a large element of common sense behind them, a decrease in fish populations due to lack of extensive macrophyte habitat is still primarily a hypothesis (Bain and Boltz, 1992, and Marshall and Rutschky, 1974). The effects of plant removal and its impacts on habitat for fish and invertebrate animals will be discussed in subsequent chapters.

- **Potential impact on numbers**

  Field studies with both algae and macrophytes indicate that the numbers of these plants can be strongly affected by the use of 2,4-D at concentrations that are typically used in the field. Shearer and Halter (1980) cite a number of studies which indicate that use of 2,4-D DMA and 2,4-D BEE at normal field rates, often cause planktonic blooms. These blooms could be due to the general stimulatory effect that 2,4-D products appear to have on algal growth in the laboratory experiments described above; but are more likely to be due to a change in nutrient levels due to the decay of aquatic weeds during the first four to eight weeks after treatment. Effects of 2,4-D on increases in phytoplankton can begin as quickly as 1-day after treatment and may persist for a short period of time or until the water body has been entirely cleared of the decaying weed mass.

  Kobraei and White (1996) and Patnaik and Das (1991) treated waters in Lake Kentucky and an Indian fishpond, at 2 mg/L (11.25 –to 45Kg a.e./L) 2,4-D DMA and 6 Kg a.i./ha 2,4-D sodium salt, respectively. The treatments were necessary for the control of *Myriophyllum spicatum* (Eurasian watermilfoil) and *Euryale ferox* (thorny lily) in Lake Kentucky and the Indian fishpond, respectively. These treatments resulted in immediate changes in nutrient levels accompanied by a phytoplanktonic bloom. In both cases, the limiting nutrient appears initially to be nitrogen in the form of nitrate at Lake Kentucky and ammonia at the Indian fishpond. Later in the season, phosphate concentration may become the limiting nutrient at the Indian fishpond site but nitrogen (nitrates) appears to still be the limiting nutrient at Lake Kentucky. Cell densities increased ~100% (from 4,500 to 8,500 cells/L in the Kentucky Lake and 30% (Table 15) in the Indian fishpond. The numbers of phytoplankton in the Indian fishpond continued to increase to levels ~200% above pretreatment levels in the Indian fishpond for eight weeks, but the cell counts returned to control levels within two to four days in Lake Kentucky. While the cell counts did not increase in Lake Kentucky after 8 days, the chlorophyll a concentrations increased to their highest levels. At the Indian fishpond site there is a proportional rise in the zooplankton count probably due to the increase in the phytoplankton count which serves as a food source.

The numbers of macrophytes in treated ponds decreased dramatically in ponds that were treated for aquatic weed control. Eighty-percent clearance of spiny lily was observed in Indian fishponds treated with 6 to 8 Kg a.i./ha within 8-weeks. In Gunthersville Reservoir, Alabama, treatment with 2,4-D DMA at concentrations of 2 mg/L, resulted in nearly complete clearance of Eurasian watermilfoil and native weeds in about 44-days. Changes in the fish populations were not observed in either case (Bain and Boltz, 1992; Patnaik and Das, 1991).

In a Wisconsin pond treated with 2,4-D BEE at 100 Kg product/ha (21 Kg a.e./ha) for control of Eurasian watermilfoil, milfoil was almost completely eliminated from the treated coves in 3-6 weeks. Native species of plants also declined significantly within the first 5 weeks (Helsel et al, 1996).

- **Potential impacts on diversity**

The dominant species of phytoplankton can change after treatment with 2,4-D DMA. Wojtalik et al. (1971) found that after treatment with 22-45 Kg a.i./ha (20-40 lbs. a.i./ha), that entire genera were eliminated within 24 hours of treatment but returned after two weeks. Kobraei and White (1996) found a Kentucky lake treated with 2,4-D DMA had conditions that were ideal due to the secondary effects of water temperature and nutrient concentrations, as a result of milfoil lysis, for the growth of Chlorophyta, Pyrrhophyta and Bacilariophyta.

The dominant macrophytes in coves of Beulah Lake, Wisconsin changed after treatment with 2,4-D BEE (112 Kg product/ha = 21 Kg a.e./ha). Before treatment, Eurasian watermilfoil was the dominant species. Five weeks after treatment in May, 1993, Eurasian watermilfoil was eliminated from the treated coves. By August, 80% to 120% of the pre-treatment standing crop of native species had returned. Two growing seasons after treatment, less than 5% of the standing crop consisted of Eurasian watermilfoil, while water celery, *Elodea*, and naiads dominated covering 95 to 100% of the total treatment area (Helsel et al, 1996). However, those species that are tolerant or more difficult to control (Robinette, 1998 and 1999 and Westerdahl and Getsinger 1988) like coontail, fanwort, various pondweeds, hydrilla, naids, water buttercup, widgeongrass or water celery or alligator weed have the potential to become a dominant species within any habitat if other measures of control are not pursued.

- **Naturally occurring re-growth of reproduction of non-noxious or non-invasive plants**

Most noxious plants like milfoil are substantially reduced upon treatment with 2,4-D. Nevertheless, it is clear that while native and desirable pondweed species do recover (Helsel et al, 1996), some of the more difficult to control species like coontail, fanwort, various pondweeds, hydrilla, naiads, water buttercup, widgeongrass, water celery or alligator weed have the potential to dominate the biomass after treatment (Helsel et al, 1996; Robinette, 1998-1999; and Westerdahl and Getsinger, 1989).

The selectivity of 2,4-D for Eurasian watermilfoil in the presence of other plant species is a primary reason 2,4-D is currently the herbicide of choice for milfoil control in some states. This selectivity has been covered in the literature (Gangstad, 1977, Gangstad, et al, 1976 and Wojtalik et al, 1971 as cited in Shearer and Halter,

1980). Although non-target native plants can be affected by the application of 2,4-D for the control of Eurasian watermilfoil, they have a quick recovery and tendency to dominate the biomass for a period of time after treatment (Helsel, 1996). Results from Loon Lake, Washington indicate that in open water ways, these native species may not be affected directly by 2,4-D (Parsons, 1999). Given the stimulatory effects of sub-lethal 2,4-D concentrations, it would not be surprising to learn that the growth of milfoil or non-target plants not killed by the herbicide treatment could be enhanced. Certainly there is some potential for this observation in relationship to the relative effects of 2,4-D on Eurasian watermilfoil and Sago pondweed (Sprecher et al, 1998).

- **Post treatment plantings of non-noxious or non-invasive species**

  In a general review article, Frank (1972) recommended the planting of non-noxious, and non-invasive native plants after the elimination of exotic noxious and invasive plants. However, some scientists have found efforts to reestablish native plant species are often unsuccessful. He indicated that such plantings would be competitive with the faster growing exotics once they have been eliminated. These native species can serve as both food and habitat for waterfowl, fish food organisms and fish. For a further discussion of the effect of the effects of 2,4-D on numbers and diversity of aquatic animals (please see Section 4.3.2.3).

- **Effects on aquatic plants: potential impacts of single versus multiple applications**

  Initial elimination of exotic plants should increase habitat for fish (Bain & Boltz, 1992). Growth and reproduction of fish may be more due to general metabolic stimulation of benthic microorganisms and subsequent greater availability of fish food stock than a precise control of the amount of habitat available (Sarkar, 1991).

4.3.1.4 Effects on Endangered Plant Species

The current literature does not discuss the effects of 2,4-D on endangered species. However a few general comments can be made. 2,4-D is normally applied as a granule (2,4-D BEE) or at or below the water surface (2,4-D DMA); thus accidental "drift" exposure to upland vegetation during application would be minimal with the exception of emergent aquatic plant communities bordering the treated area. If any proposed "sensitive" plants or candidate species under review for possible inclusion in the state list of endangered or threatened species occurs along the banks of waterways to be treated with 2,4-D products, the applicator should leave a protective buffer zone between the treated area and the species of concern (Ecology, 1989). Sensitive upland plant species could potentially be damaged if treated water was improperly used for irrigation or extensive flooding from irrigation canals treated with 2,4-D 2EHE or 2,4-D DMA occurred before herbicide degradation had occurred. Use of treated water for irrigation is normally prohibited for the Aqua-Kleen® and Navigate® products. To protect endangered aquatic plants, some knowledge must be gained on the toxicity of 2,4-D to these plants, or 2,4-D must not be applied in areas that will impact the habitat or population of these plants adversely. In the case of threatened aquatic plants, the Endangered Species Act does not allow for the control of noxious weeds to take precedence over the protection of endangered species. However, if conditions indicate that removal of noxious weeds will improve habitat for threatened/endangered plant

species, removal of the noxious species by chemical or other means should be considered. The permit for treatment of water bodies to control noxious or invasive plants may be denied or amended if "Ecology" believes that populations of plants may be adversely impacted by treatments to control these weeds (McNabb, 1999 and Dorling, 1999 personal communications).

Endangered plant species that are either fully aquatic, palustrine or riparian are as follows: Ute Ladies' Tresses, Golden paintbrush, and Nelson checker mallow are the only terrestrial species of native plants that are currently listed as endangered in the State of Washington; water howellia and marsh sandwort are the only species of aquatic plants that are currently listed as endangered in the State of Washington.

4.3.1.5 Risk Analysis for Aquatic Species of Plants

It is not standard procedure to conduct a Risk Assessment with a herbicide for aquatic plants and algae. Since blue-green algae are often important for nitrogen fixation, it is important that the risk be low for these species. Although *Anabaena flos-aquae* is at high risk (R = 2.28) when exposed to 2,4-D BEE based on laboratory experiments, low solubility and rapid hydrolysis leads low risk (RQ undeterminable) from 2,4-D acid), and may mitigate the effects of 2,4-D BEE to this species. *Anabaena flos-aquae* is not extensively important in the nitrogen fixation process. However, *Anabaena doliolum* and *Nostoc spp*., which are important to nitrogen fixation, are at very low risk (RQ = <0.1) when exposed to 2,4-D acid. Therefore, nitrogen fixation is not likely to be disrupted by use of 2,4-D BEE.

The most sensitive species of diatom appear to be at risk from exposure to all commercial products of 2,4-D (R = >0.67). However, field studies do not confirm this observation; 2,4-D DMA has been observed to cause, directly or indirectly, increased growth of the diatoms, *Melosira spp*. and *Synedra spp*. in the field.

Green algae do not appear to be at high risk from the exposure to 2,4-D. Although the most sensitive species (*Selanastrum capricornutum*) is moderately at risk (RQ = 0.17) from exposure to 2,4-D BEE, the low solubility of 2,4-D BEE and rapid conversion to 2,4-D acid mitigates this effect so that low risk (RQ = 0.07) based on Petersen's scale is more likely.

Very little work has been done with aquatic macrophytes, but 2,4-D would be expected to cause high risk to most species including *Lemna gibba* and *Synapsis alba* (RQ = >1.0). However, 2,4-D can be very selective in the field. Concentrations that will greatly reduce the biomass of Eurasian watermilfoil have been shown in the field to spare pondweed, American waterweed, *Vallisneria spp*., water stargrass, and *Chara spp*. or allow for their rapid recovery. Low use rates of 2,4-D can control Eurasian watermilfoil while allowing sago pondweed to recover by the end of the season.

In experiments designed to mimic the field situation, *Myriophyllum spicatum* (Eurasian watermilfoil) is susceptible to concentrations of 2,4-D acid that are lower (1.0 to 2.0 mg a.e./L) than the expected EEC (2.9 mg a.e./L) when the exposure time is longer than 36 hours (Green & Westerdahl, 1988). Exposure of Eurasian watermilfoil to field concentrations of 2,4-D as low as 1.26 mg a.e./L at the surface and 4.0 mg a.e./L near the at the bottom of the water column, provided control in British Columbia lagoons in cases

with no input from lotic systems. However, at other sites treated in a similar manner, and Eurasian watermilfoil control was highly variable.

Other laboratory studies with sago pondweed (*Potamogeton pectinalus*) treated with up to 2.0 mg /L showed that this species of native pondweed is capable of withstanding this concentration of 2,4-D that will control Eurasian watermilfoil (Sprecher et al, 1998). With treatment early in the year, milfoil is expected to be rapidly controlled with subsequent re-growth of damaged sago pondweed from tubers and rhizomes as well as plants. Helsel (1996) found similar effects in the field when Beulah Lake, Wisconsin was treated for control of Eurasian watermilfoil. Parsons, et.al. (1998) found that use of 2,4-D to control Eurasian watermilfoil had little or no adverse impact on other species indigenous to Loon Lake, Washington. The evidence from these laboratory and field studies is that after Eurasian watermilfoil is eliminated from the aquatic habitat that native species will regrow rapidly and quickly dominate the habitat.

4.3.2 Effects of 2,4-D on Aquatic Animals

*Summary: 2,4-D DMA is generally safe to fish, free-swimming aquatic invertebrates and benthic invertebrates. E.g., 2,4-D DMA is practically non-toxic to fish and free-swimming aquatic invertebrates (acute LC50 = >100 mg a.i./L). However, some of the more sensitive species are benthic invertebrates like estuarine shrimp (*Palaemonetes spp.*) and seed shrimp appear to be acutely sensitive to 2,4-D DMA (acute LC50 0 0.15 to 8.0 mg a.i./L for estuarine shrimp and seed shrimp respectively).*

Although 2,4-D DMA appears to be safe for use in aquatic ecosystems, 2,4-D BEE has a very high acute toxicity to the aquatic biota (acute LC50 = 0.3 mg a.i./L for rainbow trout, Daphnia magna *(~4.0 mg a.i./L) or bright scud (0.44 mg a.i./L). Concentrations of 2,4-D BEE would appear to be high enough for adverse impact to the aquatic biota, but its low solubility and rapid hydrolysis to the slightly to practically non-toxic 2,4-D acid mitigates 2,4-D BEE's toxic effects. 2,4-D acid appears to be practically non-toxic to fish and free-swimming invertebrates (LC50 = 20 to >100 mg a.i./L). However, while 2,4-D acid has a low toxicity to most species of benthic invertebrate (LC50 = >37 mg a.i./L to Cyclops vernalis and others), the most sensitive species (*Gammarus fasciatus*) is affected moderately by 2,4-D acid (LC50 = 3.2 mg a.i./L).*

The chronic toxicity (NOEC) for 2,4-D DMA is also low with the predicted or empirical long-term NOECs ranging from 5.56 mg a.i./L for rainbow trout to 27.5 mg a.i./for Daphnia magna*. The more sensitive benthic species appear extremely sensitive to chronic exposure to 2,4-D DMA (estimated chronic NOEC = 0.0083 mg a.i./L for glass shrimp), although for 80 of the species tested 2,4-D DMA can be classified as chronically non-toxic.*

Similar to the acute effects, 2,4-D BEE appears to be toxic to the biota (predicted or empirical long-term NOEC = 0.017 mg a.i./L for rainbow trout to 0.29 mg a.i./L to Daphnia magna*). However, Risk Assessments would indicate that these NOECs are higher than typical long-term EECs 0.010 mg/L; and therefore risk should be low for fish and free-swimming aquatic invertebrates. However, while the predicted NOEC (0.024 mg a.i./L) for the most sensitive benthic organisms is low enough that adverse impact may be avoided from exposure in the water column, sediment exposure may be high enough to cause adverse impact.*

Since 2,4-D BEE appears to have low chronic toxicity to the aquatic biota, it is likely 2,4-D acid, which is known to have low acute toxicity to the aquatic biota, will also have low chronic toxicity to the aquatic biota. The predicted or empirical long-term NOEC for 2,4-D acid is 1.1 mg a.e./L for the most sensitive species of fish (common carp), ~30 mg a.e./L for Ceriodaphnia dubia *and 0.18 mg a.e./L for* Gammarus fasciatus. *While these values indicate some toxicity, Risk Assessments indicate that these NOECs are well above the chronic EEC values likely to be encountered in the field (0.01 mg /L for water and 0.06 mg/L for sediment). Field studies with 2,4-D acid at maximum use rate, while eliminating milfoil allowed tolerant macrophytes like water celery and American waterweed to dominate the water body for up to two growing seasons.*

Laboratory exposure of Coho, sockeye, and pink salmon at a rate of 1.0 mg/L for 24 hours does not appear to interfere with the parr to smolt metamorphosis. Furthermore, exposure of Coho salmon at concentrations up to 200 mg/L also does not appear to interfere with the parr to smolt metamorphosis. Although other anadromous fish species like steelhead or sea-run cutthroat trout have not been tested for their ability to osmoregulate after exposure to 2,4-D and transfer from fresh to salt water or visa versa, based on the work done with salmon smoltification, this is not believed to be a problem.

Behavioral effects have been observed with 2,4-D DMA and 2,4-D BEE. Rainbow trout have been observed to avoid 2,4-D DMA at concentrations that would be encountered in the field (1 to 10 mg/L). Avoidance of 2,4-D BBE has been observed with grass shrimp, sheepshead minnow and mosquito fish. Absence of Uca uruguayensis *from areas treated with 2,4-D iso-BEE may also indicate that this species is capable of avoiding 2,4-D. However, it is unclear if fish or invertebrates would or could avoid 2,4-D in actual field situations.*

Field studies indicate that treatment with 2,4-D DMA appears to have no direct effects on numbers or diversity of free-swimming or benthic invertebrates in ponds or ditch banks. However, secondary effects such as oxygen depletion and the release of nutrients into the water column due to treatment with 2,4-D BEE can have significant impact (positive or negative) on zooplankton and benthic invertebrates. Reduction of dissolved oxygen concentration to nearly zero for one week does not affect the numbers or diversity of benthic organisms, but may cause a shift in the dominant organisms from obligate aerobes like Odonata *and* Ephemeroptera *to facultative anaerobes like Oligochaete worms and* Tendipedidae *(midge). Treatment with 2,4-D acid at levels higher than 0.38 mg/ha/month for 12 months may cause significant increases (~20%) in the biomass of the benthic biota and a short-term depression of phytoplankton populations. These changes in biomass of benthic organisms and plankton can also produce changes in the survival and biomass of associated fish. Bottom feeding fish have increased survival and increased yield (biomass) since their nutrition has been improved by increases in benthic organism biomass. Planktovoric fish have a corresponding decrease in survival and biomass due to decreases in the levels of phytoplankton and zooplankton. Other field studies using 6.0 Kg/ha 2,4-D acid, were observed to increase nutrients within the water body and caused substantial increases (>2-fold) heterotrophic bacteria, and zooplankton in less than eight weeks of phytoplankton. However, the levels of sediment associated bacteria appeared to decrease substantially.*

Fish species like largemouth bass, sunfish and others are not adversely affected by typical field concentrations of 2,4-D DMA. There was no adverse effect on numbers (including recreational or commercial fish catch) and no adverse effect on mean total

length, condition, movement within the treatment area or nesting behavior. Although the use of 2,4-D BEE should have an adverse impact on fish and aquatic invertebrates based on the results of laboratory studies, field studies indicate that, under the conditions of typical application, fish do not appear to be adversely impacted.

Sensitive, endangered and threatened species of aquatic animals that may need protection through mediation include Coho salmon, chum salmon (summer chum), Chinook salmon, sockeye salmon, bull trout, steelhead trout, cutthroat trout, Coastal cutthroat trout, Olympic mudminnow, mountain sucker, lake chub, leopard dace, Umatilla dace, and river lamprey. Other species which may need protection within Puget Sound, the San Juan Islands, and the Strait of Juan de Fuca east of the Sekiu River are Cherry Point Herring, Discovery Bay Herring, and South Pacific cod.

2,4-D applications to fully aquatic (lentic and lotic) systems may be toxic to aquatic animals (Table 22). 2,4-D DMA will generally be safe to aquatic animals (LC50 = >25 to >748 mg a.i./L = >21 to >620 mg a.e./L) for most ecologically relevant species. However, direct contact with 2,4-D BEE would be unsafe to most aquatic animals (LC50 = <4.0 mg a.i./L = <2.8 mg a.e./L). The World Health Organization recommends that 2,4-D BEE be assessed for risk based on the toxicity of 2,4-D acid since 2,4-D BEE is rapidly degraded to 2,4-D acid. The half-life for 2,4-D BEE in its degradation to 2,4-D acid is considered to be less than one day with the rate of degradation being more rapid in hard basic waters common to eastern Washington. Although 2,4-D BEE is less toxic to salmonids in hard /basic water (1.1 to 4.3 mg/L) than in soft/acid water (0.8 to 1.1 mg/L), the difference is not so great as to afford significant protection to salmonid species due to pH and hardness alone (Table 10). Additional protection from 2,4-D BEE may be due to its low solubility. Low solubility would lead to a low incidence of contact by aquatic organisms when 2,4-D BEE granules are used for aquatic vegetation control. Although laboratory tests indicate some risk to salmonids from exposure to 2,4-D BEE, field data from TVA reservoirs, Currituck Sound, NC, northeastern water and northwestern water are uniform in their appraisal of no direct toxic effects as a result of 2,4-D BEE treatments.

Although these general trends apply, there are always some exceptions for every formulation. For example, 2,4-D DMA is apparently very toxic to several species of estuarine shrimp including *Palmaemonets kadiakensis* (glass shrimp) (LC50 = 0.15 mg a.i./L = 0.12 mg a.e./L), *Cypridopsis vidua* (seed shrimp) (LC50 -= 8.0 mg a.i./L = 6.64 mg a.e./L) and possibly *Palmaemonets pugio* (grass shrimp) based on phylogenic similarity. Conversely, 2,4-D BEE does not appear to be very toxic to a variety of arthropod shellfish such as the *Orconectes nous* (crayfish) (LC 50 = 100 mg a.i./L = 69 mg a.e./L) and adult estuarine crabs (*Chasmagnathus granulata* and *Uca uruguayensis*) (LC50 = 130 mg a.i./L = 90 mg a.e./L). Similarly to 2,4-D DMA, 2,4-D acid while not toxic to most aquatic animals appears to be extremely toxic to the lined scud (*Gammarus fasciatus*) (3.2 mg a.i./L). Some of these exceptions have the potential for great ecological relevance, particularly when sediment species are involved. Since the database on these species is fragmentary they often do not respond in a manner similar to model pelagic arthropods like *Daphnia magna* which are often used as surrogates for toxicity studies with sediment organisms.

2,4-D and its formulations have a low tendency to bioaccumulate except in the case of zooplankton and benthic organisms. For zooplankton and benthic organisms, the bioconcentration factor for 2,4-D BEE has been shown to be 1 to 603 and 8,267 to

10,825, respectively in the Ft Cobb Reservoir, Oklahoma (Reinert and Rogers, 1987). However, similar concentrations were not found in fish 24 hours after treatment in Lake Seminole; Georgia. Laboratory work indicates that while 2,4-D BEE may bioconcentrate to fairly low levels in aquaria, (2 to14 in channel catfish and 6 to 21 in bluegill sunfish) (Rogers and Stallings, 1972 in Reinert and Rogers, 1987), 2,4-D BEE was rapidly hydrolyzed to the acid and excreted from these fish. 2,4-D acid and 2,4-D DMA apparently do not bioconcentrate or bioaccumulate (Biever, 1996, Biever, 1998, Plakas et al, 1992 and Gangstad, 1986). The accumulation in benthic organisms that are in some cases affected by 2,4-D BEE at concentrations that may be below the acute or chronic EEC for sediment is of potential concern. At least one species of sediment organisms (*Gammarus fasciatius*) is apparently acutely susceptible to 2,4-D acid which is less toxic than 2,4-D BEE to most species. This is of particular concern when the ratio of 2,4-D BEE to 2,4-D acid is not known and not easily predicted. However, the effects of bioaccumulation are not expected to be significant in the long term for most species. See Section 4.2.2.4 for a more detailed discussion on potential for bioaccumulation or bioconcentration in fish, aquatic, invertebrates, phytoplankton and zooplankton, birds, mammals and insects.

2,4-D BEE is applied by itself from a hopper spreader and is not combined in a tank mix with other pesticides. While 2,4-D DMA is combined with other herbicide products in some cases this is not normal in Washington State. In some cases, 2,4-D acid has been combined with other pesticides to determine if the effects of the combinations were greater than additive. Only one species of animal has been studied for synergistic effects of 2,4-D acid and the insecticides, malathion and carbaryl. Combinations of 2,4-D and these insecticides have had synergistic effects on behavioral responses with the brown planaria (*Dugesia tegrina*) (Feldhaus et al, 1998). It is unknown whether other more relevant species would exhibit synergistic effects if 2,4-D products were combined with adjuvants or other pesticides. Sub-acute and chronic effects have been studied with 2,4-D in the common carp, fathead minnow, sheepshead minnow, rainbow trout, and the grass shrimp. The sub-acute effects of 2,4-D have been seen at both environmentally relevant and non-relevant concentrations. For example, rainbow trout avoid 2,4-D DMA at concentrations as low as 1.0 to 10.0 mg/L (Folmar, 1976), and grass shrimp avoided 2,4-D BEE at concentrations as low 1.0 to 10 mg/L (Hansen et al, 1973). Avoidance may cause fish to move to marginal habitats, which may cause mortality due to predation or disease/parasites. However, authors of papers studying avoidance indicated that it was unlikely that animals exposed to 2,4-D in the field would or could avoid exposure. The estuarine sheepshead minnow and mosquito fish also avoids 2,4-D BEE (Hansen, 1969 in Hansen et al, 1973 and Hansen et al, 1972 in Shearer and Halter, 1980). Carp larvae exhibited behavioral changes, disturbances in feeding and morphological changes at 50 mg a.i. 2,4-D sodium salt (Kamler et al, 1974). The common carp is the only species that has had extensive work conducted on the acute, chronic and sub-chronic effects of 2,4-D.

Since 2,4-D is excreted by fish unmetabolized, classical synergism, with metabolic inhibitors is unlikely to occur. However, the presence of accelerators/surfactants, other "inerts", or other pesticides in either tank mix situations with 2,4-D DMA or incidental exposure from treatment with other pesticides may increase the potential for damage to the biochemistry or physiology of fishes. These potentiating effects could increase, acute or chronic (early life-stage) toxicity or increase the biochemical or pathological effects of 2,4-D in fish exposed to sub-acute dosages. A number of sub-acute effects have been noted due to the exposure to 2,4-D including apparent increase in the toxicity of 2,4-D sodium salt due to the presence of 2,4-chorophenol as a contaminant (Kamler et al,

1974). Behavioral effects such as avoidance may be potentiated or inhibited by the presence of pesticides other than 2,4-D. Various biochemical effects that are usually manifestations of physiological stress were also seen in the carp by Neskovic et al (1994) and Elezovic et al (1994) including increases in blood serum and liver glucose and glycogen levels and blood serum glutamide oxaloacetic transminase activity. Pathological changes in the tissues of common carp included vacuolization and the formation of pycnotic nuclei in the liver, edema and vacuolar degeneration of the tubular epithelial cells of the kidney, and edema and other changes in gill tissue resulting in the thinning of the respiratory epithelium. Additional pathological changes were also seen in the gill tissue (Neskovic (1994). The tench (*Tinca, tinca*) was observed to have lesions in the excretory parenchyma of the kidney, which led to necrosis following exposure of fish to 2,4-D (Larraine et al, 1999). 2,4-D is primarily excreted via the kidney and across the gill membrane (Rogers and Stalling (1972I in Gallagher, 1992). However, most of these effects can be considered to be of little importance in absence of environmental assault from sources other than the presence of 2,4-D acid at typical expected environmental concentrations (EECs) of 0.19 to 4.0 mg a.e./L. Typical EEC concentrations are much lower than the 250 to 400 mg a.e./L tested in sub-acute toxicity studies. To discover the long term effects of 2,4-D at environmentally relevant concentrations would require the conduct of multigenerational laboratory experiments with species considered to be ecologically sensitive.

Accelerators and thickening agents are rarely used with herbicides sprayed directly on the surface of a water body, but some applicators and scientists believe that surfactants like CideKick® and X-77® improve effectiveness and should be used with 2,4-D DMA products when surface (floating) weed control is necessary (Getsinger, 2000 personal communications). A thickener like Nalquatic® or Polysar® will often be used to allow a subsurface application to sink down into the water column where it will be most effective against rooted aquatic macrophytes. If the herbicide is sprayed on, thickeners also control potential drift. Although all adjuvants registered for use with aquatic herbicides should be safe to fish and other aquatic animals when used according to the label, they are not without risk to aquatic life (Watkins et all, 1985). Their 96-hour toxicity (LC50) ranges from 0.96 mg/L to > 1000 mg/L. In lakes and ponds with reasonable depth, dilution should prevent toxic effects from occurring due to the use of additives. This is particularly so if the control measure is a spot or margin treatment. A more detailed discussion of the effects of adjuvants can be found in Section 4.2.4 and in Table 12.

4.3.2.1 Acute Effects on Aquatic Animals

- **Acute effects on fish**

  Toxicity information indicates that the commercial product 2,4-D DMA is not acutely toxic to the species of fish tested (Table 2 and Table 22 and Appendix 1); that is it has an LC50 of greater than 100 mg/L (Table 12 and Appendix 2). 2,4-D DMA has a 96-hour LC50 that ranges from 100 to >560 mg a.i./L for all tested species including trout and salmon. (100 to 377 mg a.i./L), bluegill sunfish (106 to 524 mg a.i./L), smallmouth bass (236 mg a.i./L), fathead minnow (266-344 mg a.i./L), Cyprinid carp (>100 to >1000 mg a.i./L), channel catfish (119-193 mg a.i./L) and the estuarine inland silverside minnow (469 mg a.i./L). In the case of the rainbow trout, a species known for great sensitivity to pesticides, fry and juvenile tests still yielded low toxicity to 2,4-D DMA (>100 mg a.i./L).

Based on these LC50s, 2,4-D DMA can be placed in the ecotoxicological risk category of practically non-toxic (LC50 > 100 mg a.i./L). This risk category classification does not mean that 2,4-D DMA will not have an adverse impact to fish when they are exposed to the expected environmental concentration. This determination of risk compares the general toxicity of 2,4-D DMA with other registered pesticides; based on this comparison, 2,4-D has a very low acute toxicity.

The application rate for 2,4-D DMA in the United States to control aquatic macrophytes typically ranges from 2 to 4 mg a.e./L (2.4 to 4.8 mg a.i./L) (JMPR, 1997). Typical use rates in the United States are much less. WHO/FAO estimates that the typical use rate would be 1.13 mg a.e./L (1.36 mg a.i./L). Therefore, aquatic biota should be largely unaffected by these treatments.

The other commercial 2,4-D product registered and the one of primary interest in Washington State is 2,4-D BEE (Aqua-Kleen® and Navigate®). The acute toxicity of this product to fish is fairly high. However, due to its rapid degradation to 2,4-D acid researchers feel it is safe to use in the aquatic environment, except where sensitive threatened or endangered species are present and then an assumption should be made for higher risk due to use of the herbicide.

Based on the toxicity of 2,4-D BEE to fish this 2,4-D ester is placed in the Ecotoxicological Risk Categories of highly toxic (0.1 to 1 mg/L) for salmon fry and smolts, moderately toxic (>1 to 10 mg/L) for salmonid juveniles, catfish juveniles, fathead minnow juveniles and bleak, and categories ranging from highly toxic (0.1 to 1 mg/L) to moderately toxic (>1 to 10 mg/L) for bluegill sunfish. The exact acute categories are not of great importance since the LC50 exceeds expected concentrations in the environment. However, exposure to 2,4-D BEE is likely to be negligible due to its low solubility and rapid conversion to 2,4-D acid even though the concentrations immediately after application ranges from 0.19 mg/L at the surface to 3.25 mg/L at the bottom in the root zone.

When the level of concern (0.1) is exceeded so dramatically with all species, the use of the compound would not be acceptable unless mitigating factors could be considered. An acceptable mitigating factor would be to follow the WHO/FAO suggestion that 2,4-D acid be considered the toxin of concern for the reasons elaborated above.

After hydrolysis of 2,4-D BEE, 2,4-D acid is not significantly toxic to the fish species tested; that is the LC50 is typically >40 mg a.e./L for all environmentally relevant species. Based on the toxicity of 2.4-D acid to fish, it is placed in the Ecological Risk Category of slightly toxic (>10 to 100 mg/L). Therefore, 2,4-D acid and 2,4-D BEE are both unlikely to be acutely toxic to the resident fish biota. A formal risk assessment in Section 4.3.2.5 supports the conclusions of this toxicity review. For a detailed risk assessment and evaluation of potential risk of 2,4-D DMA, 2.4-D BEE and 2,4-D acid on fish, see Section 4.3.2.5.

- **Acute effects on aquatic invertebrates**

  Toxicity information indicates that the commercial product 2,4-D DMA is not acutely toxic to most species of invertebrates tested (Table 2 and Appendix 2). Exceptions to this appear to be with sediment (benthic) organisms like glass shrimp (*Palaemonetes kadiakensis*) and seed shrimp (*Cyridopsis vidua*). Another species, which may be sensitive, is the grass shrimp (*Palaemonets pugio*) based on phylogenic similarities. 2,4-D DMA LC50s that range from >100 to >1,000 mg a.i./L for all free-swimming invertebrate species tested except the sediment (benthic) organisms, which have LC50s that range from 0.15 to 8.0 mg a.i./L.

  Based on these LC50s, 2,4-D DMA can be placed in the ecotoxicological risk category of practically non-toxic LC50 > 100 mg/L for the pelagic (nektonic) aquatic invertebrates and highly toxic (LC50 = 0.1 to 1.0 mg/L) to moderately toxic (LC50 = >1.00 to 10 mg/L) for sediment organisms. This risk category does not imply that 2,4-D DMA will or will not have an adverse impact on these specific groups of invertebrates when they are exposed to the expected environmental concentration. However, this determination of risk compares the general toxicity of 2,4-D DMA with other registered pesticides; based on this comparison, 2,4-D DMA has a very low acute toxicity to free swimming invertebrates and a fairly high toxicity to benthic invertebrates. The labeled application rate for 2,4-D DMA to control aquatic macrophytes in the United States typically ranges from 2 to 4 mg a.e./L (2.4 to 4.8 mg a.i./L) (JMPR, 1997). Typical use rates in the United States are much less than this and WHO/FAO estimates that this use rate would be 1.13 mg a.e./L (1.38 mg a.i./L). Typical environmental concentrations (1.36 mg a.i./L) will probably not affect free-swimming invertebrates since they are well below the LC50s (> 100 mg a.i./L) for this segment of the biota. However, these environmental concentrations will probably affect the most sensitive benthic invertebrates since they far exceed the LC50 (0.15 mg a.i./L) for the most sensitive benthic invertebrates (glass shrimp). Even if a very liberal Federal drinking water standard is used as being typical of pore water or over-lying water concentrations, the most sensitive benthic species may still be affected by environmental concentrations of 2,4-D DMA since the LC50 exceeds the EEC by only two-fold. Even though 2,4-D DMA does not partition significantly to the sediment layer, enough of the herbicide may reach the sediment under heavy treatment scenarios to adversely affect the most sensitive species of benthic organism. For example, Wojtalik et al, (1971 in Shearer and Halter, 1980) found 0.100 to 0.450 mg/L 2,4-D in the Jagger Branch of the Gunthersville Reservoir, Alabama for up to three months after heavy treatment.

  It appears likely that 2,4-D DMA will have adverse impact to benthic invertebrates even if further analysis is conducted. However, depending on half-life considerations 2,4-D DMA may prove to be safe to the free-swimming (zooplankton) biota. The concentrations of 2,4-D DMA found in water shortly after treatment can vary considerably depending on the treatment rate, rate of uptake and release from plant material and mass of water movement through the treatment area. For example, the 2,4-D concentrations seen in water of TVA reservoirs can vary from virtually zero (0.05 to 0.5 mg/L) 24 hours after treatment to 5 mg/L five days after treatment with the variability being primarily due to the amount of water exchange that occurred (Shearer and Halter, 1980). For a determination of risk see Section 4.3.2.5.

The other 2,4-D product with aquatic use and the one of primary interest in Washington State is 2,4-D BEE (Aqua-Kleen® and Navigate®). The acute toxicity of this product to fish and aquatic invertebrates is fairly high. However, due to 2,4-D BEE's low water solubility and rapid degradation to 2,4-D acid in water, researchers feel it is safe to use in the aquatic environment.

2,4-D BEE has the highest toxicity of all the 2,4-D formulations on acute basis. However, this does not take into account special characteristics of the 2,4-D BEE formulation. 2,4-D BEE is a slow release formulation and when properly applied the concentrations in the water column will range from 0.19 mg/L at the surface to 3.25 mg/L at the bottom in the root zone. Furthermore, 2,4-D BEE is rapidly hydrolyzed to 2,4-D acid. The hydrolysis of 2,4-D BEE is usually less than one day and the acid degrades to non-toxic constituents. Under these conditions the persistence of 2,4-D BEE in Canadian waters at concentrations of greater than 0.1 mg/L was 2 to 6 days (Gallagher, 1992). Due to the low solubility, short hydrolysis time and rapid degradation of 2,4-D BEE to 2,4-D acid, WHO/FAO (JMPR, 1997) recommends that the toxicity of 2.4-D acid is more relevant to actual exposure.

Based on the toxicity of 2,4-D BEE to free-swimming zooplankton this 2,4-D ester is placed in the ecotoxicological risk categories of highly toxic (0.1 to 1 mg/L) for estuarine crab, scuds and chironomids, moderately toxic (1.0 to 10.0 mg/L) for *Daphnia magna*, various species of estuarine shrimp, *Cypridopsis vidua*, stonefly, eastern oyster and the copepod (*Nitocra spinepes*), slightly toxic (10 to 100 mg/L) for the crayfish and practically nontoxic (>100 mg/L) for the juvenile and adult estuarine crabs and adult stoneflies.

The exact toxicity categories may not be of great importance since the evaluation presented here does not constitute a risk assessment and exposure to 2,4-D BEE is likely to be negligible for most species even though potential exposure concentrations exceed the LC50 in most species tested. Furthermore, *Daphnia magna*, because of its habits, will be exposed to high concentration of 2,4-D (3.25 mg a.i./L) when seeking shelter from predators, but while feeding and during most normal activity, this species is exposed to a very low EEC (0.19 a.i./L) which may not be toxic to this species since the LC50 is 4.0 mg a.i./L).

The most sensitive species appear to benthic and sediment invertebrates to which 2,4-D BEE is extremely toxic. For example, *Gammarus spp*. has an LC50 of ~0.44 mg a.i./L, *Chironomus plumosus* has an LC50 of ~0.40 mg a.i./L; the estuarine crab (1st zoel) (*Chasmagnathus granulata*) is the most sensitive species with an LC50 of 0.3 mg a.i./L. These benthic and sediment species will not be protected from 2,4-D BEE unless mitigating circumstances occur. This information may place these species at risk since under conditions of poor time release, the sediment concentration of 2,4-D BEE can be high (from 0.95 to 56 mg a.i./L) for at least 4-days post treatment (Shearer and Halter, 1980).

When environmental concentrations exceed the LC50 so dramatically with all species, the use of the compound would not be acceptable unless mitigating factors could be considered. Fortunately, this is not considered to be a typical exposure scenario. Concentrations of 2,4-D generally dissipate in water to levels of 0.100 mg/L with a half-life of less than six days (Gallagher, 1992). More typical residue levels in sediment were much lower than previously described. E.g., concentrations

of 2,4-D from application of 2,4-D BEE pellets were approximately 0.100 mg/L for one week at Lake Seminole, Georgia (Whitney et al, 1973 in Shearer and Halter, 1980) and 0.200 to 0.650 mg/L for three weeks at Currituck Sound, North Carolina (Daly, 1974 in Shearer & Halter, 1980). In the Northwest (Lake Okanogan, B.C.), concentrations of 2,4-D were measured at 0.050 to 0.460 mg/L immediately after treatment and residues remained present at day 8 (Lim and Lozoway, 1978). These concentrations are far more acceptable. But even these more reasonable rates, the most sensitive species may not be protected. A formal risk assessment in Section 4.3.2.5 supports the conclusions in this toxicity review.

However, at Lake Okanogan, B.C., the most sensitive species (*Gammarus fasciatus*) may be protected since the LC50 is significantly higher than the EEC. The most strict interpretation of risk would still find this EEC level to pose a potential risk to the invertebrate biota. It is useful to note that 2,4-D sodium salt appeared to be somewhat less toxic than 2,4-D acid to the species tested and the LC50s for this salt are great enough so that the risk quotient level of concern are not expected to exceed (LC50 = 932 to 2400 mg a.m./L for *Daphnia magna* and *Macrobranchium spp*., respectively. Therefore, using the 2,4-D salts as surrogates for the acid may add valuable information in assessing acute risk of 2,4-D against aquatic invertebrates.

4.3.2.2 Chronic Effects of 2,4-D on Aquatic Animals

- **Chronic effects on fish**

To this date, the amount of chronic or early life-stage effects data for 2,4-D on aquatic animals (fish) is rather minimal (Table 2 and Appendix 3). Most studies deal with early life-stage (egg, egg to sac-fry, egg to fry). There are studies that deal with the early life stage (egg to fry) toxicity of 2,4-D BEE in the Chinook salmon, with early life-stage (egg to fry) toxicity of 2,4-D DMA, 2,4-D BEE, 2,4-D 2-EHE and 2,4-D acid in fathead minnow, and a 10 month life-cycle toxicity study of 2,4-D BEE with fathead minnow (Table 24).

Not all of the above listed studies are of good enough design to pass current EPA guidelines as early life-stage studies. The most sensitive and well-designed studies are an egg-fry Chinook salmon study with 2,4-D BEE (Finlayson & Verrue in Ecology, 1989) and egg-fry fathead minnow studies by DOW (1990 in Brian, 1999). The studies conducted by Hiltibran (1967) provide good supplemental data but were not conducted for a long enough period to be considered chronic studies. However, Hiltibran's data will be considered definitive if it is the only data available with a particular product and a particular species. In these studies the NOECs ranged from 17 to 40 mg a.i./L with 2,4-D DMA. The EEC for 2,4-D DMA is less than 4.8 mg a.i./L since the highest concentration at the time of application on a United States site could be no higher than the maximum use rate. Since the NOEC is higher than the EEC, our most credible studies indicate that these species should not be affected by proper use of 2.4-D at the maximum use rate.

However, since the database is so small, one cannot say that no credible risk exists for chronic exposure of fish to these products. Further research to expand this database on the chronic toxicity of 2,4-D DMA to fish needs to be conducted to give the chronic toxicity NOECs greater credibility. Typical tests that would be conducted are early life-stage tests with fathead minnow, rainbow trout and sheepshead

minnow. These species are easy to rear in the laboratory and the procedures for conducting early life-stage tests are accepted by state and federal regulatory agencies.

With 2,4-D BEE, the amount of chronic data that has been generated is extremely limited. Only a few studies with fathead minnow and Chinook salmon have been conducted. The range of NOECs for fish was 0.040 mg a.i./L for Chinook salmon in an 86-day early life-stage tests to 0.3 mg a.e./L in a 10 month life-cycle test with fathead minnow. In field residue studies with 2,4-D BEE, the residue levels started out at concentrations that were 0.19 mg/L at the surface of the water column and 3.25 mg/L in the root zone (bottom of water column) after treatment with 2,4-D BEE, but had decreased to <0.100 mg/L within two to six days. Within 5 to 22 days, the concentration in treated open waterways of the Okanogan Valley had decreased to <0.001 mg/L [Canadian ministry of the Environment (1980 in Gallagher, 1992)]. This concentration can be considered the EEC for public waterways. Since long term concentrations in the environment are much lower than the NOEC concentration, these species would probably not be adversely affected. However, it is difficult to draw conclusions on safety to the biota. Early life-stage tests conducted with species of known sensitivity like rainbow trout, fathead minnow and sheepshead minnow would improve the credibility in determination of risk.

Determination of the chronic toxicity of 2,4-D acid is not necessary since chronic toxicity of the commercial aquatic products is low enough for the protection of the fish biota. However, the chronic NOECs for the acid range from 29 mg a.i./L for the Medaka and 63 mg a.i./L for the fathead minnow. The NOECs are difficult to determine in surrogate studies done with 2,4-D sodium salt and potassium salt because they were not standard statistical values but LC1s for tests with rainbow trout, goldfish and largemouth bass. However for the most sensitive species (rainbow trout) the LC1 for an egg toxicity test was ~0.027 mg a.i./L (0.025 mg a.e./L). Since the long term NOEC is greater than the EEC <0.001 mg/L risk to these fish species is probably low. However, early life-stage tests conducted with species of known sensitivity like rainbow trout, fathead minnow and sheepshead minnow would improve the credibility of this risk analysis. The formal risk assessment in Section 4.3.2.5 supports the conclusion of this toxicity review.

- **Chronic aquatic invertebrate toxicity**

The amount of data that has been generated on life-cycle effects of 2,4-D against aquatic invertebrates is minimal (Table 2 and Appendix 4). Twenty-one day life-cycle tests have been conducted on 2,4-D DMA, 2,4-D 2-EHE, 2,4-D BEE and 2,4-D acid with *Daphnia magna*; also 4 and 7 day life-cycle tests have been conducted on 2,4-D acid with *Ceriodaphnia dubia*; and 28 day chronic tests have been conducted on 2,4-D Iso-BEE with the estuarine crabs (*Chasmagnathus granulata* and *Uca uruguayensis*).

The life-cycle NOEC for 2,4-D DMA is 27.5 mg a.i./L on Daphnia magna. This is well above the maximum exposure rate of 4.8 mg a.i./L (4.0 mg a.e./L) expected after the initial application of 2,4-D DMA. The EEC for 2,4-D DMA is less than 4.8 mg a.i./L since the highest concentration at the time of application on a United States site could be no higher than the maximum use rate, 2,4-D DMA is not likely to have chronic affects on Daphnids at typical use rates.

However, since the database is so small one cannot say that no credible risk exists for chronic exposure of invertebrates to 2,4-D DMA. Further research on the chronic toxicity 2,4-D DMA to aquatic invertebrates needs to be conducted to give the life-cycle NOECs greater credibility. Additional tests that should be conducted include life-cycle tests with *Ceriodaphnia dubia* and the mysid shrimp. These species are easy to rear in the laboratory and the procedures for conducting life-cycle studies are accepted by state and federal regulatory agencies.

With 2,4-D BEE, the amount of chronic and life-cycle data that has been generated is extremely limited. Only estuarine crabs (*Chasmagnathus granulata and Uca uruguayensis*)(28-day chronic toxicity studies) and *Daphnia magna* (21-day life cycle studies) have been conducted. The LC50 for 28-day chronic estuarine crab studies was over 50 mg a.i./L for adult crabs; this species appears to be extremely tolerant of 2,4-D Iso-BEE. However, the NOEC for 21-day life-cycle studies with *Daphnia magna* was 0.29 mg a.i./L. In field residue studies with 2,4-D BEE, the residue levels started at concentrations of 0.19 mg/L at the surface of the water column and 3.25 mg/L in the root zone (bottom of water column) after treatment with 2,4-D BEE but had decreased to <0.100 mg/L within two to six days. Within 5 to 22 days, the concentration in treated open waterways of the Okanogan Valley had decreased to <0.001 mg/L [Canadian Ministry of the Environment, 1980 (in Gallagher, 1992)]. This concentration can be considered the EEC for public waterways, which leads to the conclusion that typical use concentration will not affect the species that have been tested. However, the data is extremely limited making it is difficult to draw conclusions on safety to the biota. Additional life-cycle tests conducted with species of known sensitivity like *Ceriodaphnia dubia* or mysid shrimp would improve the credibility of this risk analysis.

Risk analysis for 2,4-D acid is not necessary since chronic toxicity of the commercial products has shown acceptable risk quotients for the protection of the fish biota. However, the life-cycle NOECs range from 26 mg a.e./L in a 7 day *Ceriodaphnia dubia* life cycle test to 79 mg a.e./L in a 21 day *Daphnia magna* life-cycle test. Again using the "Chronic" EEC of 0.001 mg/L allows for the conclusion that typical environmental concentrations will not affect the species tested. However, life-cycle tests conducted with species of known sensitivity like *Daphnia magna, Ceriodaphnia dubia* and mysid shrimp would improve the credibility of this risk analysis. A formal risk assessment in Section 4.3.2.5 supports the conclusion of this toxicity review.

4.3.2.3 Impacts of Single Versus Multiple Applications

It is extremely rare for lakes in Washington State to be treated with 2,4-D products more than once in a season. Therefore, very little practical field knowledge is known on this subject. However, some laboratory work with insects (Ahmed and Ali, 1994) and some fish-pond farm work in India (Sarkar, 1991) to control general weeds involved multiple exposures to 2,4-D. Additional laboratory work indicates that chronic exposure at high concentrations of 2,4-D acid (mimicking multiple exposures) may cause both pathological and biochemical signs of stress in the common carp (Neskovic et al, 1994 & Elezovic et al, 1994) and the tench (Gomez et al (1999). These exposure were very high (150 to 400 mg/L for 12 to 14 days); so levels of acute toxicity (96 hour LC50s~300 mg/L) were being approached in these studies. Such exposures are high and probably unreasonable as a multiple exposure model based on theoretical exposure rates. However, even low exposures may cause additional stress making sensitive species more susceptible parasites, disease, predators, and other pesticides.

Exposure rates that would be typically encountered in the field for 2,4-D DMA and 2,4-D acid do not demonstrate acute or obvious chronic effects (Table 2, Table 23, Appendix 1, Appendix 2, Appendix 3, and Appendix 4) nevertheless, fathead minnow exposed continuously to concentrations of 2,4-D DMA that ranged from 0.12 to 2.0 mg/L exhibited somewhat subtle chronic effects. After exposure for two months, no effects were found on growth, survival, egg production or fry survival, but the exposed animals spawned one month prematurely due to a general increase in metabolic rate as determined by a separate radio-iodine uptake test with the same fish. Other physiological changes noted included a reduction in bone collagen levels (Mayer, et al, 1977 in Shearer and Halter, 1980). The significance of these changes in reproductive timing and bone collagen levels is not entirely understood but is presumably indicative of some degree of chronic physiological stress.

Similar studies conducted with the fathead minnow after exposure to 2,4-D BEE at concentrations ranging from 0.7 to 1.8 mg/L did not effect the histopathology, sexual condition or development time of eggs and fry (Mount and Stephan, 1967 in Shearer and Halter, 1980). Except for mortality of eggs and fry, even the highest concentrations did not adversely affect fathead minnow. The NOEC for this life cycle study was determined to be 0.3 mg/L (Table 2 and Appendix 3). Since the chronic exposure EEC could range between <0.1 mg/L and <0.001 mg/L in Northwestern waters (Gallagher, 1992 cites Canada, 1976), these natural field rates should be safe for chronic exposure of this species.

Cumulative effects of 2,4-D acid on the development time in the southern house-mosquito has also been noted. The effects seem to run counter to those observed in the fathead minnow. The cellular generation time as determined by the mitotic index was observed to increase in mosquito larvae exposed to concentrations as low as 1.0 mg 2,4-D acid/L. This effect at the cellular level resulted in increases in the larval duration time from 180 hours to 200 hours after one generation to 230 hours after three generations with an exposure time of 4-hours per generation (Ahmad and Ali, 1994).

Such differences in the development time between predator and prey species has a potential to produce adverse effects in wild populations. If the spawning time is early for the predator species and the development time is late for the prey species, the prey may not be developed to an appropriate size when the predator fish fry need nutritional input most.

- **Potential impacts on numbers**

  Shearer and Halter (1980) reviewed a number of field studies on the effects of 2,4-D DMA and 2,4-D BEE on aquatic invertebrates (mainly benthic invertebrates). For example, 2,4-D acid which is probably the main concern from treatment with 2,4-D BEE appears to be extremely toxic to the lined scud (LC50 = 3.2 mg a.e./L, Table 23). While the lined scud appears to very tolerant of 2,4-D DMA (LV50 = >100 mg a.i./L = 86 mg a.e./L, Table 23), there are estuarine benthic organisms (*Palaemonetes spp*. and *Cypridopsis vidua*) that appear to be very susceptible to 2,4-D DMA (LC50 = 0.15 mg a.i./L to 8.0 mg a.i./L, Table 23). While these laboratory studies suggest that 2,4-D in the form of 2,4-D BEE may adversely impact benthic invertebrates, field trials do not support this conclusion. Given the absence of noted effects, the number of studies reported, and the 20 to 30 year collection period, the assumption

that any measurable direct effects on invertebrate populations would have been detected by this time seems reasonable.

Four studies have reported on the effects of 2,4-D following the treatment of invertebrate habitat. Brooker (1974 in Shearer and Halter) monitored the invertebrate populations of an English drainage ditch for six months after application of a mixture of 2,4-D DMA and dalapon to emergent ditch-bank vegetation. Since no undesirable effects were noted, the fact that a mixture of herbicides was used was not a complication. Sediment cores and net sweeps were taken at two-week intervals, and density fluctuations in the five major taxonomic groups present were considered normal. There was no change in the population density of 49 species, increases in 7 species and serendipitous appearances of 29 species in either the control or treatment streams.

In a rather limited experiment conducted in Stone Valley Lake, Pennsylvania Marshall and Rutschky (1974) found that there was a decrease in the numbers of benthic organisms five-weeks after treatment. There was also a shift in the dominant species from dragonfly, damselfly and mayflies to oligocaete worms and tendepedid midges. This study was complicated by emergence of the insect species and a drop in the dissolved oxygen content of the hypoliminion which no doubt causes a shift of species from those which require water with a high oxygen content to those which can tolerate a low oxygen content.

Effects of 2,4-D on bottom fauna of fishponds were measured by Sarkar (1991) "Commercial grade" 2,4-D was applied at rates of 0.42, 0.20, 0.375, 0.524, 0.708 and 0.875 Kg/Ha (0.038, 0.18, 0.33, 0.48 0.63 and 0.78 lbs/acre) as liquid uniformly splashed over pond surfaces for a total of 12 applications. Subsequent applications were made every 30 days for a total of 12 applications. During every month, bottom fauna were collected with a bottom sampler from eight areas in the ponds and were analyzed. Populations of bottom organisms were reported for total annual herbicide applications of 0.5, 2.5, 04.45, 6,5, 8,5 and 10.5 kg/ha/year (0.45, 2.23, 4.0, 5.8, 7.6 and 9.4 lbs/acre/year). At 2,4 D applications of 0.5 and 2.5 Kg/ha/year, the mean biomass was not significantly different from the control (no herbicide application). The higher 2,4-D treatments (4.5 to 10.5 Kg/ha/year) resulted in mean biomass increases of 22.15, 20.40, 21.30 and 21.1 percent of the control values, respectively. Dominant forms of bottom fauna identified during the study included *Chironomus lobaticceps* (27.9%), *Branchiura sowerbyi* (17.5%), *Planorbis exustus* (5.3%), unidentified *Odonota* (4.3%), *Viviparus bengalensis* (5.0%) *Lymnaea leuteola* (6.0%) *Branchiodrilus hortensis* (14%) and *Chaoborus spp*. 20%). However, effects on species diversity from the 2,4-D applications were not reported. This increase in benthic fauna was attributed to an increase in benthic (sediment) bacteria numbers and an unspecified enhancement in metabolic capacity due to the exposure to 2,4-D. The increase in benthic bacteria numbers was attributed to an increase in nutrient levels due to mass mortality of phytoplankton in the early stages of development and an unspecified stimulatory effect of 2,4-D and its metabolites.

In a similar, shorter term experiment, Patnaik and Das (1991) found that unspecified zooplankton increased about three-fold in eight weeks after treatment with Fernoxone® (2,4-D sodium salt) at rates of 6 Kg a.i./ha (5.4 lbs a.i./acre). This increase in zooplankton count mimicked the nutrient levels of nitrate and phosphate, which appeared to stimulate the growth of heterotrophic bacteria and phytoplankton,

found in the water column (Table 15). This stimulation of the growth of microorganisms provided an increased food supply for the resident zooplankton and a subsequent increase in their numbers.

There is not a large literature base concerning negative or positive impacts of 2,4-D treatment on numbers of fish and invertebrates in natural ecosystems. There is data on the effect of failure to remove weeds when they become so dense they interfere with the action of indigenous fishes, but even this data is ambiguous. Klussmann et al. (1988 in Bain and Boltz, 1992) found that catch rates for largemouth bass was greatest when the plant densities were highest, while Colle et al. (1987 in Bain an Boltz, 1992) found that largemouth bass catches were unaffected by a reduction in plant density. Ideal plant cover of about 36% appears optimal for production of largemouth bass (Ware and Gasaway, 1978 in Bain & Boltz, 1992) and complete removal of aquatic plants can cause a major decline in forage fish and largemouth bass abundance (Moxley and Langford, 1985 in Bain & Boltz, 1992). There can be a decrease in the numbers of certain size classes (intermediate size largemouth bass) and not others (large largemouth bass) if foliage is entirely removed (Klussmann et al., 1988 in Bain & Boltz, 1992).

A detailed study on the numbers and diversity of fish conducted by Olaleye et al. (1993) concluded that areas heavily infested with waterhyacinth contained a very low number (8 per unit area) of the *Ctenopoma kinglayae* (Anabantid), and no other fish species. However, if no waterhyacinth or other weeds were present, the numbers of this Anabantid went up to 30 per unit area and eight other families of fish were present at low levels. Since this work was conducted in Nigeria, it may not be directly applicable to the State of Washington.

The toxic potential of 2,4-D BEE as measured in the laboratory is apparently not realized under the 2,4-D BEE concentrations and environmental conditions present during actual field use. The fairly rapid hydrolysis of 2,4-D BEE to 2,4-D acid in nature is probably the key factor responsible for this observed lack of environmental toxicity. See Section 3 for details on the environmental fate of 2,4-D BEE. Shearer and Halter (1980) have reviewed the effects of 2,4-D on a number of species. Studies monitoring field application of 2,4-D BEE have been unable to show direct adverse effects on fish populations as a result of 2,4-D BEE treatments. Such studies have generally consisted of holding caged fish in treatment areas, plus systemic or random net-capture surveys of fish populations at various time periods post treatment. Various reports (all cited by Shearer and Halter, 1980) by Smith and Ison (1967) in TVA reservoirs, Whitney et al, (1973) in Currituck Sound, Ganstad (1978) in southern waters of the United States, Pierce (1960, 1961) in the northeast and Lim and Lozoway (1978) in British Columbia are uniform in their appraisal of no observable direct effects on fish populations as a result of 2,4-D BEE treatments.

Additional review by Shearer and Halter (1980) on the field effects of 2,4-D DMA found results that were similar to those achieved with the 2,4-D BEE product. Schultz (1973 in Shearer and Halter, 1980) exposed bluegill sunfish, largemouth bass and channel catfish in outdoor plastic pools to one time dosages of 0.5, 1.0 and 2.0 mg/L 2,4-D DMA for 84- days, and observed no adverse effects on fish. Similarly, no fish died when Stallings and Huckings (1978 in Shearer and Halter, 1980) used the same protocol to study 2,4-D DMA dynamics in bluegill sunfish. Schulz and Harmon (1974 in Shearer and Halter, 1980) reported no fish mortality but successful

bluegill reproduction in ponds treated with 2,4-D DMA. According to Scott et al (1978 in Shearer and Halter, 1980), bluegill sunfish in ponds treated with 2 mg/L 2,4-D DMA showed no toxic effects but did grow faster than fish in control ponds. Whether this was due to indirect or direct effects of 2,4-D was not stated.

In a recent study by Bain and Boltz (1992), the dominant species of aquatic weeds Eurasian watermilfoil (*Myriophyllum spicatum*), and also incidental coontail, Uruguayan waterprimrose, giant cut grass and alligator weed were removed by application of 2,4-D DMA at 2 mg/L in nearshore waters of the Gunthersville Reservoir, Alabama. Overall, the results of this study indicate that there is no evidence that localized herbicide application changed the abundance, size structure, condition or movement of largemouth bass.

A study conducted on fishponds in India evaluated the effects of "commercial grade" 2,4-D application on bottom fauna productivity and on bottom- and column-feeding fish species (Sarkar, 1991). Application of 2,4-D at rates ranging from 0.5 to 10 kg/ha/year (0.45 to 9.38 lbs/acre/year) over a 1-year period increased the bottom-fauna biomass. Survival rates of each fish species (*Labeo rohita, Gibelion catla, Cirrhinus mrigala* and *Cyprinus carpio*) were measured at the end of the 1-year period when the ponds were drained (Table 26). Survival rates of different species of fish treated with 2,4-D applications at rates of 0.5 to and 2.5 Kg/ha/year did not differ significantly from the control. However, at 4.5 Kg/ha/year (4.0 lbs/acre/year) application rates, survival rates of *L. rohita, G. catla, C. mrigala* and common carp increased by 14.1%. 17.4%, 14.4% and 30% of the control, respectively. At 6.5, 8.5 and 10.5 Kg/ha (5.8, 7.6 and 9.4 lbs/acre/year), the survival rate of *L. rohita* significantly decreased 13.4%, 29.6% and 34.1) from the control rate, and that of *G. catla* decreased by 64%, 13.0% and 19.2%. There were increases in the survival rate of *C. mrigala* and common carp at these higher dosages. Although there was a significant increase in the yield of bottom-feeding fish by 0.5 to 60% and 35.6 to 141.7 in *C. mrigala* and common carp, respectively, there was a significant decrease in the yield of species that typically feed in the water column. Sarkar (1991) concluded that there was no clear evidence of a direct influence from 2,4-D on bottom feeding fish growth in ponds. Nevertheless, the increase in benthic microbes and hence benthic invertebrates provided a greater amount of fish food organisms; subsequently, bottom fish consumed increased bottom fauna and significantly increased yields. Sarkar (1991) assumed that planktivorous fish viability was affected by phytoplankton die-off resulting from higher 2,4-D exposure (both *L. rohita* and *G. catla* are planktivorous). Because 2,4-D appears to adversely affect phytoplankton at higher concentrations (400 to 1200 mg/L), it is unlikely that planktivorous fish are adversely affected by 2,4-D. These concentrations approximate concentrations that are known to adversely affect algae (Table 19).

A similar short term study conducted by Patnaik and Das (1991) indicated that the use of 2,4-D sodium salt at application rates of 6 Kg/ha to control the thorny lily (*Euryale ferox*) did not affect the healthy condition of resident fish populations, which included *L. rohita, G. catla* and *C. mrigala*. Furthermore the successful use of fish from treated ponds as broodstock for seed production suggests no long-range effects of 2,4-D on fish.

2,4-D has been shown to have an impact on insects associated with waterhyacinth (*Eichhornia crassipes*) control. Using the 2,4-D amine salt at concentrations up to

2.0 to 2.2 while not killing the plants, decreased the hardness of the leaves and thus may increase the effectiveness of the biocontrol agents such as *Sameodes albiguttalis* (*Lepidoptera: Pyralidae*) and *Neochetina eichhornia* and *N. bruchi* (*Coleoptera*: *Curculionidae*) (Wright and Bourne, 1990).

Incremental treatment of Calf Pond, Florida at 2.2 Kg/ha starting August, 1985, resulted in an increase in hyacinth weevil density of approximately 3-times that observed prior to treatment and the number of feeding scars doubled in that time frame. The resulting damage reduced plant density and biomass severely and by April 1987, no live waterhyacinth plants remained in the lake. After the elimination of waterhyacinth, Calf pond was invaded by water lettuce, and as of November 1990 was extensively colonized by this aquatic weed. Effective control of waterhyacinth at this site was obtained within two years, employing the combined stresses of insect feeding damage and space limitations. Other workers have reported control or elimination of waterhyacinth within 9-months to 6-years. This time frame is dependent on both nutritional quality of the waterhyacinth plants and their past history with respect to initial weevil colonization and subsequent use of herbicides (Haag and Habeck, 1991).

- **Potential impacts on diversity**

As described in the Nigerian work with waterhyacinth, high infestation rates with weeds can effect both fish numbers and fish diversity (Olaleye et al, 1993). Details on the effects of weed infestation on numbers and diversity of these fish can be found in potential impacts on numbers section.

Information on the changes in numbers of fish and invertebrates due to 2,4-D treatment has been gathered, but the changes in diversity have not been addressed. As reported previously, an English drainage channel was monitored by Brooker (1974 in Shearer and Halter, 1980) for six months after it had been treated with a combination of dalapon and 2,4-D DMA to control ditch bank vegetation. The maximum concentration of 2,4-D in channel water was 0.029 mg/L at the height of the summer season. Neither the total numbers as indicated above nor the diversity was affected. Marshall & Rutschky (1974) conducted a similar, more limited, study in a small cove in Stone Valley Lake, Pennsylvania. Five-weeks after treatment with 2,4-D BEE granules the diversity had not changed, but the numbers and species composition had changed from one dominated by species that require a high dissolved oxygen content [*odonata* (dragonflies and damselflies) and *ephemeroptera* (mayflies)] to a population dominated species that could tolerate low dissolved oxygen content. This was not surprising in light of the drop in oxygen content from 6.5 mg/L prior to treatment to ~0.0 mg/L in the hypoliminion one week after treatment. Although not addressed directly, Marshall and Rutschky cited others including Pierce (1958 & 1960), Cowell (1963), Fish (1966), Gilderhaus (1967) and Price (1967) that indicated that aquatic herbicides affect non-target zooplankton community structure. Also the planktivorous fry of largemouth bass and bluegill sunfish could be affected by a drop in the number of zooplankton. In dealing with commercial fishponds Sarkar (1991) found that “Adoption of (the) recommended 2,4-D application rate will accelerate the growth of bottom fauna and production of bottom–feeding fish and also will help fish farmers in many other ways.”

- **Potential impacts on habitat use for spawning, rearing and growth**

  - **Effects on Trout and other salmonids**

    2,4-D, has been shown to have low acute and early life-stage toxicity to freshwater trout in the forms that trout are likely to encounter. 2,4-D DMA has an acute toxicity to trout (LC50 = 100 to 377 mg a.i./L). Since 2,4-D DMA is not likely to be encountered at concentrations higher than 4.8 mg a.i./L immediately after treatment, this product is unlikely to cause toxicity in freshwater trout. Due to its very short half-life salmonids are not likely to be exposed to 2,4-D BEE. Therefore, the toxin of concern would be 2,4-D acid. Shortly after initial exposure, it is estimated that the EEC for 2,4-D acid would be 0.19 mg/L at the top of the water column and approximately 3.25 mg/L at the bottom of the water column. Concentrations of 2,4-D acid would be expected to be at or below 0.100 mg/L within two to six days (Gallagher, 1992). Typical LC50s with 2,4-D acid for salmonids is 40 mg/L for 2,4-D acid although the lowest credible reported value is 25 mg/L for cutthroat trout. Under most conditions it is not likely that salmonids would be exposed to concentrations of 2,4-D acid that are high enough to cause acute toxicity.

    Although trout fry have been noted to avoid 2,4-D DMA at concentrations that are environmentally relevant (1 to 10 mg/L) (Folmar, 1976), avoidance may not be possible in real field treatment situations. Trout driven from a habitat by avoidance behavior, may not be able to obtain necessary resources for survival in other habitats. These resources could include, food, refuge, mates and appropriate egg-laying (substrate). It has been reported that fish appeared to be driven out of an area after field application of 2,4-D to TVA Reservoirs (Smith & Ison in Shearer and Halter, 1980), which would reduce the potential of adverse impact to fish species. No other field studies have confirmed this avoidance behavior with freshwater trout.

    Probably, the greatest concern is managing aquatic plants so that maximum fish breeding opportunities can occur. Although it typically takes concentrations of ~0.300 mg/L 2,4-D BEE to effectively manage aquatic foliage (Helsel, 1996), prolonged exposure (starting in June of any given year) of 0.1 mg/L might eliminate *Myriophyllum sibericum* and *Potamogeton pectinatus* from prairie ponds (Forsythe et al, 1997) by the end of the growing season. The consequences of eliminating such plants from a habitat can have tremendous consequences. Due to the effects of erosion by floods, the character of a habitat may be changed from one suitable for the reproduction of sunfish to one suitable for the reproduction of salmonids.

    If water that contains 2,4-D at effective concentrations passes, from a lake or pond into a river or stream, the rooted aquatic macrophytes may be destroyed. This can have a substantial impact during the next high water event. Normal spring floods in absence of rooted aquatic macrophytes can dig up and kill large numbers of benthic organisms while summer spates can completely denude streams of benthic biota.

    Most biota avoid floods either by migrating to calm back waters or by having life cycles which are terrestrial or aerial at these times. However when floods occur

at unusual times the fauna may be severely depleted and require several years to recover (Goldman & Horne, 1983).

Larger organisms, like salmonids, normally choose to ascend rivers or streams during high water because there are fewer shallow water barriers. Severe floods are detrimental to smaller biota if they leave only rocks and gravel. However, these floods may improve fish migration by removing major obstacles. Smaller floods can improve the environment for salmonid mating and egg survival by removing excessive silt. These benefits cannot occur if the lotic system has been dammed by aquatic weeds.

- **Effects on salmon smoltification**

  Evidence for effects on salmon smoltification is of great potential concern with herbicides that are applied in Northwestern waters. A variety of seawater challenges have been performed with salmon species that have been exposed to ecologically relevant concentrations of 2,4-D. For example, 1.0 mg/L 2,4-D BEE kills all of the salmon smolts exposed for 96 hours to this concentration. However, exposure to this concentration (1.0 mg/L) for 24 hours prior to a seawater challenge test did not affect 96 hour survival of smolts in clean seawater. Species tested included Coho salmon (*Oncorhynchus kisutch*), sockeye salmon (*Oncorhynchus nerka*) and pink salmon (*Oncorhynchus gorbuscha*) (Martens, 1979 in Shearer and Halter, 1980).

  2,4-D DMA also does not kill Coho salmon smolts at concentrations up to 200 mg/L. Fish exposed to these concentrations of 2,4-D DMA survived seawater challenge tests and no ATP-ase effects were observed with sublethal dosages of 2,4-D DMA. This is an important observation since ATP-ase is believed to be essential in maintaining osmoregulation during the parr to smolt metamorphosis (Lorz et al, 1979 in Shearer and Halter, 1980).

  These results indicate that the main 2,4-D products used for control of aquatic weeds are not likely to put salmon species at risk during the smotification process. Although most of the Northwestern relevant species have been tested, Chinook salmon, which has been shown to be sensitive of Aquathol® (Ligouri et al, 1984) and Hydrothol® (Serdar and Johnson, 1995), has not been tested in a seawater challenge test with the most common 2,4-D products.

- **Effects on sea-run cutthroat trout**

  No work was found on the effect of 2,4-D on sea-run cutthroat trout other than acute toxicity data with 2,4-D 2-EHE and 2,4-D acid (LC50 = >50 mg a.i./L for 2,4-D 2-EHE and LC50 = 40 mg/L 2,4-D acid). Since the EECs for 2,4-D 2-EHE and 2,4-D acid are estimated to be 0.116 and 4.0 mg a.i./L, respectively, this species is likely to be unaffected by the 2,4-D products that have been tested. Based on the toxicity of 2,4-D DMA to other salmonids (LC50 = 100 to 377 mg a.i../L), 2,4-D DMA is likely to be practically non-toxic to sea-run cutthroat. Although 2,4-D BEE, based on its toxicity to other salmonids (LC50 = 0.3 to 3.67 mg a.i./L), is expected to have high toxicity to sea-run cutthroat trout, it is not believed that exposure in the field is likely due to low solubility and rapid breakdown of 2,4-D BEE to 2,4-D acid. However, additional information on the

acute toxicity and chronic toxicity of 2,4-D products to cutthroat trout would be useful to aid in risk assessment with either this or related species.

A potential complicating factor with sea-run cutthroat trout and steelhead trout (*Oncorhynchus mykiss*) is similar to the parr to smolt metamorphosis except that these sea-run trout may go through this process several times in their lifetime including each time the adults migrate to the sea and the initial parr to smolt metamorphosis. Based on the seawater challenge tests with several salmonid species, this seawater to freshwater to seawater metamorphosis is not expected to be an issue. Depending on the degree of confidence that we have that sea-run cutthroat trout are similar to other salmonids in this transformation, further research may not be warranted.

- **Effects on other species (sunfish, minnow and catfish)**

  The acute and chronic toxicities of 2,4-D DMA and 2,4-D acid (hydrolysis product of 2,4-D BEE) are very low in fish. The acute LC50s are generally greater than 100 mg a.i./L for most ecologically relevant species. The LC50 of 2,4-D acid is greater than 20 mg a.i./L for the most sensitive environmentally relevant species (common carp). The chronic NOECs for these forms of 2,4-D are greater than 17.1 mg a.i./L for all fish species tested. Since the acute and chronic toxicity for these forms of 2,4-D are low, 2,4-D DMA and 2,4-D acid are unlikely to adversely impact wild fish.

  Although 2,4-D BEE is acutely toxic to most fish species (LC50 = 0.3 to 4.3 mg a.i./L), fish are unlikely to come into contact with 2,4-D BEE due to its low solubility and rapid hydrolysis to 2,4-D acid. In the species tested (Chinook salmon and fathead minnow), the chronic NOEC (0.040 to 0.3 mg a.i./L) is typically lower than the long-term EEC. Therefore, the chronic impact of 2,4-D BEE on fish is likely to be low.

  A potential concern, are the effects of 2,4-D on behavior and metabolic responses in wild fish. A relatively high concentration of BEE (110 mg/L) impaired swimming performance in green sunfish (*Lepomis cyanellus*) within 1-hour after exposure (Moore, 1974 in Ebasco, 1993). In the common carp, 2,4-D at high concentrations also impaired swimming (96 hour LC50 = 135 mg/L) causing irregular movement, erratic jerks, sluggishness followed by hyper-excitability, and finally cessation of all activity. However, unlike the green sunfish, no abnormal swimming behavior was observed during the first 24- to 48-hours of exposure (Sarkar, 1990).

  In a field study evaluating behavior of sunfish guarding their nests during 2,4-D DMA surface spray application (~4 mg/L), fish abandoned their nests in six of nine trials with bluegill sunfish and in four of seven trials with redear sunfish (Bettoli and Clark, 1992). Abandonment typically occurred within 30 seconds following applications and lasted up to 15 minutes. However, since the sample size was very small (6 controls and 7 to 9 2,4-D treatments), the effects on treated fish were not significantly different from untreated fish. Therefore, further investigation to verify these conclusions needs to be conducted. These findings may be of significance since 66% to 88% of the 2,4-D DMA treated cohorts abandoned their nests and only 20 to 50 % of the water treated cohorts

abandoned their nests. This is an important potential issue since congener predators in 100% of the treatments attacked abandoned nests. Such a dramatic impact could have a marked influence on the number of young of the year surviving to the free-swimming stage. However, once fish returned to the nest, parenting behavior (such as rim circling, fanning or agonistic displays) did not appear to be affected.

As discussed previously, Bain and Boltz (1992) found the removal of aquatic weeds with 2,4-D DMA had no significant effects on abundance, size structure, condition or movement of largemouth bass within the Gunthersville Reservoir, Alabama. This may not be the case for all species of fish and invertebrates since rainbow trout (Folmar, 1976), sheepshead minnow (Hansen, 1969 in Hansen et al, 1973) and mosquito fish (Hansen et al. 1972 in Shearer and Halter, 1980) will avoid 2,4-D when they are exposed to environmentally relevant concentrations within the laboratory environment. However, all investigators working on avoidance response stated that it was unlikely that significant numbers of exposed fish or invertebrates would or could avoid exposure to 2,4-D.

Effects on the metabolism of fish have also been discussed. The growth of carp species may be stimulated by the treatment of fish ponds with 2,4-D commercial products because the growth of bacteria and the benthic organisms that feed upon them. However, this effect was not apparent with species that fed higher up in the water column (like *Labeo rohita* and *Gibelion catla*) since 2,4-D did not have a strong effect on phytoplankton in the field (Sarkar, 1991). However, in a similar environment, Ptanaik and Das (1991) found that 2,4-D stimulated the growth of bacteria, phytoplankton and zooplankton but that these effects on fish food organisms had no effect on the resident fish population. Use of fish from treated ponds as broodstock for seed production also suggests no long range effect of 2,4-D sodium salt on fish.

- **Effects on invertebrates**

  Data on the field effects of 2,4-D against aquatic invertebrates is not extensive. From the laboratory data it is apparent that the free-swimming species are not normally affected adversely by 2,4-DMA. Typical field concentrations vary after application depending on the particular situation.

  In California, application of 2,4-D DMA at 5.7 Kg a.i./ha to control mats of aquatic weeds did not result in concentrations of 2,4-D that exceed the Federal drinking water standard (0.07 mg/L) outside the weed mat area for more than 2 hours after application (Anderson, 1982 in Gallagher, 1992). However, more typical concentrations of 2,4-D DMA are seen in ponds treated for waterhyacinth control in Florida and Georgia. Concentrations in water varied depending on application rate, but for the highest rates (8.96 Kg a.e./L), the maximum concentrations that occurred were 0.345 to 0.692 mg a.e./L (0.416 to 0.837 mg a.i./L) three days after application. These concentrations are not likely to adversely affect free-swimming aquatic invertebrates (LC50 = 100 mg a.i./L for *Daphnia magna*). However, some of the benthic species may be adversely affected. For example, since the seed shrimp (LC50 = 8.0 mg a.i./L) and the glass shrimp (LC50 = 0.15 mg a.i./L) are less than tenfold higher than the EEC, benthic organisms may be adversely affected by even a low concentration of 2,4-

D DMA. Since only one species of free-swimming invertebrate was tested in a chronic life-cycle test, the chronic safety of 2,4-D to the biota is difficult to ascertain. However, since the NOEC (27.5 mg. a.i./L) is very high for the one reviewed study, safety of 2,4-D DMA to the free-swimming invertebrate cannot be rejected without further analysis.

Similar comments for 2,4-D BEE can be made in regard to its toxicity to free swimming invertebrates and safety to the biota. Since the highest bottom water concentrations that are likely to be encountered are 3.25 mg/L (Gallagher, 1992) and 2.0 mg/L (Shearer and Halter, 1980), there is a potential for benthic fish to be adversely affected by 2,4-D BEE. If benthic species like *Gammarus fasciatus, Gammarus lacustris*, brown shrimp, nymphal stoneflies, aquatic sowbug, chironomid midge, seed shrimp or glass shrimp encounter these concentrations of 2,4-D BEE immediately after application, the result could be fatal. However, Shearer and Halter (1980) state that "the toxic potential of 2,4-D BEE, as measured in the laboratory, is apparently not realized under the 2,4-D BEE concentrations and environmental conditions present during actual field use. The fairly rapid hydrolysis of 2,4-D BEE to 2,4-D acid in nature is probably a key factor responsible for this generally observed lack of environmental toxicity…" See Section 3 for a detailed discussion of persistence of 2,4-D BEE in the environment.

Chronic risk is acceptable with 2,4-D BEE for all tested species since chronic EEC levels are unlikely to be higher than 0.001 mg/L; 2,4-D BEE applied to open water areas has typical maximum concentrations of 3.25 ml/L in bottom waters and 0.19 mg/l in surface waters. Typically these residues will dissipate to concentrations of <0.100 mg/L in 2 to 6 days and <0.001 mg/L in 5 to 22 days (Canadian Ministry of the Environment, 1977-1978 in Gallagher, 1991).

As discussed previously, there is strong evidence that effects of 2,4-D on invertebrate breeding may be complex and site specific. Zooplankton and benthic organisms, while not directly stimulated by 2,4-D to increase their numbers through more rapid breeding do appear to increase in number with chronic short-term (8-week) and long-term exposure.

For example, the numbers of zooplankton organisms in short-term fishpond studies in India increased in number almost immediately after treatment with 2,4-D sodium salt at 6 kg a.i./ha; increases in zooplankton numbers were documented by a pretreatment population of 432 zooplankton/L before treatment to 624 zooplankton/L immediately after treatment and 1260 zooplankton 8 weeks after treatment. The improvement in this pond as a zooplankton breeding site was directly correlated with a similar increase in phytoplankton counts (324 to 480 to 924 phytoplankton/L) and heterotrophic bacterial counts. This could have been due to and increase in nutrient levels which stimulated the phytoplankton and heterotrophic bacteria to grow (Patnaik and Das, 1991) (Table 9). Another possibility is that 2,4-D stimulated phytoplankton to grow by direct metabolic stimulation (Wang et al, 1991).

Sarkar (1991) found similar effects on benthic organisms after long term, repeated treatments of a fishpond in the country of India at 4.5 to 10.5 Kg/year with a commercial grade of 2,4-D. The benthic organisms increased ~21% over

the control values. This obviously improved the suitability of this breeding habitat and Sarkar (1991) explained it as stemming from an increase in the bacterial growth rate due to the availability of a readily utilized carbon source (2,4-D) and its metabolic products. The increase in the number of bacteria may also have been due to the mass mortality of phytoplankton populations and the subsequent release of nutrients that could be utilized by the benthic microbes and other organisms. The bacteria apparently improved the nutrition of the benthic organisms, allowing them to multiply.

Similar effects were not seen with zooplankton. The mass mortality of phytoplankton actually may have prevented the growth of organisms that feed upon them. Although the decrease in survival and growth of fish that feed directly on phytoplankton (Table 26) was addressed, the effects on zooplankton numbers was not directly addressed by Sarkar (1991).

Dissolved oxygen levels can effect the suitability of breeding habitat. Marshall and Rutschky (1974) found that treatment of Stone Valley Lake, Pennsylvania with 2,4-D BEE caused a decrease in the benthic dissolved oxygen concentration to anoxic levels within one week of treatment. The authors' believed that this caused a shift in dominant organisms from odonates (dragonflies and damselflies) and mayflies, which require a high oxygen content to complete development, to oligochaete worms and tendipedid midges, which can complete development in water with low dissolved oxygen content.

According to Shearer and Halter (1980), "invertebrate populations are not permanently damaged by the direct toxicity due to 2,4-D treatments has been the conclusion offered by most reports describing past large-scale plant control programs (Gangstad, 1978; Whitney et al, 1973; Wojtalik et al, 1971; and Smith & Isom, 1967 in Shearer and Halter, 1980). While this assessment may not be inaccurate, the amount of data collected to support this contention seems, in every case disproportionately low in relation to the total scope, in the particular project." Pierce (1960 and 1961 in Shearer and Halter, 1980) studied a treatment pond for several years before the application of 2,4-D, and after two seasons of work concluded that benthic organisms and zooplankton were not affected by 2,4-D. Lim et al (1978 in Shearer and Halter, 1980) found that snail populations were immediately reduced by 2,4-D treatments but that 12 months later, the populations appeared to have returned to normal in a large indoor model ecosystem containing both plants and gastropods. Furthermore, Wojtalik et al, 1971 in Shearer and Halter, 1980) documented the persistence of 2,4-D residues in the invertebrate biota of TVA reservoirs they found that 2,4-D residues were not acutely toxic to plankton and mussels.

The data set presented here indicates that 2,4-D treatments do not cause adverse effects on the invertebrate biota. In some cases, growth may actually be enhanced due to direct or indirect (nutrient releases) stimulatory effects from the 2,4-D treatment.

- **Interaction of water quality with 2,4-D products and their commercial recommended adjutants**

  Water quality encompasses many parameters, but the toxicity of chemicals to fish is most often influenced by water hardness, pH and the inter-related factors of temperature and dissolved oxygen.

  Hardness does not usually effect acute toxicity. At least three studies have reported that acute toxicity is not affected by water hardness (Shearer and Halter, 1980 cite Woodward and Mayer, 1978, Inglis and Davis). However, salmonids seem to be particularly affected by the interaction of toxicity of 2,4-D and water hardness. In a study using three types (soft/slightly acidic, hard /alkaline and a mixture of the two), bioassays were performed on juvenile Coho salmon, pink salmon and rainbow trout (Wan 1991). Water types used were city tap water (total alkalinity 4.4 mg/L, pH 6.3), British Columbia lake water (total alkalinity 80.5 mg/L, pH 8) and a mixture (total alkalinity 38.9 mg/L, pH 7.5). Ninety-six-hour LC50 results indicate different responses to 2,4-D diethanolamine (originally misidentified in Ebasco, 1993 as 2,4-D DMA), 2,4-D 2-EHE (with petroleum solvent plus emulsifier) and water type (Table 10, Wan, et al, 1991). The ester formulation appears to be 3 to 20 times more toxic than the diethanolamine salt under all conditions.

  In Washington State, hard water/higher pH conditions are generally found in eastern Washington lakes relative to western Washington lakes. Because the 2,4-D 2-EHE formulation appear to be more toxic to juvenile coho salmon, pink salmon and rainbow trout in hard water environments, potential adverse effects with 2,4-D 2-EHE on these species are more likely to occur in eastern Washington lakes. By contrast, 2,4-D diethanolamine appears to be of low toxicity (LC50 = 409 to 744 mg/L) in both soft and hard water. It therefore appears unlikely that use of 2,4-D diethanolamine or other amine salts would have adverse acute effects in either eastern or western Washington lakes. However, testing of 2,4-D DMA and other amine salts of potential commercial use in hard and soft water could be of importance in evaluating the best treatment practice.

  Wan et al (1990) evaluated the effects of 2,4-D BEE and 2,4-D dichloropropionic acid BEE (2,4-DP BEE) by themselves and in combination (Weedone® = 11.9% 2,4-D BEE; and 11.7% 2,4-DP BEE and 76.4% Carrier A) or Weedone® plus 10% diesel oil. Carrier A and diesel oil were also tested by themselves so that any supra-additive effects could be ascertained. Test conditions were similar to those of the Wan (1991) study summarized above. Results are summarized in Table 24. These data indicate that all herbicide formulations and mixtures are highly toxic to salmonids. However, exposure to 2,4-D BEE does not occur due to low solubility and the rapid conversion of 2,4-D BEE to 2,4-D acid (Section 3). 2,4-D acid has been shown to be of low acute toxicity to salmonids including rainbow trout, cutthroat trout, and lake trout (LC50 = 40 to >1000 mg a.i./L). Diesel oil and Carrier A appear to be less toxic than the herbicides alone and in mixtures with Weedone®. In this study, softer, more acidic water appears to increase 2,4-D BEE and 2,4-DP BEE toxicity to all three salmonid species in contrast to the harder more alkaline water increasing toxicity in the 2,4-D 2-EHE study (Wan et al (1991). Wan et al (1990), concluded that in soft water

conditions, toxicity to juvenile salmonids increases from Weedone® < Weedone® plus diesel oil <2,4-DP BEE < 2,4-D BEE. Across the three hardness types, 2,4-D BEE, and 2,4-DP BEE toxicity cannot be considered significantly different. The addition of 10% diesel oil to Weedone® only significantly increased toxicity to salmonids in soft water.

Because all products of 2,4-D dissociate to 2,4-D acid with time (often very rapidly), the toxic effects of 2,4-D can be affected by pH alone. It has been shown by Finlayson and Verrue (1985 in JMPR, 1997) that pH does affect the 96-hour LC50 of 2,4-D acid (Table 11). 2,4-D acid is acutely non-toxic to rainbow trout at pHs higher than 8.48 (LC50 = >1000 mg/L). However, 2,4-D acid has some toxicity to rainbow trout at pHs lower than 4.54 (LC50 = <100 mg/L). Therefore, even after 2,4-D BEE has hydrolyzed to 2,4-D acid under typical conditions, it may have some toxicity to the more sensitive species of fish and invertebrates at lower pHs. Species which are sensitive to 2,4-D acid in standard tests, (pH 6.8 to 8.2) would probably be more sensitive at lower pHs. In those species where the reported acute LC50 or chronic NOEC are less than ~10 mg/L, further work needs to be done with varying pHs to determine if the lower pHs (e.g., particularly those associated with western Washington lakes) will have adverse effects on certain segments of the biota. For example, amongst the invertebrates, seed shrimp, and *Palaemonetes spp*. appear to be particularly sensitive to 2,4-D DMA (LC50 = 8.00 to 0.15 mg/L and the lined scud appears to be particularly sensitive to 2,4-D acid (LC50 = 3.2 mg/L). Of the environmentally relevant species of fish, only the bleak and possibly cutthroat trout appear to be sensitive to 2,4-D sodium salts (LC50 13 mg/L for the bleak) and 2,4-D acid (LC50 = 25 to 64 mg/L for cutthroat trout). In chronic tests, only a few species have been tested but rainbow trout, largemouth bass and even the goldfish appear to be sensitive to chronic exposures to 2,4 D potassium salts (LC1 = 0.027, 6.47 and 12.7 mg/L respectively). None of the tested species of invertebrates appear to be sensitive in chronic exposure to 2,4-D DMA or 2,4-D acid although the data base is too small to effectively the determine the degree of sensitivity that might be expected from chronic exposure of invertebrates to various formulations of 2,4-D. Although low pH could theoretically effect the toxicity of 2,4-D, Shearer and Halter, (1980) do not believe that it is likely to be of significance under normal environmental conditions. However, under environmental conditions that tend to acidify water decreased pH may have an impact on the aquatic toxicity of 2,4-D. Such conditions include runoff from peat bogs, acid rain, mine leachate, low aquatic plant growth and the presence of evergreen trees adjacent to the water body. Nevertheless, pH does influence the rate of chemical hydrolysis of 2,4-D BEE to 2,4-D acid, and at higher pH 2,4-D BEE would be detoxified more readily than at lower pH (Section 3).

Conditions of low oxygen, or high temperature would be expected to accelerate the death rate in fish at higher toxic concentrations of 2,4-D but not necessarily change the final toxicity. In other words the fish would simply die more quickly but at the same 2,4-D concentrations (Brown et al, 1973 in Shearer and Halter, 1980). Even at concentrations that could be expected in the environment, high temperatures can cause respiratory distress in sunfish when the water has been treated with as little as 3 mg/L 2,4-D. Choleostasis was seen in water with moderate temperature that had been treated with 10 mg/L 2,4-D (Sarkar, 1990). Under conditions of marginal oxygen, temperature and 2,4-D concentrations, the

acute or chronic toxicity of 2,4-D cannot be predicted. The interactions of low dissolved oxygen concentration, high temperatures and 2,4-D concentrations would have to be determined empirically through experimentation.

Because very little work has been done on the effects of pesticide combinations it is unclear whether other pesticides, applied for other purposes, could substantially enhance the toxicity and persistence of 2,4-D.

In the State of Washington, 2,4-D products are rarely mixed with other products. Since 2,4-D BEE is a granular product that is broadcast and then allowed to sink to the root zone for control of watermilfoil, adjuvants would not be typically used with this product. 2,4-D DMA and 2,4-D 2-EHE may occasionally be used in conjugation with an oil or other carrier for the control of floating or emergent weeds. A number of surfactants are registered for use with water-soluble herbicides like 2,4-D DMA when they are applied to floating or emergent plants. Professional researcher (Kurt Getsinger) believes that when a pesticide is applied to floating or emergent vegetation that a surfactant and/or drift control agent should be used. It is not necessary to use adjuvants with subsurface injections of 2,4-D (2,4-D DMA) or when using granular products of 2,4-D. However, a thickener is often used with liquid products applied by subsurface injection to allow the treatment to sink more deeply into the water column where it can be most effective.

When liquid 2,4-D products (2,4-D DMA) are used to control floating weeds by direct contact with a spray, the use of a surfactant and a thickening agent are recommended. The surfactant should be used to allow for better wetting of the floating weeds and the thickening agent should be used to prevent drift. There are a number of adjuvants registered for aquatic use in Washington State. Most surfactants should be mixed at 0.25 to 0.5% by weight of application solution when 2,4-D is being applied to floating (surface) aquatic macrophytes. The toxicity of these adjuvants to bluegills, rainbow trout and daphnia has been well documented. None of these aquatic adjuvants should be toxic to fish or aquatic invertebrates when applied at labeled rates. However, it has been noted by Watkins et al (1985) that some aquatic adjuvants have a potential to be toxic to aquatic organisms when applied to shallow water. For example: 1) If Spar-Mate® is applied at the labeled use rates to water with a depth of less than 1.5 meters, it can be toxic to bluegill sunfish. 2) If Cide-Kick®, X-77®, Formula 403®, or IVOD® are applied at the labeled use rate to water with a depth of less than 0.1 meters, they may be toxic to fish. Since the depths given are for concentrations of the adjuvant that will kill 50% of the treated animals an additional safety factor of ~10-fold would need to be added to assure safety of the adjuvant to the biota. Details of the toxicity and depth considerations for a number of aquatically applied adjuvants can be found in Table 12. Although adjuvants are typically considered to be “nearly inert”, they are not entirely inert. Adjuvants can either enhance, diminish, or have no effect on the activity of herbicides. Although acute aquatic testing has been done on a number of adjuvants, insufficient data exists to appropriately evaluate risk from the toxic effects of adjuvants when mixed with herbicides and applied to the aquatic ecosystem.

One possible exception is the surfactant, Syndets® Abdelghani et al (1997) indicate that the surfactant is from 40 to 85 times more toxic than 2,4-D. Lethal concentrations of surfactant plus 2,4-D were found to be lower than the recommended field applications to control right-of-way weeds. However, it was found that hazardous amounts of this surfactant would rarely move from the target to roadside ditches where the presence of large volumes of water is likely to provide dilutions to levels that pose no threat to aquatic life.

Not all formulations of 2,4-D have a similar toxicity on an acid equivalence (a.e) basis. We know that 2,4-D DMA is practically non-toxic to most species of fish and invertebrates while, the ester compounds are more toxic. However, the esters are rapidly converted to 2,4-D acid in natural water systems and 2,4-D acid is relatively non-toxic to most species of fish and invertebrates. The conversion of the esters to 2,4-D acid is so rapid that JMPR (1997) recommends the use of the acid toxicity data to generate appropriate risk quotients if the values for the ester products are adverse. The "inert materials" and contaminants may interact with the pesticide to give antagonistic, additive, cumulative or synergistic effects against target aquatic plants and algae, and non-target fish and aquatic invertebrates (Kamler et al., 1975). For example: former commercial preparations of 2,4-D sodium salt containing 2 or 3% of 2,4-dichlorophenol are toxic in 24 hour to carp sac-fry at concentrations of 1600 mg/L 2,4-D sodium salt. However, technical preparations not containing the impurity have only a minimal toxicity (20% mortality in 48 hours). Current products used in the United States are not believed to contain these high levels of 2,4-dichlorophenol.

4.3.2.4 Effects on Endangered Species

There are a number of species that have been classified as sensitive, endangered or threatened. These include several species of salmon, 13 species of rockfish, 2 species of herring, 2 species of dace, and 8 species of amphibians. A list of these species can be found in Appendix 5. Chinook salmon (*Oncorhynchus tshawytscha*) is one of the more sensitive species both for acute (LC50 = 0.32 to 0.38 mg a.i./L for 2,4-D BEE) (Table 22 and Appendix 1) and early life-stage (NOEC = 0.04 mg a.i./L for 2,4-D BEE) (Appendix 4) toxicity. In several tested salmon species there does not appear to be any significant effects from 2,4-D products including 2,4-D BEE and 2,4-D DMA on the smoltification process.

Evidence for effects on salmon smoltification is of great potential concern with herbicides that are applied in northwestern waters. Although they were completed, quite some time ago, a variety of seawater challenges have been performed with salmon species exposed to ecologically relevant concentrations of 2,4-D. For example, 1.0 mg/L BEE kills all of the salmon smolts exposed for 96 hours to this concentration. However, exposure to 1.0 mg/L for 24 hours prior to a seawater challenge test did not affect 96-hour survival of smolts in clean seawater. Species tested included coho salmon, sockeye salmon, and pink salmon (Martens, 1979 in Shearer and Halter, 1980).

2,4-D DMA does not kill coho salmon smolts at concentrations up to 200 mg/L. Fish exposed to these concentrations of 2,4-D DMA survived seawater challenge tests and no ATP-ase effects were observed with sub-lethal dosages of 2,4-D DMA. This is an important observation since ATP-ase is believed to be essential in maintaining

osmoregulation during the parr to smolt metamorphosis (Lorz et al, 1979 in Shearer and Halter, 1980).

These results indicate that the main 2,4-D products used for control of aquatic weeds are not likely to put salmon species at risk during the smoltification process. Although most species relevant to the northwest have been tested, Chinook salmon has not been tested in a seawater challenge test with the most common 2,4-D products.

4.3.2.5 Risk Analysis for Aquatic Species

Summary: Based on the acute toxicity determine in the laboratory, 2,4-D BEE may adversely impact the aquatic biota. However, field data indicates that this is not the case. 2,4-D BEE behaves in the field in a manner similar to 2,4-D acid which does not adversely impact fish, free-swimming invertebrates or benthic invertebrates. Secondary effects of 2,4-D BEE or 2,4-D acid may impact the aquatic biota in either a positive of negative manner. Reduced dissolved oxygen content can affect the dominant species present although there is no good evidence that it effects either numbers or diversity. Increases in nutrient levels can lead to an increase in the number of phytoplankton, heterotrophic and benthic bacteria present which can lead to an increase in the number of zooplankton and benthic organisms present. Short-term decreases in the number of phytoplankton present have also been observed; this decrease in the phytoplankton count can lead to decreases in the numbers and biomass (yield) of planktovoric fish. Increases in the numbers of benthic invertebrates present can lead to increases in numbers and biomass of benthic feeding fish.

A great deal of data relevant to the risk analysis was discussed in Sections 4.2.3.2.1 Acute Effects and 4.2.3.2.2 Chronic effects. However, no formal risk analysis was actually performed in that section.

Certain mitigating behavioral and toxicity factors can improve the Risk Analysis picture. A number of species of fish and invertebrates are known to be able to avoid environmentally relevant concentrations of 2,4-D in the laboratory. For example, rainbow trout fry can avoid 2,4-D DMA at concentrations as low as 1 mg a.i./L (Folmar, 1976), sheepshead minnow and mosquito fish can avoid 2,4-D BEE (Hansen, 1969 in Hansen et al, 1973 and Hansen, 1972 in Shearer and Halter, 1980), and grass shrimp can avoid 2,4-D BEE at concentrations of 1.0 mg a.i./L (Hansen et al, 1973). Grass shrimp has also been reported to be able to select between low and high dosages of 2,4-D in the laboratory. However, even though these species can distinguish between polluted and unpolluted waters, the investigators conducting the work believed that it was unlikely that they would or could avoid water polluted by pesticides.

However, the estuarine crab (*Uca uruguayensis*) may be able to avoid areas of Samborombon Bay most highly contaminated with 2,4-D Iso-BEE and ethyl-parathion (Rodriguez and Lombardo, 1991). This observation was based on the absence of Uca uruguayensis from an area known to be contaminated with high levels of 2,4-D and ethyl-parathion and the fact that this species has a much lower “resistance” to these toxicants than are other species of estuarine crab (*Chasmagnathus granulata*). Therefore, this may or may not be a true resistance due to selection pressure. Species with a short life-span may develop resistance fairly rapidly to pesticides. However, (Chambers et al, 1977, and Fabacher and Chambers, 1974 in Shearer and Halter, 1980) noted that the mosquito fish (*Gambusia affinis*) appeared to be cross-resistant to 2,4-D due to prior selection with

chlorinated hydrocarbons other than 2,4-D. This may also be the case with *Chasmagnathus granulata* since exposure to other chlorinated hydrocarbons for species living in Samborombon Bay is well documented (Rodriguez and Romano (1994).

Although there have been reports of bluegill and redear sunfish temporarily abandoning their nests due to the application of 2,4-D DMA, the effect was not significantly different from the control (Bettoli and Clark, 1992). The concentrations of 2,4-D DMA used in this experiment (4.0 mg a.i./L) would be unlikely to have acute or chronic effects on sunfish. (Acute LC50 =106 to 524 mg a.i./L and estimated chronic toxicity would be approximately 5.9 to 29 mg a.i./L) based on an acute to chronic toxicity ratio of 18.0 (Table 14). Furthermore, no laboratory avoidance tests have been conducted with these species to determine if they are capable of detecting and avoiding 2,4-D at environmentally relevant concentrations.

There has been only one field report of avoidance of 2,4-D. Smith and Ison (1967 in Shearer and Halter, 1967) reported that application of 2,4-D to TVA reservoirs "appeared to result in some movement of lake fish out of the treated area." This report has been repeatedly cited as evidence that fish may avoid 2,4-D treated areas and thus reduce the potential for incurring adverse effects. However, in a much more sophisticated research program using radio-tracking of largemouth bass in treated and control areas, Bain and Boltz (1992) found that "movement of radio-marked largemouth bass in Gunthersville Reservoir (Alabama) were limited and largely confined to study sites. Differences among sites, presence of herbicides (2,4-D DMA) in the water and effect of reduced vegetation coverage had no measurable effect on movement of adult largemouth bass."

According to Gallagher (1992), "Canadian work by the Ministry of Environment using 2,4-D BEE showed rapidly decreasing herbicide residues in open water. This report presents data on concentrations and persistence of 2,4-D (in the water column) found near 15 treatment areas. Maximum sample concentrations of 2,4-D in open water treatment areas were 0.190 ppm in surface water and 3.250 ppm in bottom water samples. Herbicide residue values decreased to levels well below 0.100 ppm (International Drinking Water Standard), which in the United States has been changed by EPA to 0.070 mg/L,) within two to six days after treatments. Persistence of detectable residues (>0.001 mg/L) in open water treatments ranged from five days to less than 22 days. In an enclosed treatment site, concentrations as high as 1.230 mg/L (surface samples) and 4.000 mg/L (bottom samples) were measured. Detectable resides were recorded for as long as 43 days but less than 49 days." Similar findings were discussed by Frank (1972) in ponds treated with 1.33 mg/L 2,4-D BEE where 0.024 to 0.034 mg/L of 2,4-D was found in water 1 to 4 days after application, which declined further to 0.001 mg/L within 36 days of application. More recent work also verified these observations; Helsel et al (1996) found water concentrations of 2,4-D of 0.190 to 0.330 mg/L one day after application and ~0.030 to 0.090 mg/L thirty days after treatment in coves of Beulah Lake, Wisconsin where the treatment rate was 112 Kg formulation/ha (100 lbs formulation/acre) and water movement had been restricted by the installation of a polyvinylchloride curtain at the cove mouth.

Use of 2,4-D DMA at 4.12 to 8.96 Kg/ha to control of waterhyacinth produced water concentrations of 2,4-D that ranged between 0.65 and 1.00 mg/L in the first two weeks of application. The maximum concentrations were not seen until three to fourteen days had passed (Frank, 1972 and Gangstad, 1986). Further information from Gangstad (1986)

indicates that water concentrations of 2,4-D dropped to 0.005 mg/L 14- to 28-days after application of 8.96 kg a.e./L or less of 2,4-D DMA to ponds in Georgia and Florida.

Since these results are fairly consistent, the expected environmental concentrations (EEC) for 2,4-D BEE have been estimated to be 0.100 mg/L for short term acute effects and 0.001 mg/L for long term chronic effects. For 2,4-D DMA, the EECs have been estimated to be 1.00 mg/L for short-term acute affects and 0.005 for long term chronic effects.

Due to non-linear dissipation, the dissipation rate of 2,4-D in the field is difficult to determine. Therefore the EECs used in these risk assessments are based on observed environmental concentrations for short periods (2 to 6 days) and for longer periods (5 to 30 days). These time frames approximately correspond to time frames that are considered to be acute and chronic exposures, respectively.

- **Acute risk assessment**

  In general risk assessment for protection of the biota takes into consideration two factors.

  1) The typical environmental concentrations of the pesticide that the biota will be exposed to for short periods of time (1 day to 1 week).

     For 2,4-D DMA, the most typical environmental concentration that fish and most invertebrates will be exposed to for short periods is ~1.0 mg/L (Frank, 1972). However, JMPR (1997) indicates that the most typical use rate within the United States is 1.13 mg a.e./L (1.36 mg a.i./L). We will be using this slightly higher exposure rate to generate our acute risk assessment. However, for benthic (sediment) organisms, the most typical environmental concentration that benthic organisms (catfish, carp, scuds, various estuarine shrimp and aquatic sow bugs) could be exposed to is 0.100 to 0.450 mg/L for up to three months after heavy treatment in a TVA reservoir (Wojtalik et al, 1971 in Shearer and Halter, 1980).

     For 2,4-D BEE, the most typical environmental concentration that fish and most invertebrates will be exposed to for short periods (2-6 days) is 0.100 mg/L (Gallagher, 1992 cites Canadian Ministry of Environment, 1980). These values are believed to be representative of northwest waters with an open treatment area. Although impounded waterways may exhibit higher concentrations of 2,4-D (up to 0.330 mg/L), it is not believed that this data is relevant to public waterway control practices in Washington State. JMPR (1997) indicates that the most typical use rate within the United States is 1.36 mg a.e./L. However, for benthic (sediment) organisms, the most typical environmental concentration that benthic organisms (catfish, carp, scuds, various estuarine shrimp and aquatic sow bugs) could be exposed to is 0.05 to 0.46 mg/L found immediately after treatment at Lake Okanogan, B.C. 2,4-D persisted in the sediment for at least 8 days but had disappeared from the sediment by 17 days (Lim and Lozoway, 1978 in Shearer and Halter, 1980).

  2) The 96-hour toxicity (LC50) of the pesticide to the most sensitive environmentally relevant species.

For 2,4-D DMA, the most sensitive environmentally relevant fish size class and species are juvenile (fingerling) Chinook salmon, bluegill sunfish and Hamilton's carp *(Cirrhina mrigala hamilton*) with LC50s of ~100 mg a.i./L. For aquatic invertebrates the most sensitive size class and species are mature glass shrimp and juvenile grass shrimp with LC50s of ~0.15 mg/L.

For 2,4-D BEE, the most sensitive size class and species of fish and invertebrate are rainbow trout fry and the estuarine crab (*Chasmagnathus granulata*), which both have LC50s of 0.30 mg/L.

Since 2,4-D BEE is rapidly converted to 2,4-D acid, risk assessment should be done with primary emphasis on this metabolite of 2,4-D BEE. For 2,4-D acid, some of the most sensitive environmentally relevant species of fish are the common carp, cutthroat trout, white perch, and lake trout with LC50s of 20, 40, and 45 mg a.e./L respectively. There are other species that are more sensitive to 2,4-D acid like *Rasbora nielgereinsis* (LC50 = 5.6 mg a.i./L) and *Labeo boga* (LC50 = 3.4 mg/L). However, these species are not environmentally relevant to northwest waters. The most sensitive environmentally relevant species of invertebrate is the lined scud (*Gammarus fasciatus*) which has an LC50 of 3.2 mg a.e./L.

- **Acute risk assessment with 2,4-D DMA**

2,4-D DMA does not appear to have significant toxicity to the most sensitive fish species tested (Table 22). The risk quotient for the most sensitive fish species tested is less than the level of concern (0.1) for protecting the biota (RQ = EEC/LC50 = 0.014 = 1.36 ppm a.i/100 ppm a.i.) (Table 25). Even if the highest United States use rate of 4.0 mg a.e./L (4.8 mg a.i./L) is substituted for the most common use rate, the risk quotient for the most sensitive fish species tested is less than the level of concern (0.1) for the protection of the biota.(RQ = EEC/LC50 = 0.048 = 4.8 ppm a.i./100 ppm a.i.).

In US EPA evaluation of pesticides under FIFRA, an acute Risk Quotient of higher than 0.1 is interpreted as exceeding the Level of Concern, and leads to the conclusion that the risk may be unacceptable unless further analysis shows otherwise (Urban and Cook, 1985). The general practice in risk assessment is to estimate the Expected Environmental Concentration (EEC) based on the highest expected initial concentration and the most representative half-life. Therefore, 2,4-D DMA should be safe to fish when used at or below the maximum use rate.

However, the more sensitive species of invertebrates in the biota may be at risk. Environmental exposure of the most sensitive species of aquatic invertebrates could be fatal. This conclusion is apparent since the EEC and the LC50 for glass shrimp are 1.36 mg a.i./L and 0.15 mg a.i./L, respectively (Table 23). Although Hanson (1973) found that grass shrimp (a related species) was capable of avoiding 2,4-D BEE at concentrations as low as 1.0 mg/L. However, the conclusions of the authors are that this related species of shrimp is extremely sensitive to pesticides and is unlikely to avoid water polluted with pesticides. The grass shrimp is important to the food web where it occurs, and related species in Washington estuaries may also be of importance to the food web. Since the Risk Quotient for this species is above the level of concern (0.10), benthic organisms within the biota may be adversely

affected; RQ = EEC/LC50 = 9.1 = 1.36 ppm a.i./0.15 ppm a.i.). Even levels of 2,4-D bound (0.100 to 0.45 mg/L with a geometric mean of 0.21 mg/L) to the sediment due to treatment with 2,4-DMA have the potential to adversely impact this segment of the biota since the Risk Quotient for the most sensitive species is above the level of concern (0.1) as described by Urban and Cook, 1985 in Giddings (1999); RQ = EEC/LC50 = 0.67 = 0.100 ppm a.i/0.15 ppm a.i.

- **Acute risk assessment with 2,4-D BEE**

  2,4-D BEE appears to have significant toxicity to both fish and invertebrates in laboratory experiments. The most sensitive animals tested are rainbow trout (fry) and the first zoel of the estuarine crab (*Chasmagnathus granulata*) (LC50 for both species = 0.3 mg a.i./L) (Table 22 and 23). Other sensitive species include various salmon species (LC50 = 0.32 to 1.92 mg a.i./L) and scuds (*Gammarus spp*.) (LC50 = 0.44 to 0.76 mg a.i./L). If these species were exposed to the concentrations found at the bottom of Lake Okanogan immediately after treatment (EEC =3.25 mg/L) death could be the result as the LC50s are considerably below the EEC.

  Since 2,4-D concentrations dissipate to <0.100 mg/L within two to six days, this value can be considered the EEC for acute purposes. The risk quotient for the most sensitive fish and free-swimming invertebrate species tested using this field generated EEC for 2,4-D BEE is still higher than the level of concern (0.1) for protecting the biota (RQ = EEC/LC50 = 0.33 = 0.100 ppm a.i./0.300 ppm a.i.) (Table 25). However, a risk assessment conducted for 2,4-D acid is probably more realistic because of 2,4-D BEE's rapid degradation to the acid (Shearer and Halter, 1980, and JMPR, 1997). This approach has been recommended by JMPR (1997) and the normal field findings for a lack of observed toxicity for applied 2,4-D BEE are consistent with this approach.

  2,4-D acid is not toxic to fish species typically tested for acute toxicity. The most sensitive species is the common carp which has an LC50 of 20 mg a.e./L for 2,4-D acid, and the mullet, which have LC50s of 20 mg a.e./L and 12 mg a.e./L (13 mg a.i./L for 2,4-D sodium salt) respectively (12 mg a.e.)L. Other species (like cutthroat trout, white perch, lake trout, and rainbow trout) which most people believe are more sensitive than common carp were less sensitive in the studies we evaluated. However, since the highest concentration of 2,4-D in bottom water immediately after applications is 3.25 mg/L, the level of concern for protection of this segment of the biota is exceeded as the risk quotient is greater than 0.10 (RQ = EEC/LC50 = >0.16 = 3.25 ppm a.e./ 20 ppm a.e.). An identical conclusion for the invertebrate segment of the biota can be made since the LC50 of the most sensitive species is less than that for the common carp; the risk quotient for the scud (*Gammarus fasciatus*) is >0.1 (RQ = EEC/LC50 = 1.0 = 3.25 ppm a.e./3.2 ppm a.e.). Both common carp and lined scud are predominantly benthic in their habits. Therefore, there is an exposure risk to 2,4-D BEE and its acid for these species immediately after application.

  Nevertheless, the biota may still be safe from the effects of 2,4-D acid because of the rapid dissipation of 2,4-D to EECs that do not exceed 0.100 mg/L. If Lake Okanogan concentrations (0.100 mg/L) are considered to be the EEC, the risk quotient for the most sensitive organisms tested is <0.1, which is below the level of concern for protection of the biota (RQ = EEC/LC50 = 0.031 = 0.1 ppm a.e./ 3.2 ppm a.e.). This data indicates that when sensitive species are exposed to the ultimate product from

2,4-D BEE applications that the biota is not likely to be adversely affected. This does not imply that all species will be unaffected but that a very large percentage of them (95%) are likely to be unaffected by 2,4-D treatments. For safety of endangered biota, the level of concern is 0.05 (0.031 is below this value). Therefore, endangered species within the biota are likely to be protected under the application scenario.

The safety of 2,4-D BEE and its degradate (2,4-D acid) to some sediment organisms can be argued since the sediment will often exhibit concentrations of 2,4-D in that are much higher than in the water. Concentrations of 2,4-D in the sediment of up to 56 mg/L have been seen for four days or longer in situations where 2,4-D BEE pellets were misapplied or improperly formulated. However, more recent formulations have shown concentrations in the sediment at Lake Okanogan, B.C. of 0.05 to 0.460 mg/L (geometric mean = 0.15 mg/L) in the sediment with residues remaining for at least 8 days. Also, higher concentrations of 2,4-D (0.200 to 0.65 mg/L) have been observed for up to three-weeks at Currituck Sound, North Carolina. These concentrations are of potential concern in risk assessment since a risk assessment with the most sensitive sediment dwelling organisms would yield risk quotient that exceeds the level of concern (0.10). Using the lined scud and the most likely sediment concentration of 2,4-D acid likely to be encountered at Lake Okanogan, it appears that a risk assessment with the most susceptible species within the biota will not exceed the acute level of concern (0.10); RQ = EEC/LC50 = 0.05= 0.15 ppm a.e./3.2 ppm a.e.). If the majority of the 2,4-D is in the form of 2,4-D BEE, the likelihood of adverse risk is even greater since the acute level of concern (0.10) is exceeded; RQ = EEC/LC50 = 0.34 = 0.15 ppm a.i./0.44 ppm a.i. using *Gammarus* spp. as the most sensitive species).

Free-swimming invertebrates (like daphnids, freshwater prawns, and *Cyclops vernalis)* appear to be very tolerant to 2,4-D DMA and 2,4-D acid and its salts. The LC50 for these free-swimming invertebrates is greater than 37 mg a.i./L. Therefore, the free-swimming invertebrates should not be affected by even the maximum expected environmental concentration of these products (~0.100 to 1.36 mg a.i./L) during a 4-day exposure period since the risk quotient is below the level of concern of 0.1 for protection of the biota (RQ for 2,4-D DMA = 1.36 ppm a.i./>135 ppm a.i. = >0.01 for *Daphnia magna*; RQ for 2,4-D acid = 0.10 ppm a.i./37 ppm a.i. = 0.002 for C*yclops vernalis*; and RQ for sodium salt = ~0.09 ppm/932 ppm a.i. = 0.0001 for *Daphnia magna*.

Although 2,4-D DMA found at EEC levels in water and sediment and 2,4-D BEE and 2,4-D acid found at EEC levels in sediment, have the potential to damage benthic sediment invertebrates, the available field data indicates that most species are not likely to be harmed by the direct effects of 2,4-D. However, secondary effects like oxygen depletion may cause a shift in the dominant species present, while not adversely impacting the number of benthic organisms present or their diversity (Brooker, 1974 and Pierce, 1960, 1961 in Shearer and Halter, 1980, Marshall and Rutschky, 1974, Sarkar, 1991, Patnaik and Das, 1991 and Frank, 1972). See Section 4.3.1.3 for further details.

- **Chronic risk assessment**

  In general, chronic risk assessment for protection on the biota takes into consideration two factors.

1) The typical environmental concentrations of the pesticide that the biota will be exposed to for "chronic" periods of time. Chronic exposure is typically considered to be from 7 to 28 days. What is considered chronic exposure for a species, in part depends on the length of its life cycle. For example, a chronic exposure for *Ceriodaphnia dubia* may be considered to be four to seven days since this organisms is able to complete its full life cycle within this time frame. However, a chronic exposure for *Daphnia magna* is generally considered to be 28 days since it takes this much time for this species to go from a neonate to the completion of its reproductive period. Fish on the other hand, can take 30 to 90 days to go through a sensitive portion of their life cycle which is generally from egg to free-swimming fry (smoltification).

   For 2,4-D DMA, the typical environmental concentration that fish and most invertebrates will be exposed to for chronic periods is fairly low. However, the patterns of residue persistence are not predictable, especially during the first three weeks after treatment. However, it appears unlikely, that 2,4-D DMA will persist at concentrations higher than 0.3 to 0.4 mg/L for more than two-weeks (Gangstad, 1986, Schultz and Gangstad, 1975) at which point the concentration falls to approximately 0.005 mg a.e./L (0.006 mg a.i./L). Typical dosage rates for controlling waterhyacinth in Georgia and Florida are 2.24, 4.48 and 8.96 Kg a.e./ha and the mean residue concentration on day-1 after application is ~0.235 mg a.e./L. 2,4-D DMA dissociates very rapidly, and the half-life of the free acid appears to range from 4.4 days in Southern ponds (Schultz and Gangstad, 1975) to about 10 days in northern waters according to (Peterson et al, 1994). Since the degradation time is likely to be somewhat longer in Northwestern waters than in southern waters, a compromise half-life of 6.6 days was used to generate the EEC. The EEC was based on the geometric mean for the concentrations during 28-days. In 28-days, the concentration of 2,4-D dissipates from a typical 1 day mean of 0.235 mg a.e./L to a typical 28-day mean concentration of 0.012 mg a.e./L (0.014 mg a.i./L); the geometric mean over this time frame would be 0.075 mg a.e./L (0.091 mg a.i./L). However for benthic (sediment) organisms, the most typical environmental concentration that they (catfish, carp, scuds various estuarine shrimp and aquatic sow bugs, etc.) could be exposed to is 0.100 to 0.450 mg/L (geometric mean equals 0.21 mg/L) for up to three months after heavy treatment (Wojtalik et al, 1971 in Shearer and Halter, 1980).

   For 2,4-D BEE, the most typical environmental concentration that fish and most invertebrates will be exposed to for acute and chronic exposure periods ranges between <0.100 mg/L after 2 to 6 days dissipation time to <0.001 mg/L after 5 to 22 days dissipation time (Gallagher, 1992 cites Canadian Ministry of Environment, 1980). These values are believed to be representative of northwest waters with an open treatment area. Impounded waterways (Beulah Lake, Wisconsin) (Helsel et al, 1996) (may exhibit higher concentrations of 2,4-D (0.190 to 0.330 mg/L on day-1 and 0.03 to 0.090 mg/L on day 30), but this data is probably not relevant to public waterway control in Washington State. Concentrations are known to vary dramatically based on the depth of the water, temperature, and dilution factors. Although concentrations of 2,4-D may be quite high in closed ecosystems, the observed primary and secondary effects of 2,4-D applications would be reduced or undetected in a more open ecosystem (Gallagher, 1992). In the absence of a known half-life, the EEC for chronic

exposure is estimated by taking the geometric mean of the measured concentration at 2 to 6 days (<0.10 mg /L and the mean measured concentration at 5 to 22 days (<0.001 mg/L); this geometric mean value = 0.010 mg/L. For benthic (sediment) organisms, the most typical environmental concentration that they (catfish, carp, scuds, various estuarine shrimp, aquatic sow bugs, etc.) could be exposed to is 0.05 to 0.46 mg/L found immediately after application. The concentrations fell below the detection limit within 17 days of initial treatment. Assuming a geometric degradation, the half-life would not be longer than 8.5 days; therefore the concentrations in the sediment after 28 days would be estimated as 0 0.005 to 0.047 mg/L with the geometric mean EEC over a 28 day period of 0.020 to 0.18 mg/L.

2) The Chronic toxicity (NOEC) of the pesticide to the most sensitive environmentally relevant species.

   Since the database is so small, the chronic NOEC is estimated based on the geometric mean of the acute/chronic (LC50/NOEC) ratio for the 2,4-D products tested. The acute to chronic ratio and the geometric mean are presented in Table 14. The geometric mean for this ratio was calculated to be 18.0. When conducting chronic studies it is generally assumed that the chronic toxicity will be approximately 10-fold less than the acute toxicity; and the calculated acute to chronic ratio for 2,4-D indicates that it follows this estimate.

   From the acute to chronic ratio, an estimate of the chronic NOEC for the most sensitive species is made by dividing the acute LC50 by the acute to chronic ratio.

- **Chronic risk assessment with 2,4-D DMA**

  For 2,4-D DMA the most acutely sensitive species of fish are the bluegill sunfish, Hamilton's carp, Chinook salmon and the rainbow trout. For all these species the LC50 is ~100 mg a.i./L. The estimated chronic NOEC for 2,4-DMA against the most sensitive species of fish is 5.56 mg a.i./L. The estimated 2,4-D DMA EEC for a 28-day exposure period (0.091 mg a.i./L) does not exceed the level of concern (1.0) for chronic safety of 2,4-D DMA to the fish biota; RQ = EEC/NOEC = 0.016 = 0.091 ppm a.i./ 5.56 ppm a.i. Therefore, 2,4-D DMA should be safe to the most sensitive fish species within the biota. This does not imply that no species of fish will be adversely impacted by the application of 2,4-D DMA to control aquatic weeds, but that a great majority of the resident fish species will not be adversely affected.

  In US EPA evaluation of pesticides under FIFRA, a chronic Risk Quotient of higher than 1.0 (RQ = EEC/NOEC) is interpreted as exceeding the Level of Concern, and leads to the conclusion that the risk may be unacceptable unless further analysis shows otherwise (Urban and Cook, 1986). Since the level of concern for 2,4-D DMA in chronic fish testing is not exceeded, this product is believed to be safe for use even when the most sensitive species of fish are present. The general practice in risk assessment is to estimate the Expected Environmental Concentration (EEC) based on the highest expected initial concentration and the most representative half-life. Therefore, 2,4-D DMA should be safe to the fish of the biota when used at typical use rates.

For 2,4-D DMA, the most sensitive environmentally relevant invertebrate is the glass shrimp. For aquatic invertebrates the most sensitive size class and species are mature glass shrimp and juvenile grass shrimp with LC50s of ~0.15 mg/L. The estimated chronic NOEC for 2,4-DMA against the most sensitive species of invertebrate is 0.0083 mg a.i./L [LC50/(acute to chronic ratio) = 0.15 ppm a.i/18 ppm a.i.]. Since the estimated EEC for DMA after a 28-day exposure period is 0.091 mg a.i./L, the level of concern (1.0) for chronic safety of 2,4-D DMA to the invertebrate biota is exceeded; RQ = EEC/NOEC = 11.0 = 0.091 ppm a.i./ 0.0083 ppm a.i. Therefore, 2,4-D DMA may not be safe the most sensitive benthic invertebrates. *Palaemonetes spp*. are often studied because of their importance to the food web, abundance and sensitivity to pesticides. Other species of estuarine shrimp and other benthic invertebrates are not as sensitive to 2,4-D DMA. For example, the seed shrimp, chironomid, aquatic sowbug, lined scud, and glass worm have LC50s of 8.0, >100, >100, >100 and 890 mg a.i./L, respectively. If the most sensitive species amongst these benthic or partially benthic species is evaluated, the Risk Quotient is below the level of concern (1.0) for protection of this segment of the biota. The estimated chronic NOEC for seed shrimp is 0.44 mg a.i./L [LC50/(acute to chronic ratio] 8 mg a.i./18 depending) and therefore the chronic risk quotient is <1.0 (RQ = EEC/NOEC = 0.021 = 0.092 ppm a.i./0.44 ppm). Since 10 of the 11 species would not be at risk based on an chronic RQ of less than unity, the great majority of the species in this segment of the biota would be protected if 2,4-D DMA is used at typical use rates. If the fish species are also included, 19 of the 20 species would not be at risk base on a chronic RQ of less than unity for all but one of these species.

A typical sediment contains 0.1 to 0.45 mg/L of 2,4-D DMA for three months after application. If the average sediment is considered to contain a typical concentration, the EEC can be estimated to be 0.21 mg/L. Therefore the risk quotient will again be above the level of concern for chronic protection of the biota from typical applications of 2,4-D DMA. However, even if one species in 20 is not discounted, 95% of the species in the biota will still be protected when 2,4-D DMA is used in a typical manner. E.g. chronic RQ for seed shrimp exposed to sediment = 25 = 0.21 ppm a.i./0.0083 ppm a.i.; and chronic RQ for pink shrimp 0.47 = 0.21 ppm a.i./0.44 ppm a.i.

- **Chronic risk assessment to 2,4-D BEE**

For 2,4-D BEE, the most sensitive size class and species of fish and invertebrate are rainbow trout fry and the estuarine crab (*Chasmagnathus granulata*) first zoels, which both have LC50s of 0.30 mg/L. Therefore, the estimated chronic NOEC for the most sensitive species would be 0.017 ppm a.i. (acute LC50/chronic NOEC = 0.017 mg a.i./L = 0.3 ppm a.i./18). As discussed previously, a typical chronic EEC for open northwest waters was calculated as 0.01 mg/L using the geometric mean of the short term environmental concentrations (<0.100 mg/L) and the long term environmental concentration (<0.001 mg/L). Using this estimated NOEC, the chronic Risk Quotient is below the level of concern (1.0) for chronic exposure (RQ = 0.59 = 0.01 ppm a.i./0.017 ppm). Therefore, the biota should be able to withstand the effects of 2,4-D BEE if it is applied at a treatment level typical of that described for Canada by Gallagher (1992). However, if exposure through contact with sediment is a serious issue, the more sensitive species could be adversely affected by chronic exposure to 2,4-D BEE.

As noted earlier, the chronic sediment EEC ranges between 0.019 to 0.19 mg/L in the waters of Lake Okanogan. If the geometric mean of these two values is considered to be the most typical concentrations, the chronic EEC becomes 0.06 mg/L. Since first zoel estuarine crabs may not be significantly exposed to sediment, a sensitive benthic (sediment) organism was chosen for risk analysis with 2,4-D BEE. Scuds (*Gammarus spp*.) are almost as sensitive to 2,4-D BEE as first zoel estuarine crabs (LC50 = 0.44 mg a.i./L). Therefore, the estimated chronic NOEC for *Gammarus spp*. would be 0.024 mg a.i./L (0.44 ppm a.i./18) using the estimated chronic NOEC for scuds, the calculated chronic risk quotient is above the level of concern (1.0) for protection of this segment (benthic organisms) of the biota from the effects of properly applied 2,4-D BEE (RQ = EEC/NOEC = 2.5 = 0.06 ppm a.i./0.024 ppm a.i.).

Since sediment appears to be risk factor for the biota when 2,4-D BEE is used to control weeds, an additional risk analysis follows with 2,4-D acid, as recommended by JMPR, 1997 when risk analysis with the ester indicates an unacceptable risk.

Since 2,4-D BEE is rapidly converted to 2,4-D acid, risk assessment should be done with emphasis on this metabolite of 2,4-D BEE. For 2,4-D acid, the most sensitive environmentally relevant species of fish is the common carp with an LC50 of 20 mg a.i./L. There are other species that are more sensitive to 2,4-D acid like *Rasbora nielgereinsis* (LC50 = 5.6 mg a.i./L) and *Labeo boga* (LC50 = 3.4 mg/L). Other species usually considered sensitive (like cutthroat trout, white perch, lake trout, and rainbow trout) were not as sensitive as common carp in the evaluated studies. However these species are not environmentally relevant to northwest waters. The most sensitive environmentally relevant species of invertebrate is the lined scud which has an LC50 of 3.2 mg a.e./L. Estimated chronic NOECs for these species are 1.11 and 0.18 mg a.e./L (1.11 = 20 ppm a.e./18 and 0.18 = 3.2 ppm a.e./18), respectively and the EECs are considered to be the same as previously described under 2,4-D BEE since it is not apparent from the literature source whether the expressed values are for 2,4-D BEE, 2,4-D acid or total 2,4-D. In all cases, the estimated chronic NOEC is higher than the calculated EEC. Therefore, the level of concern is not exceeded with this active principal of 2,4-D BEE (2,4-D acid) and most sensitive tested species. For the lined scud, the chronic Risk Quotient is less than unity for water and sediment (RQ water = EEC/NOEC = 0.056 = 0.01 ppm a.e./0.18 ppm a.e.; and RQ sediment = 0.33 = 0.06 ppm a.e./0.18 ppm). Therefore, biota exposed to 2,4-D acid through the proper applications of 2,4-D should be protected from adverse affect. This does not imply that all species will not be affected but that the great majority of them will not be affected. When the level of concern is not exceeded by the risk quotient, it is generally assumed that 95% of the species in the treated biota will tolerate treatment with the studied pesticide.

It is note worthy that the free-swimming invertebrates are chronically more tolerant than benthic organisms to 2,4-D DMA, 2,4-D BEE and 2,4-D acid and its sodium salt. For free-swimming invertebrates, the chronic risk quotient is above the level of concern of 1.0 for protection of the biota. For example: RQ of 2,4-D DMA = 1.36 ppm a.i./27.5 ppm a.i. 0.005 for *Daphnia magna*; RQ of 2,4-D acid = 0.01 ppm a.i./26 and ppm a.i. = 0.0003 for *Ceriodaphnia dubia*.

Although risk analysis indicates the presence of risk to biota from the use of 2,4-D BEE in both acute and chronic tests, its breakdown product (2,4-D acid) appears to present an acceptable risk when 2,4-D BEE is used according to typical application

practices. As 2,4-D BEE rapidly degrades to 2,4-D acid, it can be safely used as an aquatic herbicide in Washington State. Summaries of the Risk Assessments used in this section are provided in Table 25.

4.3.3 2,4-D Potential Impacts to Terrestrial Wildlife and Plants

The goal of this section is to discuss the effects of single applications/exposures and chronic applications/exposures to terrestrial wildlife (birds and mammals) and terrestrial plants exposed to aquatic herbicides containing 2,4-D (Aqua Kleen® and Navigate®) which are registered for use in the State of Washington. Possible effects on the food chain and threatened and endangered species will also be discussed as well as ways to mitigate exposure of these organisms to the treatment. The information presented summarizes toxicological studies to determine the effects of 2,4-D containing products on plant and animal species.

Aqua Kleen® and Navigate® both contain 2,4-D butoxyethyl ester (BEE) which is rapidly converted to the acid form of 2,4-D in the environment. Therefore, for the purposes of discussing the impacts of aquatic applications of 2,4-D on terrestrial plants and animals in Washington State, these are the forms of 2,4-D that will be discussed in detail.

4.3.3.1 Effects on Amphibians

Acute data for 2,4-D DMA salt and 2,4-D acid are available for several species of amphibians (Table 26). The acute 96-hour LC50 for 2,4-D DMA ranged from 287 mg a.i./L for the frog (*Limondynastes peroni*) to 337 mg a.i./L for the Leopard frog (*Rana pipiens*). The acute 96-hr LC50 for 2,4-D acid was 359 mg a.i./L for the Leopard frog and 8.05 mg a.i./L for the Indian toad (*Bufo melanostictus*). These data indicate that 2,4-D DMA is relatively non-toxic to amphibians while 2,4-D acid is relatively non-toxic to the Leopard frog and moderately toxic to the Indian toad. Although this data is limited to only a few studies it appears that 2,4-D acid may be more toxic in these species than 2,4-D DMA (JMPR, 1997).

4.3.3.2 Effects on Terrestrial Animals (Birds, Mammals and Insects)

Studies have been conducted to assess the toxicity and other impacts of 2,4-D acid and the 2,4-D BEE containing products Aqua Kleen® and Navigate® on various animal groups. Acute oral (LD50), acute dietary (LC50) and chronic dietary studies are presented.

4.3.3.2.1 Effects on Birds

- **Acute effects on birds**

  Acute oral data for 2,4-D acid and 2,4-D BEE are available for several different species of birds (Table 27). The acute oral LD50 of 2,4-D acid ranges from 200-400 ppm for chukar (*Alectoris graeca*) to >5,000 ppm for Japanese quail (*Coturnix japonica*). The lowest acute oral LD50 for 2,4-D BEE listed is > 2,000 ppm for bobwhite quail (*Colinus virginianus*). This data indicates that the 2,4-D acid is moderately toxic to practically nontoxic to birds when orally dosed and that 2,4-D BEE is practically nontoxic to birds when orally dosed.

Acute dietary (LC50) data is available for 2,4-D acid and 2,4-D BEE for both bobwhite quail and mallard ducks (*Anas platyrhynchos*). The LC50 for 2,4-D acid for both mallard ducks and bobwhite quail is > 5,620 ppm. The lowest LC50 for 2,4-D BEE is >5,000 ppm for bobwhite quail and > 5,620 ppm for mallard ducks. These data indicate that both 2,4-D acid and 2,4-D BEE are practically nontoxic to mallard ducks and bobwhite quail when consumed in the diet.

- **Chronic effects on birds**

  No data was found on the chronic effects of 2,4-D acid or 2,4-D BEE on birds. However, Puvanesarajah and Bliss (1992) studied the metabolism of 2,4-D acid in poultry and found that after dosing at 18.3 ppm in the diet for seven days 81% - 114% of the dose was recovered in the excreta and <0.1% was found in the eggs and poultry tissues. This data indicates that doses of up to 18.3 ppm 2,4-D acid in the diet is not chronically toxic.

- **Possible effects on the food chain**

  The potential of 2,4-D acid or 2,4-D BEE to accumulate in birds and mammals has not been well studied. However, it is unlikely that bioaccumulation will occur due to the low K_{ow} values for both 2,4-D acid and 2,4-D BEE. 2,4-D BEE is converted to acid quickly in animals and 2,4-D acid is rapidly eliminated (Puvanesarajah and Bliss, 1992 and Krautter and Downs, 1996).

4.3.3.2.2 Effects on Mammals

- **Acute oral**

  Acute oral rat data is available for 2,4-D BEE (LD50 866 mg/kg) (Table 28). Acute oral data is available for more than one mammalian species for 2,4-D acid and LD50 values range from 100 ppm in guinea pigs to between 400-800 ppm in mule deer. This data indicates that 2,4-D BEE is slightly toxic and that 2,4-D acid is moderately to slightly toxic.

- **Subchronic and chronic effects on mammals**

  A 2-generation rat reproduction study (WIL Research Laboratories Inc, 1984) was conducted using 2,4-D acid. The no observable effect level (NOEL) was 5 mg/kg/day based on slight decreases in pup body weights seen at the next highest dose level. The results of a one year dog dietary study with 2,4-D acid (Dalgard, 1993) had a NOEL of 1 mg/kg/day based on decreased weight gain and poor feed consumption at the next highest dose level. The results of a 2-yr dietary study in mice using 2,4-D acid (Johnson, 1995) found no statistical differences between controls and treated animals in any of the parameters tested at any dose levels including the high dose of 125 mg/kg/day. The results of these tests indicate that terrestrial species may be effected by long term exposure to 2,4-D acid in the diet. However, use patterns for aquatic applications of 2,4-D in the State of Washington make long term exposure unlikely.

- **Mitigation of effects on birds and mammals**

  - **Mitigation measures specific to 2,4-D**

    There are two common routes of exposure of livestock and terrestrial wildlife to aquatic applications of 2,4-D products. The two routes are exposure through drinking water treated with products containing 2,4-D or eating aquatic plants, fish or other aquatic organisms from the treatment site. Based on the acute and chronic studies listed above 2,4-D BEE and its breakdown product 2,4-D acid (used as an aquatic herbicide) do not pose a significant acute or chronic risk to terrestrial birds or mammals. However, in order to mitigate possible problems with the watering of dairy animals, the labels for these products do not allow applications to water bodies that are used for this purpose. The best mitigation or control for wild animals and birds is to follow the label directions. Many studies have been run on these products to ensure their safety to wildlife and the label directions and warnings reflect the results of these studies. Therefore, if the chemicals are applied according to the label, the effect on terrestrial wildlife should be minimal.

  - **General mitigation measures**

    Although 2,4-D BEE and 2,4-D acid do not pose significant risks to terrestrial wildlife, the following measures should be considered prior to all aquatic herbicide applications. One possible mitigation measure would be not allowing applications if large populations of birds use shorelines or islands in the water body to be treated for nesting until after nesting is complete. Another mitigation measure would be to time applications to avoid migratory waterfowl and other bird species that use certain water bodies during migration. Efforts to avoid effects on migratory and nesting birds would best be coordinated between the permit writer and The Washington State Department of Fish and Wildlife (WDFW) prior to granting the permit.

- **Effects on endangered terrestrial plants, birds and mammals**

  A list of endangered terrestrial plants, birds and mammals is located in Table 32. Minimal effects to these organisms are expected from application of aquatic herbicides containing 2,4-D. Mitigation of possible effects on listed endangered species is best accomplished by following the mitigation sections above for terrestrial plants, birds and animals. As stated in the previous mitigation sections, the best way to mitigate possible effects on all terrestrial species is to follow the directions listed on the label.

  Other mitigation measures involve the contact of WDFW by the issuer of the permit to ascertain if any endangered species may be affected by the application of the chemical to the water body in question. Questions asked by the permit granter would ascertain if any resident endangered bird or animal species are known to use the water body in question or its shorelines or islands as breeding or forage areas, or if the application coincides with the migration of any endangered species. If endangered species are present mitigation measures may involve postponing application until after the breeding season or postponement of application until after migration of the species in question. Use of an alternate means of control (i.e.

mechanical, manual, or biocontrol) may also be an option if the risk is determined to be too great to the species in question.

4.3.2.3 Effects on Terrestrial Plants

2,4-D is a herbicide and plant growth regulator. Therefore, it can affect a wide variety of terrestrial plants. In general, the most susceptible species are dicot broadleaf plants while monocots (grasses, grains, and other narrow leaf plants) are the most resistant. Adverse effects to terrestrial plants depend on a wide variety of factors including application rate and number of applications, soil absorption and the susceptibility of the species in question.

Crops (especially broadleaf plants) exposed to irrigation water containing 2,4-D may be adversely affected. However, when water containing 0.025 to 0.061 mg a.e./L was used to irrigate certain crops, levels found in the crops were below the limit of detection or below the FDA tolerances for these crops. For example, levels found in or on potatoes, grain sorghum, carrots, romaine lettuce and onions were 0.03-0.012, <0.05 to 0.12, 0.02 to 0.06, 0.11 to 0.33 and <0.01 mg/Kg, respectively.

Another study found that most tested crop plants (potatoes, grain sorghum, soybeans, carrots, Romaine lettuce, onions, sweet corn, dwarf corn and cotton) are not adversely effected when irrigated with water containing up to 5.5 mg a.e./L 2,4-D. However, grapes, sugar beet seedlings and possibly red Mexican beans may have irreversible damage if 2,4-D is present at concentrations as low as 2.21 mg a.e./L. Most other crop plants will exhibit some signs of damage after irrigation but will not suffer any significant reduction in yields or significant residues in their edible portions. It should be noted that not all crops in the study were irrigated at the highest rates of 2,4-D containing water but were irrigated at levels believed to be the maximum typically encountered during irrigation.

- Mitigation of effects on terrestrial plants

  The main route of exposure for terrestrial plants to aquatic herbicides are through spray/drift and the use of treated water as irrigation. The two 2,4-D labels currently registered for aquatic use in the State of Washington do not have an over spray/drift risk component as they are granular products. If the label restrictions are followed, the risk of terrestrial plants to aquatic applications of 2,4-D in Washington State should be negligible.

4.4 ADDITIONAL POTENTIAL DIRECT AND INDIRECT IMPACTS OF HERBICIDE USE ON WET LAND ENVIRONMENTS

Summary: The presence of 2,4-D products at concentrations effective against weeds in wetland environments may adversely effect these environments. Dilution effects should mitigate the effects of 2,4-D so that it does not effect aquatic plants or non-target animals in marshes, bank and estuarine areas. The presence of 2,4-D in the lotic environment, due to outflow from a lake or pond, may cause the destruction of aquatic plants that are favorable to the production of appropriate habitat for sunfish, minnows and bass. The subsequent habitat, with a low level of areal aquatic weed cover and a benthos consisting primarily of sand and gravel, would be more appropriate to the production of salmonids.

*The estuarine environment may be affected by the use of 2,4-D. Some of the most susceptible species of invertebrates are estuarine species including grass shrimp, glass shrimp, and seed shrimp. The estuarine crab (*Uca uruguayensis*) has been eliminated from some estuarine areas due to the effects of 2,4-D. It is unclear if this is due to an avoidance response or an acute or chronic toxicity response. The presence of estuarine crabs and estuarine shrimp like those mentioned above are critical since they are important to the maintenance of the food web that attracts both game fish and fish of commercial importance. Anaerobic sediment typically found in estuaries can lead to the production of 2,4-chlorophenol or 4-chorolpehnol which are both very toxic to some species of aquatic macrophytes, marine phaeophytes, various beneficial fungal species and amphipods.*

Failure to control emersed, floating, marginal and bank weeds can cause the native vegetation to be crowded out producing dense monoculture stands of noxious and invasive weeds, leading to the degradation of natural habitats and an economic burden for residents who must keep water flowing or navigable.

Pasture land flooded with water containing 2,4-D may lead to the destruction of various turf plants. In addition, sensitive crop plants like Mexican red beans, lentils, peas, grapes and tomatoes, may be irreversibly damaged by the presence of 2,4-D in irrigation or floodwater. Other non-target plants, as discussed in Table 13, may be adversely impacted.

Because of the manner in which 2,4-D products are applied, significant impact to other wetland environments is unlikely. There may be some tendency for drift into other wet land environments or a flow of water into estuarine, palustrine, riparian, lentic or lotic environments. However, it is not anticipated that the impact would be measurable due to dilution effects, as treated ponds, lakes, and canals normally flow into streams and rivers and ultimately into estuaries.

4.4.1 Estuarine (Intertidal) Environments

Water from a stream or river containing 2,4-D may flow into an estuary. However, dilution effects from the water already present in the estuary and diurnal tides should dilute 2,4-D to levels where it is not significant in the water column. Some estuaries have sediment that is anaerobic, and there potentially could be a build up of 2,4-D in this anaerobic sediment. It has been demonstrated that 2,4-D in anaerobic conditions does not degrade readily (Boyle et al, 1999 and Elder (1980 in JMPR, 1997) and Kuhlmann & Kaczmarzcyk (1995 in JMPR, 1997). Under anaerobic conditions, these sediments either did not significantly degrade 2,4-D or reductively dechlorinated 2,4-D or 4-chlorphenol through a 2,4-chlorophenol intermediate or 4-chlorophenyl acetate. Contaminated estuarine sediments from San Diego, California and Arthur Kill, New York/New Jersey degraded essentially 100% of the 2,4-D present in approximately 80- to 140-days days. Uncontaminated estuarine sediments from Tuckerton, New Jersey and Flax Pond, New York demonstrated no significant degradation of 2,4-D after >160 days which may have been due to the lack of bacteria that were physiologically able to use 2,4-D as a carbon source (See Section 3). The addition of sulfate as an electron acceptor, hydrogen as an electron donor and acetate as a carbon source increased the rate of 2,4-D degradation in contaminated San Diego sediment by almost 50%. It was suggested that the addition of sulfate, hydrogen and acetate to contaminated sediments could be considered as a viable

remediation strategy in contaminated marine and estuarine systems. However, addition of sulfate may adversely impact marine species.

The production of 2,4-dichorophenol and ultimately 4-chorophenol may have ecotoxicolgical ramifications. 2,4-chlorophenol was not present in the environment at high concentrations since it was metabolized to 4-chlorophenol as an ultimate product. 2,4-chorophenol is toxic to a variety of species. 2,4-chlorophenol has been reported to be toxic to certain fungal propagules at concentrations below those detected in soil (Short et al, 1991); hyphomycetes, zygomycetes and ascomycetes fungus would not grow on Martin's media that had been treated with 0.050 mg/L. 2,4-dichlorophenol has been shown to be toxic to a number of species of plants, animals and microbes. 2,4-dichlorophenol is toxic to *Lemna gibba* at 9.2 μM/L (~1.5 mg/L) (Ensley et al, 1994), the marine phaeophyte (*Phyllospora comosa*) early zygotes at less than 0.0001 mg/L (Burridge et al, 1995), the marine amphipod (*Allocrestes compressa*) at ~1.0 mg/L, goldfish at approximately 20 mg/L (Kamler et al, 1974), fathead minnow at 3.1 mg/L (Holcombe and Phipps, 1979 in Shearer and Halter, 1980); with all values expressed as LC50s. The toxic symptoms were usually death but fathead minnow exhibited reduced growth with exposures of 3.1 mg/L. The intrinsic toxicity of 2,4-dichlorophenol is often higher than 2,4-D acid or 2,4-D DMA. However, 2,4-dichlorophenol is degraded very rapidly to 4-chlorophenol and other less toxic products, it is unlikely to be a problem in the environment. However, since 4-chlorophenol persists for at least 80 days in laboratory experiments with anaerobic estuarine sediment (Boyle et al 1999). Therefore, a demonstration of its toxic properties would be of value in assessing risk to the resident biota from the use of 2,4-D.

For 2,4-D BEE the estuarine/marine organisms had LC50s that ranged from 0.30 mg a.i./L for *Chasmagnathus granulata* first zoels to 3370 mg a.i./L for the adults; but more typically the LC50s ranged around 1.0 to 2.2 mg a.i./ L for various species of estuarine shrimp. For 2,4-D DMA, the estuarine/marine species had LC50s that ranged from ~0.15 mg a.i./L for *Palaemonetes spp*. to >1000 mg a.i./L for fiddler crab with typical LC50s ranging from 100 to 200 mg a.i./L. Estuarine shrimp are often tested for toxicity because of their importance in the aquatic food web, their great abundance and sensitivity to pollution and pesticides.

Some of the estuarine/marine species are more susceptible than the freshwater species. For example, Hansen (1973) noted that estuarine shrimp were chosen for his avoidance experiments with 2,4-D BEE and other pesticides (DDT, Endrin, Dursban, Malathion, and Carbaryl) as shrimp may be very vulnerable to pollution because the are (1) extremely sensitive to pesticides and (2) are generally unlikely to avoid water polluted by pesticides. Consequently, it is important that pesticides destined for use in and near estuaries be tested to determine their toxicity to shrimp and the capacity of shrimp to avoid them. This is particularly important since some species of estuarine shrimp are important in the food web and particularly abundant in the ecosystem.

Failure to properly assess the cumulative properties and toxicity of a pesticide prior to its wide spread use can lead to situations as described for Samborombon Bay, Argentina where extensive contamination of the Rio de la Plata estuary has eliminated *Uca uruguayensis* (Estuarine crab) (Rodriguez and Lombardo, 1991). The removal of this species was attributed to known high levels of aldrin and DDT and possibly ethyl-parathion and 2,4-D Iso-BEE due to their extensive use in the region. It was pointed out that 2,4-D is able to persist in sediment for several months at high concentrations and that

herbicides account for 60% to 70% of the total pesticides applied on crops in Argentina. (Rodriguez et al, 1994). Concern was expressed surrounding the effects of 2,4-D on estuarine crabs because "all stages of its life cycle are important in the aquatic trophic web, which also includes many fish species with great commercial and sport fishing value (Rodriguez and Lombardo, 1991)."

However, this observation was based on the absence of *Uca uruguayensis* from an area potentially contaminated with high levels of 2,4-D and ethyl-parathion and the fact that this species has a much lower "resistance" to these toxicants than the other species of estuarine crab (*Chasmagnathus granulata*). This may or may not be true resistance due to selection pressure. However, species with a short life span may rapidly develop resistance to pesticides. However, (Chambers et al, 1977, and Fabacher and Chambers, 1974 in Shearer and Halter, 1980) noted that the mosquito fish (*Gambusia affinis*) appeared to be cross-resistant to 2,4-D due to prior selection with chlorinated hydrocarbons other than 2,4-D. This may also be the case with *Chasmagnathus granulata* since exposure to other chlorinated hydrocarbons for species living in Samborombon Bay is well documented (Rodriguez et al, 1994).

Even with extensive dilution, the more sensitive species may be adversely affected if they inhabit an estuarine environment where the anaerobic sediment incapable of degrading 2,4-D. However, there are no verified field examples of the effects of 2,4-D on estuarine species.

4.4.2 Palustrine (Marshy) Environments

Extensive growth of rooted aquatic macrophytes may effectively dam a marsh and increase the depth of the palustrine system by several fold. In this way aquatic macrophytes assist in spreading waters onto the surrounding land to increase its fertility and provide additional areas for fish and amphibians to feed and spawn (Goldman & Horne, 1983). Even without flooding, these plants may have an effect on habitat suitability for wild birds, mammals and other terrestrial organisms.

The dominant plants found in palustrine environments are emersed (emerged). Most emersed plants are not likely to be adversely impacted at the concentrations of 2,4-D BEE used to control fully aquatic weeds. However, floating and rooted submersed plants, that are typically found in a palustrine environment may be impacted by water that enters these areas from lakes and ponds. If these rooted macrophytes are destroyed due to a herbicide, there will be less tendency for the marsh to flood and therefore potential habitat will be lost to fish and amphibians. Also, if these plants are lost, and flooding does not occur, loss of suitable habitat for wild birds and mammals may occur.

4.4.3 Riparian (Margin and Bank) Environments

2,4-D products are used to treat the submerged margins of lakes and ponds to eliminate weeds and algae that interfere with recreational use. Both 2,4-D DMA and 2,4-D 2-EHE are used to control weeds along rights-of-way, ditch banks, and in the case of 2,4-D DMA broad leaf, floating, emersed, marginal and bank weeds on ponds and lakes.

The application rates of these herbicides has the potential to impact any non-target broadleaf species that they come in direct contact with, particularly those species that are described in Table 9. Also, according to Ebasco (1993), these 2,4-D products have

particular utility in control of purple loosestrife (*Lythrum salicaria*) which proliferates in wetland habitats, invading wet meadows, pasture wetlands, cattail marshes, stream and river banks, lake shores, irrigation ditches, drainage ditches and storm water retention basins. Purple loosestrife prefers moist organically rich soils. The species crowds out native vegetation, creating dense monoculture stands that provide poor habitat for native wildlife. In producing thick stands, it chokes out waterways, slowing flow and promoting siltation. Invasion of this noxious weed results in degradation of natural habitats for both native vegetation and wildlife as well as causing economic burden on farmers whom must keep irrigation water flowing.

2,4-Dichlorophenoxyacetic acid, particularly 2,4-D DMA and 2,4-D 2-EHE, under a wide range of environmental conditions is used primarily along rights-of-way, and on the banks of irrigation canals, drainage ditches, lakes and ponds. To control annual and perennial broad leaf weeds along irrigation canals, 2,4-D DMA should be applied at 1.1 to 2.2 Kg a.e./ha (0.95 to 1.9 lbs a.e./acre); when used for controlling bank weeds, this herbicide should be dissolved in 20 to 100 gallons of water per acre. When using 2,4-D 2-EHE for similar control purposes along drainage ditches, 2,4- D 2-EHE should be applied at 1.0 to 2.4 Ka a.e/ha (0.89 to 2.1 lbs a.e./acre). Although 2,4-D 2-EHE should not be applied directly to water, 2,4-D DMA is applied directly to water to control waterhyacinth at 2.12 to 4.24 Kg a.e./ha (1.9 to 3.8 lbs a.e./ha) and in TVA reservoirs to control Eurasian watermilfoil at 10.6 to 42.6 Kg a.e./ha (9.5 to 38 lbs a.e./acre). 2,4-D BEE granules may be applied to lakes and ponds throughout the United States at 21 to 42 Kg a.e./ha (19 to 38 lbs a.e/acre) primarily to control Eurasian watermilfoil but also to control other species as described in Section 1.0. For the latest information on the use of 2,4-D products, the current labels and permits must be consulted.

2,4-D products may be used for other plant control situations including applications to upland forests, range and pastureland cropland, wetlands and other waterway management. For the control of weeds, 2,4-D acts as a growth regulator with auxin-like properties. At relatively low dosages or when applied by low-volume methods, 2,4-D can be sprayed on plant leaves and translocates to kill the plant root. At somewhat higher dosages, the herbicide can be applied directly to soil to be absorbed by plant root systems.

Any non-target plants and animals have a potential to be impacted by 2,4-D products as described in Sections 4.2.3.1 and 4.2.3.2. Please review these sections for information on 2,4-Ds acute (Table 2, 17 and 22) and chronic (Table 2 and 23) effects on non-target aquatic plants and animals.

4.4.4 Other Wetland Environments

Pasture, which is permanently or partially flooded, may be impacted by 2,4-D treated waters if flood waters come from lakes, ponds or canals treated with 2,4-D for weed control. Although, 2,4-D does not typically impact grasses adversely, some of the more sensitive species such as bentgrasses, carpet grass, buffalo grass, and Saint Augustine grass and also turf plants which are not true grasses, such as dichondra, alfalfa, sweet clover and other legumes, may be impacted adversely. Although no efficacy claim has been made by the manufacturer, it is difficult to determine if treated water from a lake or pond will impact a site planted with these “grasses” adversely. Many sensitive plants are not affected by irrigation, agricultural spray or flood waters that have their origin in treated water bodies**.** Section 4.2.5 deals with the effects of irrigation water, agricultural

sprays and flood water. Most of the tested crop plants are not affected by water "irrigated" with water containing normal concentrations (up to 5.5 mg a.e./L of 2,4-D). However, grapes, sugar beet seedlings and possibly red Mexican beans may suffer irreversible damage if 2,4-D is present at concentrations as low as 2.21 mg a.e/L. Most other crop plants, while exhibiting signs of damage after irrigation with 2,4-D, did not have reduced yields or significant residues in their edible tissues. Residues were either below the limit of detection, insignificant or much lower than FDA tolerances. The crops that were investigated included potatoes, grain sorghum, soybeans, carrots, Romaine lettuce, onions, red Mexican beans, sugar beets, grapes, sweet corn dwarf corn, and cotton. Not all crops were irrigated with the highest rates of 2,4-D containing water but were irrigated at concentrations that were believed to be the maximum that would typically be encountered in water used for irrigation (Gangstad, 1986).

4.4.4.1 Lentic Environment

Potential impacts on lentic and lotic environments as to the chemical ecology were discussed extensively in Section 4.2.3.1. Effects on the biota in these environments were discussed extensively in Section 4.3.

4.4.4.2 Lotic Environment

The lotic environment can be influenced by the presence of 2,4-D in water from a lake or pond outlet. If 2,4-D is present at levels that controls weeds and the outlet gate is closed, a type of habitat favorable to sunfish and amphibians will develop. If the outlet gate is open, another type of habitat more favorable to salmonids may develop. Also if protracted (seasonal) contact with water containing 2,4-D DMA at concentrations of as low as 0.1 mg/L occurs, *Myriophyllum sibericum* and *Potamogeton pectinatus* may be eliminated by the end of the growing season (Forsythe et al, 1997).

- **Closed outlet gate or absence of 2,4-D**

  If the outlet gate from a pond or lake to a river or stream remains closed, dense growths of rooted aquatic macrophytes may effectively dam rivers and streams and increase the depth of the lotic system by several fold. In this way the aquatic macrophytes assist in spreading waters onto the surrounding land to increase its fertility and provide additional areas for fish and amphibians to feed and spawn (Goldman & Horne, 1983). Similar effects may occur if the lake or pond is not treated with 2,4-D.

- **Open outlet gate in presence of 2,4-D**

  If water that contains 2,4-D at effective concentrations passes through the outlet gate of a lake or pond into a river or stream, the rooted aquatic macrophytes may be destroyed. This can have a substantial impact during the next spate or high water event. Normal spring high flows, in absence of rooted aquatic macrophytes, can dig up and kill large numbers of benthic organisms while summer spates (uncommon in Washington) can completely denude streams of benthic biota.

  Most biota avoid spates either by migrating to calm back waters or by having life cycles which are terrestrial or aerial at these times. However when floods occur at

unusual times the fauna may be severely depleted and require several years to recover (Goldman & Horne 1983).

Larger organisms, like salmonids, choose to ascend rivers or streams during spates or high water because there are fewer shallow water barriers. Severe floods are detrimental to smaller biota if they leave only inhospitable rocks and gravel. However, these increased water levels may improve fish migration by removing major obstacles. Adequate water levels can improve the environment for salmonid mating and egg survival by removing excessive silt. These benefits cannot occur if the lotic system has been dammed by aquatic weeds.

4.5 UNCERTAINTY ANALYSIS

Summary: The uncertainty analysis indicates that field studies often reflect the risk analysis that has been used to generate the label. Models that have been used since 1975 indicate that an acute risk quotient of <0.1 or a chronic risk quotient of <1.0 reflects safety of the product to exposed aquatic animals under field situations. An acute risk quotient is generated by dividing the short-term predicted environmental concentration (short-term EEC) by the acute LC50 of the most sensitive species of concern within the ecosystem. Providing a 10-fold safety factor will insure that less than 5% of the animals with similar sensitivity will be adversely affected. Dividing the long-term EEC by the chronic NOEC or chronic predicted NOEC for the most sensitive species generates a chronic risk quotient. A safety factor is not necessary in chronic risk assessment since all animals with a similar sensitivity will also not be affected by exposure to chronic EECs for the compound being evaluated. For 2,4-D DMA and 2,4-D acid, field studies indicate that risk quotients can predict the safety or lack of safety of a herbicide to the biota with reasonable accuracy. However, while 2,4-D BEE has the potential for adverse impact to the biota based on its laboratory toxicity, field studies have been unable to document direct adverse effects on fish or benthic invertebrates. Several studies in TVA reservoirs, Currituck Sound, North Carolina and British Columbia are uniform in their appraisal of no observed direct effect on fish populations as a result of 2,4-D BEE treatments. "The toxic potential of 2,4-D BEE as measured in the laboratory is apparently not realized under the 2,4-D BEE concentrations and environmental conditions present during actual field use. The fairly rapid hydrolysis of 2,4-D BEE in nature is probably the key factor responsible for this general lack on environmental toxicity (Shearer and Halter, 1980 citing various authors)." 2,4-D DMA has an acute risk quotient of <0.1, for fish, free-swimming invertebrates and 80% of the benthic invertebrates; and field studies indicate that exposure to typical use rates of 2,4-D DMA in the field will not directly effect the survivorship, numbers (recreational and commercial fish-catch), diversity, reproduction or nesting behavior in various warm water fish, free-swimming invertebrates and benthic invertebrates (Bain and Boltz, 1992, Bettoli and Clark, 1992 and Shearer and Halter, 1980 citing various authors). Conversely, 2,4-D BEE has an acute risk quotient of >1.0 in 7 of 9 fish species and 3 of 12 benthic invertebrate species; but field studies indicate that most fish and aquatic invertebrates will not be adversely and directly impacted by exposure to typical field use rates of 2,4-D BEE (Shearer and Halter, 1980 citing various authors and Marshall and Rutschky, 1974). The failure of classic risk assessment to determine the field safety of 2,4-D BEE is due to the low solubility of 2,4-D BEE and its rapid hydrolysis to 2,4-D acid. These factors tend to limit contact of the biota to 2,4-D BEE while increasing contact with the much less toxic 2,4-D acid. For this reason, JMPR (1997) suggests that risk quotients be determined with 2,4-D acid since this is the "real" toxicant involved with most 2,4-D products including 2,4-D BEE. The acute risk

quotient with 2,4-D acid and its sodium and potassium salt is <0.1 for all environmentally relevant species of fish, free-swimming invertebrates and benthic invertebrates; and treatment of commercial fish ponds with 2,4-D sodium salt (a surrogate of 2,4-D acid) produces no direct impact on the biota of these ponds although secondary effects have been seen which produce increases in heterotrophic bacterial count, phytoplankton count, zooplankton count, benthic invertebrate biomass and subsequently benthic feeding fish survivability and yield (biomass). In general, similar effects were observed with chronic risk quotients as well. Chronic risk quotients of <1.0 for 2,4-D DMA and 2,4-D acid generally predict the chronic safety of these herbicide to fish, free-swimming invertebrates and sediment invertebrates. However, while chronic risk quotients of <1.0 for 2,4-D BEE generally predict chronic safety to fish, free-swimming invertebrates and benthic invertebrates when they are in the water column, accurate prediction of chronic safety or lack of safety from exposure to treated sediment, is not possible without an understanding of bioavailability factors that could mitigate the toxic effects of 2,4-D BEE sorbed to sediment.

The assumptions of risk analysis contain specific safety factors that are discussed by Urban and Cook (1986). The model discussed by Urban and Cook has been used since 1975 and was designed to provide a safety factor that would allow for differential variability and sensitivity among fish and wildlife species.

It was assumed that the slope of the dose response curve for the effects of a pesticide on most fish and wildlife species would be unknown. Since it is impossible to test every non-target-species that might be exposed, the following factors influence whether a correct risk management decision will be made:

1) Does the model predict risk so that the biota will be protected? Statistical analysis of the effects of slope on estimating the acute LC50 indicates that an expected environmental concentration (EEC) value that is actually 10-times less than the acute LC50 would lead to 1 to 4% mortality.

2) Terrestrial organisms are believed to be less susceptible to environmental assault than aquatic species. Therefore, a less stringent acceptable EEC is used to designate unacceptable risk in these species. The less stringent acceptable EEC is 5-times less than the acute LC50 or LD50, which would lead to a field mortality of approximately 10%, is used as a level of unacceptable risk in birds and mammals. The higher safety factors listed in item 1) for aquatic organisms is believed to be necessary since aquatic organisms are less likely to be able to limit their exposure through behavioral modifications such as moving out of the treated area or switching to an alternative food source.

3) Larger safety factors are warranted for the protection of threatened and endangered species where a factor of 10-fold is insufficient to protect that segment of the biota. E.g. In cases where no mortality is acceptable an EEC of 20 times less than the acute LC50 should be sufficient to ensure protection of species in which even a single death is of special concern.

4) For chronic effects, an EEC equal to the no observed effect concentration (NOEC) or no observed effect level (NOEL) is believed to be sufficient to reduce risk to a minimum level, since statistical analysis indicates that if the EEC is less than the

NOEC there is a 95% probability that no adverse impact to long-term survival, growth or reproduction will occur.

5) The above precautions will adequately protect any species that is acutely exposed to residues 10-fold lower than the EEC. However, to protect the entire biota or a segment of the biota, the acute EEC must be 10-fold lower than the LC50 for the most sensitive species that you wish to protect and the chronic EEC must be less than the chronic NOEC of the most sensitive species that you wish to protect.

The above criteria are considered rough estimates of potential risk to non-target organisms. The model used for ecological risk assessment does not provide a mechanism for estimating absolute uncertainty or an unchangeable probability of safety to the biota.

If the tested species are representative of the biota and are sufficient in number, uncertainty can be reduced to a minimum. 2,4-D DMA, 2,4-D acid, 2,4-D sodium salt and 2,4-D potassium salt can be considered functionally equivalent for many species since all of these chemicals dissociate to the free acid almost as soon as they are dissolved in water. With very few exceptions, all of these herbicides are slightly to practically non-toxic to most environmentally relevant fish and invertebrate species. However, a factor that tends to increase uncertainty is that *Palaemonetes spp.* (grass and glass shrimp) may be significantly more susceptible to 2,4-D DMA than to the other freely dissociable 2,4-D herbicides and *Gammarus fasciatus* (lined scud) appears to be two orders of magnitude more susceptible to 2,4-D acid than 2,4-D DMA. For these freely dissociable 2,4-D materials, four-species of cold freshwater fish, more than 10-species of warm freshwater fish and 12-species of benthic freshwater fish have been tested for acute toxicity in the laboratory. Many of these invertebrates may not be relevant to this assessment since they are estuarine or marine species. However, since these estuarine and marine species are often more sensitive than their freshwater equivalents, additional sensitivity may be added to the risk assessment by including them with the other benthic invertebrates.

The case of 2,4-D BEE may be considered special, since it is insoluble in water and rapidly hydrolyzed to 2,4-D acid. These characteristics make laboratory risk assessments subject to some doubt with 2,4-D BEE. A risk assessment with 2,4-D BEE will be excessively conservative. Field data from water bodies treated with 2,4-D BEE yield results that would typically be expected for 2,4-D acid based on its laboratory effects against aquatic organisms

The Expected Environmental Concentrations (EEC), as presented in Section 4.3.2.5 (Risk Analysis in Aquatic Species), are believed to be fairly accurate based on many years of successful risk management. However, field data for individual water bodies, indicating both the acute and chronic average concentrations, could improve the ability to assess and manage risk particularly for sensitive species.

The fact that 2,4-D BEE has the potential to cause severe adverse impact to the biota, based on laboratory data, is not borne out by field tests which have shown 2,4-D BEE to have low direct impact on fish and benthic invertebrates. Mitigating factors that are inherent to the nature of the herbicidal product, like low solubility and rapid hydrolysis, must be considered in order to make a risk assessment that is of value to the user. Also, the possibility of making an incorrect risk management decision must be weighed carefully particularly if limited field data is available. Fortunately, long term use of 2,4-

D DMA and 2,4-D BEE allows for practical field experience to mitigate an adverse laboratory risk assessment with 2,4-D BEE.

4.6 ADDITIONAL INFORMATION NEEDS

Summary: The importance of the role of sediment in removing 2,4-D from the environment should be investigated along with the effects of 2,4-D in sediment on benthic organisms. Levels of granular 2,4-D BEE in the sediment is particularly important since under some circumstances, it is known to accumulate to very high levels. Whether or not these high sediment levels are biologically available to benthic species that reside on the surface or in the interstitial areas of the sediment has not been adequately evaluated. Furthermore, the effects of digestion on those species that consume sediment to extract nutrients are unknown. Due to the extreme sensitivity of certain benthic organisms to 2,4-DMA and 2,4-D BEE our risk assessment leads us to conclude that although 2,4-D products were safe to most organisms (90 to 95%), the most sensitive benthic organisms may not be protected. Therefore, the toxicity of 2,4-D in overlying water, interstitial water and whole sediments needs further investigation.

The effects of post-treatment plantings of native aquatic plants needs to be investigated to determine if this is a practical approach to revegetation after the elimination of watermilfoil. Further investigations with varying treatment rates and conditions should be conducted to determine which rates and conditions cause the greatest destruction of watermilfoil and the least damage to native aquatic plants.

Further investigations need to be conducted to determine which levels of 2,4-D are safe to sensitive, threatened and endangered species (particularly salmon and sea-run trout). Additional studies emphasizing specie indigenous to the Northwest should be conducted so that risk due to exposure can be managed more effectively. This is of particular concern for benthic organisms since regulators, registrants, the applicator community and the general public have recently expressed great concern over this issue.

4.6.1 Soil and Sediment

The concentrations of 2,4-D in sediment due to the use of granular 2,4-D BEE needs to be investigated further. The effects of partitioning (Kd) between soils and water with different soils/sediments also needs to be further investigated. Without well-determined values for how much 2,4-D a given soil/sediment type removes and how rapidly, the assumption that the persistence of 2,4-D in the water column is not strongly affected by partitioning between the water phase and the soil/sediment phase may lead to a water column half-life that is functionally too long. Also, without knowledge of the partitioning and persistence of 2,4-D in sediment, an underestimate of the EEC for sediment dwelling organisms is likely. A knowledge of the concentration of 2,4-D in the sediment is necessary so that an adequate Risk Assessment and evaluation can be made for sediment organisms. Due to the extreme sensitivity of benthic (sediment) organisms to 2,4-D BEE, 2,4-D DMA and 2,4-D acid, our risk assessment lead us to conclude that although, the 2,4-D products were safe to most organisms (90% to 95%), the most sensitive benthic (sediment) organisms may not be. Sediment quality and its effect on aquatic organisms are becoming important topics amongst representatives from industry and the regulatory community.

4.6.2 Water

The effects of water quality on the toxicity of 2,4-D products has been adequately investigated. It has been demonstrated that 2,4-D products can cause depletion of dissolved oxygen concentrations to nearly zero due to decay of treated foliage and that ammonia, nitrite and nitrate, phosphate, pH, hardness and alkalinity effect the toxicity and secondary effects of 2,4-D (Frank, 1972, Kobraei and White, 1996, Sherry, 1994, Marshall and Rutschky, 1974, Sarkar, 1991, and Patnaik and Das, 1991). These water quality parameters have been known to influence algal blooms, growth of zooplankton, growth and growth inhibition of fungal propagules and survival and dominance of a particular species (particularly amongst benthic fish and invertebrates). The reason for these changes have been attributed to both a general metabolic stimulation caused by the presence of 2,4-D and to secondary effects influenced by the release of nutrients combined temperature and dissolved oxygen content.

4.6.3 Plants

Kobraei and White (1996), Patnaik and Das (1991) and Sarkar (1991) have adequately demonstrated that dead and dying plants release nitrogen and phosphorous which is utilized by phytoplankton, heterotrophic bacteria, sediment bacteria and other sediment organisms to stimulate their growth. In the case of 2,4-D, field data indicates that the levels of nitrogen and phosphate change significantly after treatment with 2,4-D.

The planting of desirable vegetation in the aquatic environment after treatment with 2,4-D has yet to receive serious investigation. Helsel et al (1996) compared treatments of 2,4-D at 112 kg formulation/Kg (100 lbs formulation /acre) with the effects of bottom fabrics placed in a pond. However, he only examined replanting with cuttings of desirable native plants in the portion of the study where bottom fabrics were used to remove undesirable vegetation. Even in the case where bottom fabrics were used to control undesirable vegetation, the cuttings of desirable plants did not root and the bulk of them became uprooted and floated to shore. Helsel et al (1996) found that in the next two growing seasons Eurasian watermilfoil dominated the sites where fabric had been used to control this noxious species in the previous year; Eurasian watermilfoil covered 60% of areal area and no other species competed effectively with watermilfoil. However, he also found that in those sites treated with 2,4-D BEE that Eurasian watermilfoil only covered ~5% of the areal area and the remainder of the previously treated sites was covered with native species including water celery, *Elodea*, Coontail and other species (Table 16); this re-growth of native species in areas treated with 2,4-D BEE occurred naturally without artificial re-plantings of native species. Furthermore, judicious use of 2,4-D BEE at Loon Lake, Washington in 1998 allowed for the control of Eurasian watermilfoil without adverse impact to *Elodea canadensis* chara spp., naiads, various *Potamogeton spp*. and other native plants (Parsons, 1999).

Post-treatment plantings of native non-noxious and non-invasive plants could increase diversity and decrease the numbers of the less desirable 2,4-D resistant plants through competition. This would improve the habitat since a more diverse plant community would attract a more diverse animal community. The practicality and utility of post-treatment plantings of native plants and when they should be evaluated on a case-by-case basis.

4.6.4 Acute and Chronic Animal Studies

There are very few well-designed chronic toxicity studies that have been conducted with 2,4-D products. For an ideal understanding of chronic effects, early life-stage (ELS) studies need to be conducted on all end use products or their technical equivalent with rainbow trout, fathead minnow and sheepshead minnow. Since Coho salmon and Chinook salmon are so important in the Northwest, ELS studies should also be conducted with these species. To have a better understanding of the chronic effects of 2,4-D products on invertebrates, Life cycle studies should be conducted with all end use products or their technical equivalent with *Daphnia magna*, *Ceriodaphnia dubia* and *Mysidopsis bahia*. In order for proper comparisons to be made, additional acute studies should be conducted in the same time frame as the new early life-stage and life cycle studies. Ideally, the acute and chronic studies should be conducted with fish from the same parental line, or at least of the same known and specified seriotypes or germ lines.

Ideally, additional acute and chronic work needs to be done on fully aquatic and water associated animal species. These species include aquatic reptiles (turtles), amphibians (salamanders, toads, and frogs), and *lepidoptera* associated with wetland communities or used as biocontrol agents on aquatic plants (*Sameodez albiguttalis, lepidoptera: Pyralidae*).

Great concern has recently been expressed concerning the effects of pesticides on benthic (sediment) organisms. In light of the high toxicity of 2,4-D products to some benthic (sediment) organisms, additional testing needs to be conducted to determine the extent of the toxicity that is caused by 2,4-D DMA, 2,4-D BEE and 2,4-D acid within the laboratory in both acute and chronic tests. Furthermore, although the few studies conducted in the field indicate that benthic invertebrate species are, for the most part, not affected directly by 2,4-D additional field studies emphasizing the species that have demonstrated sensitivity in the laboratory to 2,4-D products is warranted. These species include, but should not be confined to, *Gammarus fasciatus, Gammarus lacustris* and the various species of estuarine shrimp including pink shrimp, glass shrimp, grass shrimp, seed shrimp and mysid shrimp (*Mysidopsis bahia*).

4.7 MITIGATION MEASURES

Summary: The use of 2,4-D may be considered a mitigation measure when Eurasian watermilfoil is present. Reduction of watermilfoil while saving native aquatic plants may improve habitat for the growth of plants, fish, zooplankton and benthic organisms.

Methods to lower levels of released phosphates during post treatment aquatic plant decay could be useful. Although chelating agents such as fly ash metal, ferric iron, aluminum and zirconium have been use to remove phosphate from eutrophic lakes, there may be some risk to the aquatic environment due to the toxicity of these chelating agents to fish and invertebrates. Also by the time excessive phosphate levels are noticed, it may be too late to prevent an algal or heterotrophic bacterial bloom.

The use of 2,4-D itself may be considered a mitigation measure when floating and submersed aquatic macrophytes are out of control. Treatment with appropriate concentrations of 2,4-D BEE granular or 2,4-D DMA liquid may improve habitat for fish, pelagic aquatic invertebrates (zooplankton) and benthic organisms (catfish, common carp and sediment dwelling organisms.

However, treatment with 2,4-D can produce side effects that need to be mitigated. For example, release of too much phosphate due the decay of treated plants or removal of phosphate during the development of aerobic conditions in a fairly shallow hypolimnion. When anaerobic conditions redevelop, phosphate and iron may be released once again and provide nutrients for growth particularly after a sediment disturbance like a mechanical weed removal or a fall overturn. In order for these releases from the hypolimnion to be useful to photosynthetic organisms, the water must be both shallow and transparent enough for photosynthesis to occur. Removal of excess phosphate may be achieved by the addition of ferric iron, metals in fly ash, or salts of aluminum or zirconium. However, these remediation techniques may have an adverse impact on sensitive aquatic animals. Therefore, the negative impact of excess phosphate must be weighed against the possible negative affects of these chelating metals on the resident biota. This method is occasionally used to clean up the phosphate from eutrophic lakes and it could be used as a remedial measure when high phosphate levels are noticed due to the decay of herbicide treated aquatic plants. However, by the time high phosphate levels are noticed, it may already be to late to prevent an algal bloom

Levels of 2,4-D that need remediation are unlikely to occur except in cases where there has been a spill. For example, if a treatment boat sinks, concentrations near the boat will be high enough to cause extensive fish kill. There is some evidence that salmonids, sheepshead minnows, mosquito fish, glass shrimp and *Uca uruguayensis* may avoid areas where the concentration of 2,4-D is higher than 1 to 10 mg/L (Folmar, 1976, Hansen et al, 1973 and Hansen, 1972 in Shearer and Halter, 1980, and Rodriguez and Lombardo, 1991). However, the ability of these species to avoid 2,4-D even if it is present at toxic concentrations has been largely rejected by investigators conducting avoidance studies. Furthermore, since the ability of threatened and endangered species to avoid exposure to 2,4-D is unknown, extra caution should be taken to allow for a level of concern of 0.05 rather than the more typical value of <0.10 for non-endangered species. Restrictions on seasonal applications is warranted to protect sensitive salmon smolts from the affects of these products, particularly 2,4-D BEE; similar season restrictions may be applied to protect fish and fisheries and prevent water use restrictions during the height of the recreational or commercial fishing seasons.

When 2,4-D products, particularly 2,4-D BEE, are being used for control of aquatic, weeds, the lowest effective concentration should be used. The use of these herbicides in open waterways where a lot of lateral mixing and dilution occur will also decrease the dissipation time and possibly the sediment concentrations and sediment persistence.

In cases where sediment or water becomes seriously contaminated, rates of degradation of 2,4-D may be improved by adding lake mud, which has had a 2,4-D degradation history (Frank, 1972). It has also been suggested that in anaerobic marine or estuarine sediments with bacteria known to degrade 2,4-D, degradative capacity can be enhanced by the addition of a non-oxygen electron acceptor like sulfate, an electron donor like hydrogen and an additional utilizable carbon source like acetate (Boyle et al, 1999).

4.8 SUMMARY AND CONCLUSIONS

Summary for 2,4-D DMA: 2,4-D DMA (2,4-D dimethylamine salt) does not appear to adversely affect tested fish, free-swimming invertebrates or benthic organisms when applied at typical use rates in the field. Acute toxicity for this compound is low for all

species of fish and free-swimming invertebrates (LC50 > 100 mg a.i./L). However, while 80% of sediment invertebrates tested were not acutely affected by concentrations of 2,4-D DMA that exceed 100 mg a.i./L, the most sensitive species (glass shrimp and seed shrimp) exhibited high (LC50 = 0.15 mg a.i./L) to moderate (LC50 = 8.0 mg a.i./L toxicity to 2,4-D DMA. Since the acute EEC is typically ~1.36 mg a.i./L (and can be as high as 4.8 mg a.i.), the acute risk quotient is above the level of concern of 0.1 for the most sensitive benthic species tested (RQ = 32 for water and 1.4 for sediment). These calculations, lead to the conclusion that the sediment biota may be at risk from acute exposure to 2,4-D DMA. The chronic toxicity values were determined by prediction or empirically. These values are very low for fish, free-swimming invertebrates and 90% of the benthic biota (predicted chronic NOEC = 5.6, 27.5 and 0.0083 mg a.i./L for rainbow trout, Daphnia magna *and glass shrimp, respectively). Therefore, the chronic Risk Quotient using an EEC of 0.091 mg a.i./L is less than the chronic level of concern of 1.0 for over 95% of the aquatic biota. The acute and chronic risk quotients do not exceed the level of concern for fish and free-swimming invertebrates (RQ = <0.016), therefore 2,4-D DMA can be used for control of aquatic weeds without significant impact to these segments of the biota. However, the sediment invertebrates may be at risk since the risk quotient for the most sensitive species exceeds the chronic level of concern of 1.0 (RQ = ~11). The available field data confirm that fish (largemouth bass, sunfish and a variety of other warm water fish) do not appear to be impacted with respect to numbers, mortality, condition, movement, reproduction, or nesting behavior by 2,4-D DMA use at labeled treatment rates. Furthermore, Brooker (1974) and Pierce (1960 and 1961) in Shearer and Halter (1980) conducted field studies, which indicate that free-swimming and benthic invertebrates are not impacted directly by mixtures of 2,4-D DMA and dalapon or 2,4-D DMA alone with respect to numbers, diversity or dominant species.*

Summary for 2,4-D BEE: Due to the low solubility and rapid hydrolysis of 2,4-D BEE (2,4-D butoxyethyl ester) to 2,4-D acid, 2,4-D is unlikely to adversely impact the aquatic biota. However, acute toxicity for this compound is very high with the LC50 for the most sensitive environmentally relevant fish species (rainbow trout) being 0.3 mg a.i./L. The LC50 for all environmentally relevant species ranged between 0.3 mg a.e./L for rainbow trout and first zoels of Chasmagnathus granulata*, to ~4.0 for* Daphnia magna *and 0.44 mg a.i./L for* Gammarus spp. *The short-term EEC is low (0.100 mg a.e./L). However, the toxicity to many species of fish and benthic invertebrates is fairly high; so that the acute level of concern (0.10) is exceeded in 6 of 9 fish species, and 4 of 13 invertebrate species tested (RQ = >0.22). Therefore, based on a classical risk assessment, 2,4-D BEE may have adverse acute impact on fish and benthic invertebrates but probably not on free-swimming invertebrates. The chronic toxicity, determined by prediction or empirically, is also fairly high for the most sensitive environmentally relevant fish species (chronic NOEC = 0.017, 0.29 and 0.024 mg a.e./L, respectively for rainbow trout,* Daphnia magna *and* Gammarus spp.*, respectively). But, the predicted chronic risk quotient is below the level of concern of 1.0 for the species tested (RQ = <0.58) if the animals remain in the water column (EEC = 0.01 mg/L). However, the long-term sediment concentration (0.06 mg a.i./L) is high enough to cause potential adverse impact to the most sensitive sediment organisms (RQ = 2.5). Therefore, use of 2,4-D BEE at 100 lbs. formulation/acre to control Eurasian watermilfoil has the potential to adversely impact the aquatic biota based on laboratory data with 2,4-D BEE.*

2,4-D BEE appears to be safe under field conditions because it has a low solubility and is rapidly hydrolyzed to 2,4-D acid, which has relatively low toxicity. 2,4-D acid is slightly toxic (LC50 = 20 mg a.e./L) to the most sensitive fish species tested (common carp) and

practically non-toxic (LC50 >100 mg a.i./L) to the free-swimming invertebrate biota (daphnids and freshwater prawns). Although 2,4-D acid has significant acute toxicity (LC50 = 3.2 mg a.i./L for lined scud), the short-term EEC (0.1 mg/L in water and 0.15 mg/L in sediment) is more than ten-times lower than the acute toxicity value. For chronic exposure, 2,4-D acid has an NOEC that is significantly lower than the long-term EEC of 0.01 mg /L. For common carp, Ceriodaphnia dubia *and* Gammarus spp.*, the predicted or empirical NOECs are 1.11, ~30 and 0.18 mg a.e./L. 2,4-D BEE has a relatively low toxicity because 2,4-D BEE is rapidly hydrolyzed to the almost non-toxic 2,4-D acid. Since the risk quotients for both acute and chronic exposure to 2,4-D acid are well below their respective levels of concern, 2,4-D BEE and its degradate (2,4-D acid) will probably not impact the general animal biota when 2,4-D BEE is used according to the label.*

When used in the field, all products of 2,4-D, are observed to have no significant adverse impact to fish, and free-swimming and benthic invertebrates. 2,4-D products have been observed in some cases to increase the general metabolic rate of bacteria, fungi, algae, fish, and invertebrates. Such effects may cause early spawning, increased survivorship and increased biomass in plants and animals exposed to 2,4-D. However, most investigators believe that these are secondary effects due to the release of nutrients from dying plant tissue that induces an algal or heterotrophic bacteria bloom, which leads to better nutrition for zooplankton, benthic organisms and eventually fish. 2,4-D appears to be rapidly eliminated and biomagnification across trophic levels does not occur, although some cases of extremely high bioaccumulation are seen in zooplankton and benthic organisms. In fish low levels of bioaccumulation may occur upon exposure to 2,4-D DMA and 2,4-D BEE. These herbicides are metabolized to 2,4-D acid and rapidly eliminated from edible fish tissue.

The risk to aquatic life from the use of 2,4-D was assessed using two methodologies. One of the methods was designed to compare chemicals for toxicity and the other was designed to determine whether or not the chemical was safe to the biota.

The first method is the U.S. EPA ecotoxicological risk categories for mammals, birds, and aquatic organisms and a summary of its criterion can be found in Table 1. For fish, birds and mammals these categories are very highly toxic, highly toxic, moderately toxic, slightly toxic, and practically non-toxic. The exact quantitative values vary considerably depending on species and exposure route (EPA, 1982, Brooks 1973 in Ebasco, 1993) (Table 1). This method classifies 2,4-D DMA (2,4-D dimethylamine salt) as practically non-toxic (LC50 = > 100 mg a.i./L) to all species fish and free-swimming invertebrates. However, while the effects on 80% of the sediment invertebrates classify 2,4-D DMA as practically non-toxic, the two most sensitive species (glass shrimp and seed shrimp) would classify 2,4-D as highly toxic ((LC50 = 0.1 to 1.0 mg/L) to moderately toxic (LC50 = > 1 to 10 mg /L), respectively. This indicates that compared to other pesticide chemicals the acute toxicity of 2.4-D DMA is extremely low to aquatic organisms in general, but that there are exceptions.

This method classified 2,4-D BEE (2,4-D butoxyethyl ester) as highly toxic (LC50 = 0.1 to 1.0) mg/L) to moderately toxic (LC 50 = >1 to 10 mg/L) with most fish species being affected at the highly toxic level, and most invertebrate species being affected at the moderately toxic level. This categorization indicates that compared to most other pesticides the acute toxicity of 2,4-D BEE is fairly high. However, the toxicity of the 2,4-D BEE hydrolysis product (2,4-D acid) classified this metabolite as moderately toxic

to practically non-toxic (LC50 = >100 mg/L) with almost all species being affected at the slightly toxic (LC50 = >10 to 100 mg/L) to practically non-toxic level. There was one environmentally relevant species that was an exception; lined scud with a 2,4-D acid LC50 of 3.2 mg a.e./L. However, 2,4-D BEE and 2,4-D acid do not appear to cause direct adverse impact to either free-swimming or benthic organisms in the field.

For the test substance to be considered safe to the biota, according to the second method (Urban and Cook, 1986), the acute LC50 must be at least 10 times greater than the 4-day weighed expected environmental concentration (EEC). For threatened and endangered species the acute LC50 must be 20 times greater than the 4-day weighted expected environmental concentration (EEC) for the test substance to be considered safe. The chronic NOEC must also be equal to, or greater than, the 28-day weighted EEC, for the test substance to be considered safe.

Risk assessments indicate that 2,4-D DMA (2,4-D dimethylamine) is acutely and chronically safe to fish and free-swimming invertebrate biota. The acute LC50 for the most sensitive species in this segment of the biota is greater than 100 mg a.i./L (Table 22, Table 23 and Table 28), and the short-term EEC is typically 1.36 to 4.8 mg a.i./L. Therefore, the risk quotient will be less than the acute level of concern of 0.1 for the protection of this segment of the biota (RQ = 0.048). For these segments of the biota, 2,4-D DMA also has a low chronic toxicity with predicted or empirical chronic NOECs ranging from 5.56 mg a.i./L for rainbow trout and all other environmentally relevant species of fish to 27.5 mg a.i./L in *Daphnia magna*. Since the long-term EEC is 0.091 mg a.i./L for typical treatment levels where 1-day environmental concentration is 0.235 mg a.e./L (0.283 mg a.i./L), the risk quotient is less than the chronic level of concern of 1.0 (RQ = <0.016) for protection of this segment of the biota.

However, some of the estuarine shrimp, which are known for their sensitivity to pollution and pesticides as well as their importance in the food chain, are very sensitive to the acute effects of 2,4-D DMA. The most sensitive benthic invertebrate species yielded acute and chronic risk assessment values that were above the levels of concern for protection of this segment of the biota (acute RQ = 9.1 in water and 1.4 in sediment and chronic RQ = 11.0 in water and 25.3 in sediment).

Field treatment with 2,4-D DMA at 2.0 to 4.0 mg a.i./L has shown minimal impact in nesting behavior, survival, growth, reproduction, recreational catch or commercial catch in warm-water fish (Bettoli and Clark, 1992, Bain and Boltz, 1992, and Shearer and Halter, 1980 citing various authors). In a manner similar to that of warm water fish, aquatic invertebrates have been shown not to be adversely impacted in either numbers, diversity or dominant species when exposed to 2,4-D-DMA in the field (Brooker, 1974 and Pierce, 1960 and 1961 in Shearer and Halter, 1980). The only endangered species tested (Chinook salmon), responds to 2,4-D DMA in a manner similar to rainbow trout; therefore, endangered species with responses to 2,4-D DMA similar to Chinook salmon should be protected. However, all organisms will not be protected; herbicide concentrations identified as not causing significant adverse impacts may still impact the more sensitive aquatic biota. Nevertheless, economically important and endangered or threatened species are expected to be protected at forecast herbicide application rates and estimated exposure concentrations. See Section 4.3.2.5 for details of the risk assessment with 2,4-D DMA.

Risk assessments indicate that 2,4-D BEE (2,4-D butoxyethyl ester) is not acutely safe to the biota based on the results of laboratory studies. In species with acute LC50s less than 1.0 mg a.e./L, exposure to 2,4-D BEE could be fatal if mitigating factors do not occur. The 4-day EEC (0.1 mg a.i. /L) is higher than the acute LC50 in most of the fish species tested (7 of 9) and a significant proportion of the benthic invertebrates tested (3 of 14). However, free-swimming invertebrates have LC50s (4.0 to 7.2 mg a.i./L that are high enough so that they will probably not be adversely impacted (acute RQ = <0.025). Therefore, the use of 2,4-D BEE could present a substantial acute risk to the biota. The acute risk quotient exceeds the level of concern of 0.1 for a significant segment of the biota. The most sensitive environmentally relevant fish (rainbow trout) and benthic invertebrate species (bright scud) have risk quotients higher than the acute level of concern (0.1) for 2,4-D BEE (RQ = 0.33 for fish and 0.23 to 0.34 for benthic invertebrates) (Table 23 and 28).

When using the predicted or empirical chronic NOEC for chronic risk assessment, 2,4-D BEE appears to be chronically safe to the most sensitive fish species and free-swimming invertebrates since the predicted or empirical risk quotient is below the level of concern of 1.0 (RQ = 0.01 ppm/ 0.017 = 0.59 for rainbow trout and 0.01 ppm/0.29 ppm = 0.03 for *Daphnia magna*) for the protection of these segments of the biota. Although chronic water column exposure of the most sensitive benthic invertebrate species is likely to be safe to this segment of the biota, chronic sediment exposure may cause adverse impact if mitigating factors are not present (RQ water = 0.01 ppm/0.024 ppm = 0.41 and RQ sediment = 0.06 ppm/ 0.024 ppm = 2.5).

The risk assessment indicates that a number of benthic species may be adversely impacted by the effects of 2,4-D BBE. Although laboratory data indicate that 2,4-D BEE has the potential to adversely impact fish and invertebrate biota, field data indicates that this probably does not occur under typical use and environmental conditions. This safety of 2,4-D BEE to the biota is probably due largely to its low solubility and rapid hydrolysis to the much less toxic 2,4-D acid.

2,4-D acid has very low acute and chronic toxicity to fish, free-swimming invertebrates and benthic invertebrates. For the most part, 2,4-D acid has an acute toxicity that is similar to that of 2,4-D DMA salt, with LC50s that exceed 100 mg a.e./L for fish and aquatic invertebrates. However, the most sensitive species of fish (Common carp), free-swimming invertebrate (*Daphnia magna*) and sediment invertebrate (lined scud) had LC50s that were 40, ~209 and 3.2 mg a.e./L, respectively. Since these LC50 values are more than 10-fold higher than the short-term EEC (0.1 mg/L), 2,4-D acid is unlikely to have acute impact on this segment of the biota. Furthermore, the estimated chronic NOEC values for 2,4-D acid are 1.1 mg a.e./L for common carp, ~11 mg a.e./L for *Daphnia magna* and 0.18 mg a.e./L for lined scud. Since these NOEC values are greater than the typical EEC for long-term water column (0.01 mg/L) or sediment exposure (0.06 mg/L) it is unlikely that 2,4-D acid will have adverse impact on the aquatic animal biota.

It is noteworthy, that 2,4-D BEE appears of have no direct impact on benthic invertebrates at treatment rates of 100 lbs formulation/acre and does not appear to affect numbers or diversity of these sensitive organisms (Marshall and Rutschky, 1974). However, due to secondary effects of an anoxic hypoliminion, dominant species may shift from species that require a high dissolved oxygen content to species that can tolerate a low dissolved oxygen content. Although 2,4-D BEE has not been observed to directly impact fish and invertebrate populations adversely, its hydrolysis product (2,4-D sodium

salt surrogate of 2,4-D acid) has been observed to cause increases in heterotrophic bacteria counts phytoplankton counts, zooplankton counts and benthic invertebrate biomass. These secondary effects caused by the release of nutrients from macrophytes and algae, sometimes resulted in increases in survivorship and growth (as reflected by yield) of fish. Adverse chronic impact is unlikely since treatment at up to 6 Kg a.i./L did not affect the future reproductive status of carp species used as seed animals (Sarkar, 1991 and Patnaik and Das, 1991).

However, the effects of granular formulations may have been underestimated for benthic organisms and overestimated for free-swimming organisms. 2,4-D BEE granular (Aqua-Kleen® and Navigate®) are believed to release 2,4-D BEE gradually into the aquatic ecosystems where its low solubility and rapid hydrolysis to 2,4-D acid reduce its potential aquatic impact. In a field experiment at Loon Lake, Washington, application of 100 lbs. formulation/acre of 2,4-D BEE pellets resulted in a maximum water column concentration of one to two mg/L in one to three days after treatment and elimination of 2,4-D from the water column within 3-days to 1-week after treatment. Similar effects have been seen in fifteen open waterways in British Columbia (Gallagher, 1992). Whether or not benthic organisms are better protected from the use of 2,4-D BEE pellets depends on the EEC in overlying water, pore water and sediment due to slow release of the active ingredient. Although herbicides found in sediments may not participate in environmental toxicity due to lack of biological availability, this assumption cannot be made without empirical evidence. High concentrations in the benthic zone may be the case because granular formulations are designed to initially create the highest concentration at the bottom near the plant roots. However, current EECs assume complete, instantaneous, mixing of all granular formulations. With additional information, the degree of risk to aquatic life from the use of granular formulations can be determined. Experience with other herbicidal products indicates that the concentration of the herbicide in the hypolimnion can be many times higher than the concentration of the herbicide at the surface of a pond of lake.

In conclusion, 2,4-D DMA (2,4-D dimethylamine) is safe to use for control of nuisance aquatic vegetation at labeled use rates and provides a large safety factor for protection of fish and free-swimming aquatic biota from acute and chronic effects. However, the more sensitive species of sediment invertebrates may be acutely and chronically impacted. However, field studies indicate that invertebrate species should not be impacted by the use of 2,4-D DMA for the control of ditch bank weeds or aquatic weeds in ponds (Brooker, 1974 and Pierce 1961 and 1962 in Shearer and Halter, 1980).

Although 2,4-D BEE (2,4-D butoxyethyl ester) has the potential to harm fish and aquatic invertebrates based on risk assessment conducted with laboratory data, field studies indicate that the use of 2,4-D BEE pellets has no direct impact on fish populations (Shearer and Halter, 1980). Limited field data with benthic invertebrates indicates a similar lack of direct effects, but indirect effects such as decreased dissolved oxygen content can result in a shift in dominant organisms to those more tolerant of low dissolved oxygen content (Marshall and Rutschky, 1974). Low solubility of 2,4-D BEE and a rapid hydrolysis of 2,4 D BEE to 2,4-D acid also improves the safety of Aqua-Kleen® and Navigate® by decreasing contact of 2,4-D BEE and increasing contact to 2,4-D acid, which appears to have low toxicity to the aquatic biota.

Aqua-Kleen® and Navigate® applied at concentration of 100 lbs. Formulation/acre will control Eurasian watermilfoil and spare most species of native aquatic vegetation. Field data indicated the use of 2,4-D products designated for aquatic use should be safe to the aquatic animal biota at use rates specified in the label.

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LIST OF TABLES

Table 1: U.S. EPA Ectoxicological Catagories[1] for Mammals Birds and Aquatic Organisms

Acute Oral Toxicity in Mammals (mg/Kg body wt)	Toxicity in Birds		Acute Toxicity in Fish and Invertebrates mg/L test solution	Toxicity Ranking
	Acute Oral (mg/Kg body weight)	Dietary mg/Kg feed		
<10	<10	<50	<0.1	Very Highly Toxic
10-50	10-50	50-500	0.1-1.0	Highly Toxic
>50-100	>50-500	>50-1000	>1-10	Moderately Toxic
>500-2000	>500-2000	>1000-5000	>10-100	Slightly Toxic
>2000	>2000	>5000	>100	Practically Non-Toxic

[1] EPA, (1982) Pesticide Assessment Guidelines, Section E: Ecological Effects, Brooks, 1973 in Ebasco, 1993.

Table 2: Toxicity of 2,4-D to Different Aquatic Species; and Sensitive Species and Stages Tested

Species Name	Common Name	Test Type	Age of Organism	Test Duration	Test Chemicals – 2,4-D Products LC50 or EC50 & (NOEC) in mg a.i./L					
					BBE	2-EHE	DMA	Na Salt	K Salt	Acid
Algae										
Anabaena flos-aquae	Blue-green algae	Static Acute	Log growth phase	5-days	6.37 (3.14)	>30 (>30)	153 (68)			>2.02 (<2.02)
Anabaena dolium	Blue-green algae	Static Acute	Log growth phase	5-days						~500 (<100)
Chlamydomonas reinhardtii	Blue-green algae	Static Acute	Log growth phase	8-days						>40 (X)
Nostoc linkia	Blue-green algae	Static Acute	Log growth phase	12-days						~500 (<100)
Nostoc calcicola	Blue-green algae	Static Acute	Log growth phase	12-days						~500 (<100)
Nostoc sp.	Blue-green algae	Static Acute	Log growth phase	12-days						~500 (<100)
Selenastrum capricornutum	Green algae	Static Acute	Log growth phase	5-days	24.9 (12.5)	>30 (15)	67 (19)			41 (34)
Chlorococcum spp.	Green algae	Static Acute	Log growth phase	10-days	75					50
Chlorella fusca	Green algae	Static Acute	Log growth phase	5-days						89 (X)
Dunaliella tertiolecta	Green algae	Static Acute	Log growth phase	10 to 20-days	75		185			75
Scenedesmus quadricauda	Green algae	Static Acute	Log growth Phase	20-days						98
Navicula pelliculosa	Freshwater diatom	Static Acute	Log growth phase	5-days	1.86 (1.76)	4.1 (3.75)	5.28 (1.70)			2.02 (<2.02)
Skeletonema capricornutum	Marine diatom	Static Acute	Log growth phase	5-days	1.66 (0.79)	0.23 (0.19)	37 (96)			2.08 (<2.08)

Table 2: Toxicity of 2,4-D to Different Aquatic Species; and Sensitive Species and Stages Tested (Continued)

Species Name	Common Name	Test Type	Age of Organism	Test Duration	Test Chemicals – 2,4-D Products LC50 or EC50 & (NOEC) in mg a.i./L					
					BBE	2-EHE	DMA	Na Salt	K Salt	Acid
Phaeodactylum tricornutum	Marine diatom	Static Acute	Log growth phase	10 to 20 days	150		362			50
Isochrysis galbana	Marine Haptophyte	Static Acute	Log growth phase	10-days	75	4.1 (3.75)				50
Sinapsis alba	macrophyte	Static Acute Root Elongation	Seedling	3-days						1.17
Sinapsis alba	macrophyte	Static Acute Germination	Seed	3-days						166
Lemna gibba	duckweed	Static Acute	3-4 leaf stage	14 days	0.58 (0.20)	0.50 (0.19)	0.58 (0.27)			0.695 (0.0581)
Fish										
Oncorhynchus mykiss	Rainbow trout	Flow Acute	Fry	4-days	0.52	7.2 (2.1)				
Oncorhynchus mykiss	Rainbow trout	Flow Acute	Smolts	4-days	0.47					
Oncorhynchus mykiss	Rainbow trout	Flow Acute	Mixed Fry, Juvenile, & NS[3]	4-days	2.0		218 (159)			
Oncorhynchus mykiss	Rainbow trout	Flow Acute	Juveniles	4-days				>100		
Oncorhynchus mykiss	Rainbow trout	Renewal Acute	Fry	4-days	0.3					
Oncorhynchus mykiss	Rainbow trout	Static Acute	Smolts	4-Days	2.1	76 (19)				

Table 2: Toxicity of 2,4-D to Different Aquatic Species; and Sensitive Species and Stages Tested (Continued)

Species Name	Common Name	Test Type	Age of Organism	Test Duration	Test Chemicals – 2,4-D Products LC50 or EC50 & (NOEC) in mg a.i./L					
					BBE	2-EHE	DMA	Na Salt	K Salt	Acid
Oncorhynchus mykiss	Rainbow trout	Static Acute	Juvenile	4-Days						163 (320)
Oncorhynchus mykiss	Rainbow trout	Static Acute	Juv/NS[3]	4-Days	1.3 (<0.85)					358
Oncorhynchus mykiss	Rainbow trout	Flow Chronic ELS[1]	Egg-Sac-Fry	23-27-Days					6.8 (0.027)	
Oncorhynchus mykiss	Rainbow trout	Static Acute	Fingerlings	4-Days						<100->1000 (~316)
Oncorhynchus gorbuscha	Pink salmon	Static Acute	Fry	4-Days	0.45	31				
Oncorhynchus gorbuscha	Pink salmon	Static Acute	Juvenile	4-Days	0.73					
Oncorhynchus kisutch	Coho salmon	Renewal Acute	Fry	4-days	0.45					
Oncorhynchus kisutch	Coho salmon	Static Acute	Fingerlings	4-day		116				
Oncorhynchus kisutch	Coho salmon	Static Acute	Juvenile	4-day	1.92		>100			
Oncorhynchus tshawytscha	Chinook salmon	Flow Acute	Fry	4-day	0.32					
Oncorhynchus tshawytscha	Chinook salmon	Flow Acute	Fry	4-day	0.38					
Oncorhynchus tshawytscha	Chinook salmon	Flow Chronic ELS	Egg-Fry	86 days	(0.040)					
Oncorhynchus nerka	Sockeye Salmon	Renewal Acute	Fry	4-days	0.45					
Oncorhynchus clarkii	Cutthroat trout	Static Acute	Juvenile/ Fingerlings	4-days		>50				40

Table 2: Toxicity of 2,4-D to Different Aquatic Species; and Sensitive Species and Stages Tested (Continued)

Species Name	Common Name	Test Type	Age of Organism	Test Duration	Test Chemicals – 2,4-D Products LC50 or EC50 & (NOEC) in mg a.i./L					
					BBE	2-EHE	DMA	Na Salt	K Salt	Acid
Salvelinus namaycush	Lake trout	Static Acute	Juveniles	4-days						45
Lepomis macrochirus	Bluegill sunfish	Flow Acute	Juvenile	4-days	0.61					
Lepomis macrochirus	Bluegill sunfish	Static Acute	Juvenile/ns	4-days	0.68	19 (10)	145 (<103)			217 (<204)
Lepomis macrochirus	Bluegill sunfish	Static Acute	Sub-adult	4-days			177			
Lepomis macrochirus	Bluegill sunfish	Static Chronic ELS	Sac-fry	12-days		(>50)	(>40)			
Lepomis cyanacellus	Green sunfish	Static Chronic ELS	Sac-fry	12-days			(>25)			
Erimyzon sucetta	Lake chubsucker	Static Chronic ELS	Sac-fry	12-days			(>25)			
Micropterus dolomieu	Smallmouth bass	Static Acute	Juveniles	4-days			236			
Micropterus dolomieu	Smallmouth bass	Static Chronic ELS	Sac-fry	12-days			(>25)			
Lepomis gibbosus	Pumpkin-seed sunfish	Static Acute	NS	4-days						95
Roccus americanus	White perch	Static Acute	NS	4-days						40
Campostnotum anomalum	Stoneroller	Static Chronic ELS	Egg-Sac-Fry	8-days		(>25)				
Alburnus alburnus	Bleak	Static Acute	NS	4-days	3.4					
Alburnus alburnus	Bleak	Static Acute	Egg	2-days				13		
Micropterus salmoides	Largemouth bass	NS[2] Acute	Juvenile	4-days						

Table 2: Toxicity of 2,4-D to Different Aquatic Species; and Sensitive Species and Stages Tested (Continued)

Species Name	Common Name	Test Type	Age of Organism	Test Duration	Test Chemicals – 2,4-D Products LC50 or EC50 & (NOEC) in mg a.i./L					
					BBE	2-EHE	DMA	Na Salt	K Salt	Acid
Micropterus salmoides	Largemouth bass	Static Acute ELS	Eggs	3.5-days					165	
Micropterus salmoides	Largemouth bass	Static Acute ELS	Sac-Fry	3.5-days					161	
Micropterus salmoides	Largemouth bass	Flow Chronic ELS	Eggs-Sac-Fry	3.5-days					93 (6.5)	
Mugil cephalus	Mullet	Static Acute	NS[3]	4-days				32		
Morone saxatilis	Striped bass	Static Acute	Juveniles/NS[3]	4-days						70
Pimephales promelas	Fathead minnow	Static Acute	Fry	4-days	2.5	>5 (10)				
Pimephales promelas	Fathead minnow	Static/NS Acute	Fry/Juv/NS[3]	4-days	4.3		314			190 (256)
Pimephales promelas	Fathead minnow	Flow Chronic ELS	Egg-Fry	32-days	(0.0805)	(0.12)	(17.1)			(63.4)
Pimephales promelas	Fathead minnow	Flow Chronic Life-Cycle	Spawn to Spawn	10 months	(0.3)					
Ictalurus punctatus	Channel catfish	Static Acute	Fry/Juvenile/NS[3]	4-days	1.02		132			
Ictalurus punctatus	Channel catfish	Static Acute	Sub-adults	4-days			193			
Menidia beryllina	Inland silverside	Flow Acute	Juvenile/NS[3]	4-days		>0.85 (0.26)	469			175 (<111)
Anguilla rostrata	American eel	Static Acute	NS[3]	4-days						301
Oryzias latipes	Medaka	NS[2] Acute	NS[3]	2-days to 4-days				>40		2780

Table 2: Toxicity of 2,4-D to Different Aquatic Species; and Sensitive Species and Stages Tested (Continued)

Species Name	Common Name	Test Type	Age of Organism	Test Duration	Test Chemicals – 2,4-D Products LC50 or EC50 & (NOEC) in mg a.i./L					
					BBE	2-EHE	DMA	Na Salt	K Salt	Acid
Oryzias latipes	Medaka	Flow Chronic ELS	Sac-Fry	28-days						28.4
Trichogaster pectoralis	Sepat Siam	Static Acute	4-6 cm	4-days						153
Clarias batrachus	Keli	Static Acute	4-6 cm	4-days						60
Lepistes reticulata	Guppy	Static Acute	NS[3]	4-days						71
Rasbora nielgeriensis	Rasbora	Static Acute	NS[3]	4-days						5.6
Labeo boga		Static Acute	NS[3]	4-days						3.8
Cyprinus carpio or *Carrassius auratus*	Carp or Goldfish	Renewal Acute	NS[3]	4-days						20
Cyprinus carpio or *Carrassius auratus*	Carp or Goldfish	Static Acute	NS[3]	4-days			>748			152
Cyprinus carpio or *Carrassius auratus*	Carp or Goldfish	Static Acute ELS	Eggs	4-days					>187	
Cyprinus carpio or *Carrassius auratus*	Carp or Goldfish	Static Acute ELS	Sac-Fry	4-days					>201	
Cyprinus carpio or *Carrassius auratus*	Carp or Goldfish	Flow Chronic ELS	Eggs-Sac-Fry	8-days					126 (12.7)	
Cirrhina mrigala hamilton	Hamilton's carp	NS[2] Acute	Fingerlings	4-days			>100			

Table 2: Toxicity of 2,4-D to Different Aquatic Species; and Sensitive Species and Stages Tested (Continued)

Species Name	Common Name	Test Type	Age of Organism	Test Duration	Test Chemicals – 2,4-D Products LC50 or EC50 & (NOEC) in mg a.i./L					
					BBE	2-EHE	DMA	Na Salt	K Salt	Acid
Fundulus diaphanus	Banded killifish	Static Acute	NS[3]	4-days						27
Daphnia magna	Daphnia	Flow Acute	<24 hrs	2-days	7.2 (<3.4)		>135			
Daphnia magna	Daphnia	Static Acute	<24 hrs	2-days	4.0	5.2 (0.6)		932		209 (<12)
Daphnia magna	Daphnia	Flow Chronic Acute	<24 hrs	21-days	(0.29)	0.13 (0.015)	176 (27.5)			235 (79)
Ceriodaphnia dubia	Daphnia	Static Acute	NS[4]	2-day						236
Ceriodaphnia dubia	Daphnia	Renewal Chronic Life-Cycle	NS[4]	4-day						81.8 (49)
Ceriodaphnia dubia	Daphnia	Renewal Chronic Life-Cycle	NS[4]	7-day						72.5 (48.8)
Crassostrea virginica	Eastern oyster	Flow Acute Shell growth	Larvae or Juvenile	4-days	2.6	1.0 (0.39)	~209			123 (30)
Mytilus edulis	Bay mussel	Static Acute Normal Tocophore Development	NS[5]	4-days						212
Mytilus edulis	Bay mussel	Static Acute EC50 attachment	NS[5]	4-days						262
Mytilus edulis	Bay mussel	Static Acute EC50 attachment	NS[5]	4-days						262

Table 2: Toxicity of 2,4-D to Different Aquatic Species; and Sensitive Species and Stages Tested (Continued)

Species Name	Common Name	Test Type	Age of Organism	Test Duration	Test Chemicals – 2,4-D Products LC50 or EC50 & (NOEC) in mg a.i./L					
					BBE	2-EHE	DMA	Na Salt	K Salt	Acid
Orconectes nous	Crayfish	NS	NS[3]	NS	100					
Gammarus fasciatus	Lined scud	Static Acute	Juvenile	4-days	0.44	2.4	>100			
Gammarus fasciatus	Lined scud	Static Acute	Mature/NS[4]	4-days/NS	5.8					3.2
Gammarus lacustris	Bright scud	Static Acute	NS[4]	2-days	0.76					
Cyclops vernalis	Cyclops	NS[2] Acute	NS[3]	NS						37
Nitocra sinepes	Copepod	NS[2] Saltwater	NS[3]	4-days	3.1					
Macrobranchium spp.	Freshwater prawns	Static Acute	NS[3]	4-days				~2300		
Palaemonetes kadiakensis	Glass shrimp	NS[2] Saltwater	NS	NS	1.18		0.15			
Palaemonetes pugio	Grass shrimp	Flow Acute Saltwater	Juvenile	4-days		>1.4 (0.003)	>0.14			
Panaeus duorarum	Pink shrimp	Flow Acute Saltwater	NS[3]	4-days			181 (65)			554
Panaeus aztecus	Brown shrimp	Static Saltwater	Juvenile	2-days	5.6	0.48				
Cypridopsis vidua	Seed shrimp	Static Acute Saltwater	Mature	NS			8.0			
Cypridopsis vidua	Seed shrimp	Static Acute Saltwater	Juvenile/NS[3]	2-days	1.99		181 (65)			
Chironomus plumosus	Midge	Static/NS Acute	Larvae	4-days	0.56		>100			
Chaoborus punctipennis	Glassworm	NS Acute	Larvae	1-day			1151			

Table 2: Toxicity of 2,4-D to Different Aquatic Species; and Sensitive Species and Stages Tested (Continued)

Species Name	Common Name	Test Type	Age of Organism	Test Duration	Test Chemicals – 2,4-D Products LC50 or EC50 & (NOEC) in mg a.i./L					
					BBE	2-EHE	DMA	Na Salt	K Salt	Acid
Aselus brevicaudus	Aquatic sowbug	Static Acute	Juvenile/NS[3]	4-days	2.46		>100			
Pteronarcys californica	Stonefly	Static Acute	Larvae	4-days	1.6					
Pteronarcys californica	Stonefly	Static Acute	Adult	4-days	>1000					
Chasmagnathus granulata	Estuarine crab	Static Acute Saltwater	1st Zoel	4-days	0.3					
Chasmagnathus granulata	Estuarine crab	Chronic Saltwater	Adult	28-days	>50					
Chasmagnathus granulata	Estuarine crab	Static Acute Saltwater	Adult	4-days	3370					
Chasmagnathus granulata	Estuarine crab	Static Acute Saltwater	Juvenile	4-days	2890					
Chasmagnathus granulata	Estuarine crab	Chronic Saltwater	Juvenile	28-days	30					
Cancer magister	Dungeness crab	Static Acute Saltwater	1st Zoel	4-days						>10
Cancer magister	Dungeness crab	Static Acute Saltwater	Adult	4-days						>100
Uca uruguayensis	Estuarine crab	Chronic Saltwater	NS[3]	28-days	>30		(>1000)			
Uca uruguayensis	Estuarine crab	Static Acute Saltwater	Adult	4-days	130					
Lumbrichulus variegatus	Oligochaete	Flow Acute	NS	4-days						122 (87)
Tubifex tubifex	Tubifex worm	Static Acute	20 mm length							161

[1] ELS = Early Life-Stage
[2] NS = Not Specified and presumed to be Static
[3] NS = Not specified but presumed to be Juveniles
[4] NS = Not specified and presumed to be <24 hour old
[5] NS = Not specified and presumed to be a late tocophore.

Table 3: Soil Erodability Factors

Surface Layer Texture	Estimated K
Clay, Clay Loam, Loam, Silty Loam	0.32
Fine Sandy Loam, Loamy very Fine Sand, Sand Loam	0.24
Loamy Fine Sand, Loamy Sand	0.17
Sand	0.15
Silt Loam, Silty Clay Loam, Very Fine Sandy Loam	0.37

Source: Barfield et al., 1981in Ebasco, 1993 and USDA. 1978a in Ebasco, 1993

Table 4: Effects of 2,4-D DMA Treated Irrigation Water on Various Crops

Crop	Concentration in Water (mg./L)	Mode of Irrigation	Phytotoxic Effect	Effects on Yield	Residue in Crop Mg 2,4-D/Kg
Sugar beets	5.5 at 1st[1] 2.2 at 2nd[1]	F[2]	Curvature of petioles, wilting, slumping chlorosis & necrosis	No effect on roots or tops	NS[3]
Soybeans	5.5 at 1st 2.2 at 2nd	F	50-20% Chlorosis	No effects on seed yields	NS
Sweet corn	5.5 at 1st 2.2 at 2nd	F	Desiccation of lower leaves	No effect on yields of fodder or shelled corn.	
Sugar beets	5.5 at 1st 2.2 at 2nd	S[4]	Drooping and wilting	Fresh weight and root yields increased. % Sugar decreased but gross-yields are higher.	NS
Soybeans	5.5 at 1st 2.2 at 2nd	F	5-20% Chlorosis	No effects on seed yields	NS
Soybeans	5.5 at 1st 2.2 at 2nd	S	Suppressed growth and early chlorosis	Yields and quality were not reduced	NS
Dwarf Corn	5.5 at 1st 2.2 at 2nd	S	None	No significant reduction in fodder or shelled corn yields	NS
Sugar beets	5.5 at 1st 2.2 at 2nd	F	NS	NS	Residues detected

Table 4: Effects of 2,4-D DMA Treated Irrigation Water on Various Crops (Continued)

Crop	Concentration in Water (mg./L)	Mode of Irrigation	Phytotoxic Effect	Effects on Yield	Residue in Crop Mg 2,4-D/Kg
Soybeans	5.5 at 1st 2.2 at 2nd	F	NS	NS	0.009-0.05 mg/Kg prior to harvest and not detected in edible tissue at harvest
Sweet corn	5.5 at 1st 2.2 at 2nd	F	NS	NS	No residues in grain at harvest. 0.08 mg/Kg in foliage 7 days after 1st irrigation
Sugar beets	5.5 at 1st 2.2 at 2nd	S	NS	NS	3.80 prior to harvest 0.009 mg/Kg at harvest in beets. None in foliage
Soybeans	5.5 at 1st 2.2 at 2nd	S	Malformed some foliage. Interfered with normal development of early squares and blooms	Total yield of cotton was significantly higher	0.52 mg/Kg in foliage and pods 2 days after treatment. None detected in edible tissue at harvest
Dwarf corn	5.5 at 1st 2.2 at 2nd	S	NS	NS	0.521 mg/Kg in foliage and pods 2 days after treatment. None detected in edible tissue at harvest

[1] 1st and 2nd irrigation separated by three to ten days
[2] Furrow irrigation
[3] Not Specified
[4] Sprinkler irrigation (Also could represent the effects of an agricultural spray to wetting treatment)

Table 5: Classification of Pesticides Based on Bioconcentration Factor

Classification	Bioaccumulation Factor[1]	Characteristics
Non-accumulative	≤10	Pesticide readily decreases when organism is removed from exposure
Slightly accumulative	60-700	Pesticide is only gradually lost when organism is removed from exposure or pesticide is degraded by organisms
Moderately Accumulative	700-8,000	Pesticide is only gradually lost when organism is removed from exposure, or pesticide is slowly degraded by organism
Highly accumulative	≥8,000	Pesticide is not lost when an organism is removed from exposure, or pesticide is not significantly degraded by organism

Source: Weber, 1977 in Ebasco, 1993

[1] As defined by Weber, 1977 in Ebasco, bioaccumulation = concentration in aquatic organism/ concentration in water. Typically, this is termed the bioconcentration factor and not the bioaccumulation factor which takes into account accumulation from all sources of exposure including food, water consumed, exposure water and if applicable, air exposure.

Table 6: Concentration of 2,4-D Residues in Pond Components after Treatment with 23 Kg/Ha of 2,4-D BEE Formulation[1]

Days after application	2,4-D Concentrations (mg/Kg)			Concentration Factor	
	Milfoil	Sediment	Water	Plant/Water	Plant/Soil
1	136	8.0	1.8	76	4.4
7	206	4.4	2.2	94	2
13	98	4.3	3.0	33	1.4
34	16	1.6	1.6	10	1
55	16	1.0	1.3	12	0.8
82	8	0.4	1.1	7.3	0.4
111	NA	0.5	0.8	NA	0.6
182	NA	0.6	0.2	NA	3.0

Source: Birmingham and Colman (1985 in Ebasco, 1993)

[1] Ponds contained about 4,000 Kg water and 290 Kg sediment. From day 1 to day 20 and day 27 onward ponds contained 0.5 Kg plant material.

Table 7: Concentration Factors in Water, Hydrosoil and Fish for 2,4-D DMA Applied to Ponds in Florida and Georgia

Pond	Time after Application Days	App. Rate Kg a.e./Ha	Water mg a.e./L	Hydrosoil mg a.e./L	Fish mg a.e./L	Concentration Factor Soil	Concentration Factor Fish
Florida	1	2.24	0.025	0.005	0.08	0.20	3.20
Florida	1	4.48	0.155	0.014	0.048	0.09	0.31
Florida	1	8.96	0.312	0.033	0.005	0.11	0.02
Georgia	1	2.24	0.025	0.018	0.005	0.72	0.20
Georgia	1	4.48	0.233	0.024	0.014	0.10	0.06
Georgia	1	8.96	0.657	0.026	0.022	0.04	0.03
Florida	3	2.24	0.005	0.005	0.005	1.00	1.00
Florida	3	4.48	0.172	0.014	0.005	0.08	0.03
Florida	3	8.96	0.345	0.046	0.005	0.13	0.01
Georgia	3	2.24	0.087	0.008	0.005	0.09	0.06
Georgia	3	4.48	0.39	0.018	0.005	0.05	0.01
Georgia	3	8.96	0.692	0.04	0.005	0.06	0.01
Florida	7	2.24	0.005	0.005	0.005	1.00	1.00
Florida	7	4.48	0.048	0.01	0.005	0.21	0.10
Florida	7	8.96	0.025	0.008	0.005	0.32	0.20
Georgia	7	2.24	0.059	0.01	0.005	0.17	0.08
Georgia	7	4.48	0.4	0.018	0.005	0.05	0.01
Georgia	7	8.96	0.395	0.042	0.005	0.11	0.01
Florida	14	2.24	0.005	0.005	0.036	1.00	7.20
Florida	14	4.48	0.005	0.01	0.005	2.00	1.00
Florida	14	8.96	0.005	0.013	0.043	2.60	8.60
Georgia	14	2.24	0.027	0.005	0.005	0.19	0.19
Georgia	14	4.48	0.008	0.005	0.005	0.63	0.63
Georgia	14	8.96	0.05	0.005	0.005	0.10	0.10
Florida	28	2.24	0.005	0.005	0.005	1.00	1.00
Florida	28	4.48	0.005	0.007	0.005	1.40	1.00
Florida	28	8.96	0.005	0.005	0.005	1.00	1.00
Georgia	28	2.24	0.005	0.006	0.005	1.20	1.00
Georgia	28	4.48	0.005	0.005	0.005	1.00	1.00
Georgia	28	8.96	0.005	0.005	0.01	1.00	2.00
	Mean	2.24	0.02	0.006	0.018	0.30	3.00
		4.48	0.118	0.01	0.009	0.01	0.90
		8.96	0.208	0.018	0.009	0.09	0.50

Table 8: Dissolved Oxygen Concentration (mg/L) at Different Temperatures

Temperature in Degrees Centigrade	Dissolved Oxygen Concentration in mg/L
0	14.2
1	13.9
2	13.5
3	13.1
4	12.7
5	12.4
6	12.1
7	11.7
8	11.5
9	11.2
10	10.9
11	10.7
12	10.5
13	10.2
14	10.0
15	9.8
16	9.6
17	9.4
18	9.1
19	9.0
20	8.9
21	8.6
22	8.5
23	8.4
24	8.3
25	8.2

Table 9: Relationship of pH and Temperature to the Percentage of Unionized Ammonia [NH4OH + NH3 (dissolved)] in Freshwater

pH	Temperature (°C)				
	5°	10°	15°	20°	25°
6.5	0.04%	0.06%	0.09%	0.13%	0.18%
7.0	0.12%	0.19%	0.27%	0.40%	0.55%
7.5	0.39%	0.59%	0.85%	1.24%	1.73%
8.0	1.22%	1.83%	2.65%	3.83%	5.28%
8.5	3.77%	5.55%	7.98%	11.2%	15.0%
9.0	11.0%	15.7%	21.4%	28.5%	35.8%

Table 10: Effects of pH and Hardness on the Toxicity of three formulations of 2,4-D[1]

Hardness (mg/L)	pH	Fish Species	96 hour LC50 (mg/L)		
			2,4-D BEE	2,4-D 2-EHE	2,4-D diethanolamine
Soft 4.2-4.8	6.1-6.3	Coho salmon	1.1	156	472
		Pink salmon	0.4	30	291
		Rainbow trout	0.8	167	409
Intermediate 38.9-43.4	7.5	Coho salmon	1.5	158	493
		Pink salmon	0.9	70	363
		Rainbow trout	1.3	164	511
Hard 80.5-86	8.0-8.1	Coho salmon	4.3	63	662
		Pink salmon	1.1	21	438
		Rainbow trout	2.2	79	744

[1] Wan et al, 1990, and Wan et al., 1991

Table 11: Effects of pH on the Toxicity of 2,4-D Acid to Rainbow Trout[1]

pH	96 hour LC50 (mg/L) for 2,4-D Acid
4.54	<100
5.6	<400
6.8	<1000
8.48	>1000

[1] Finlayson & Verrue, 1985 in JMPR, 1997

Table 12: Toxicity of Adjuvants Registered for Aquatic Use to Aquatic Animals

Adjuvant	Use	Use Rate L/ha	Depth for LC50 to be Achieved	96 hr LC50 (mg/L)		
				Bluegill	Rainbow Trout	Daphnia magna
Spar-Mate®	Surfactant	140	1.5	0.96		
R-11®	Surfactant			4.2-5.5	3.8	19
X77®	Surfactant	4.7	0.1	4.3	4.2	2.0
Cide-Kick II®	Surfactant	7.0	0.1	4.3-5.2		
Widespread®	Surfactant®			7.0	6.6	16
Induce®	Surfactant/ Accelerant			7.3	8.3	18
Super Spread 200®	Surfactant®			9.3		
Liqua Wet®	Surfactant			11.0	13	7.2
Spreader Sticker®	Surfactant/ Sticker			35	36	48
Formula 403		18.7	0.1	37		
IVOD®		18.7	0.1	37		
Passage®				52	75	17
Big Sur®		4.7	<0.1	112		
Nalquatic®	Thickener	9.3	<0.1	200		
LI-700®				210	130	170
Agri Dex®	Surfactant/ Accelerant			>1000	>1000	>1000
Polysar®	Thickener	4.7	<0.1	3600		
Herbex®		2.3	<0.1	8000		
Foamer®	Anti-Foam					
No Foam A®	Anti-Foam					
Dyne Amic®	Surfactant					
Penetrator®	Surfactant/ Accelerant					

Table 13: Plant Susceptibility to 2,4-D

Weeds – Foliar Application (Portman and Losey, 1979 in Ebasco, 1993		
Easily Killed:		
Alder*	Pigweed	Lambsquarters
Black Mustard	Dandelion	
Broadleaf Plantain*	Russian Thistle	
Usually susceptible at higher rates or with repeat application:		
Buttercup	White Clover	
Canada Thistle	Yarrow	
Control not practical:		
Annual Bluegrass	Reed Canary Grass*	Sandbur
Barnyardgrass	Russian Knapweed	Western Bracken
Moss	Cattails*	Wildoat
Quackgrass	Fiddleneck (mature)	
Crops:		
Easily killed:		
Grapes	Red Clover	Mint
Tomatoes	Alfalfa	Sugar beets
Beans	Tree fruits	Hops
Lentils	Peppers	Strawberry
Peas and vetches	Sweet clover, Crimson clover	
Less easily killed:		
Corn	Asparagus	Flax
Potatoes	Bulb crops	
Generally not killed or injured:		
Grains (wheat, oats, barley, rye)		
Grasses (annual and perennial)		
Non-crop Terrestrial Plants – 2,4D Ester (BLM, 1987 in Ebasco, 1993)		
Susceptible:		
Willow		
Susceptible to Intermediate:		
Cottonwood	Serviceberry	Lupine
Quaking Aspen	Snowberry	
Resistant:		
Indian ricegrass	Thickspike wheatgrass Western	Idaho fescue
Bluegrass	wheatgrass	Spike fescue

* Identified with wetland environments

Table 14: Acute to Chronic Ratio for Aquatic Organisms

Species	Length of Chronic Test	2,4-D Formulation	Acute Toxicity (LC50 mg/L)	Chronic Toxicity NOEC[1] (mg/L)	Ratio[2]
Chinook salmon	86 days	2,4-D BEE	0.32-0.38 (0.35)[2]	0.040	8.75
Fathead minnow	32 days	2,4-D BEE	2.5-3.25 (2.85)	0.0805	35.4
Fathead minnow	Life Cycle 10 months	2,4-D BEE	2.5-3.25 (2.85)	0.3	9.5
Fathead minnow	32 days Early Life-Stage	2,4-D 2-EHE	>5	0.12	>41.7[3]
Fathead minnow	32 days Early Life-Stage	2,4-D DMA	266-355 (307)	17.1	18.0
Rainbow trout	23-27 days Early Life-Stage	2,4-D K Salt	358	LC50 = 4.2-11[4] (6.79)[5]	52.7
Goldfish	8 days	1,4-D K Salt	>187 to >201 (>194) or >164[6,7,8]	LC1 = 8.9-18.2[4] (12.7 a.i. 10.8 a.e.)	>15.2
Largemouth bass	7.5 days	2,4-D K Salt	161-165 (163)	LC1 = 3.2-13.1 (6.5)	25.1
Fathead minnow	32 days	2,4-D Acid	133-320 (206)	63.4	3.2
Medaka	28 days	2,4-D acid	2780	27.2-30 (28.6)	97.2
Daphnid	21-day Life-Cycle Flow	2,4-D BEE	7.2[9]	0.29[9]	24.8
Daphnid	21-day Life-Cycle	2,4-D 2-EHE	5.2[9]	0.015[9]	346.7[3]
Daphnid	21-day Life-Cycle	2,4-D DMA	184[9]	27.5[9]	6.69
Daphnid	21-day Life-Cycle	2,4-D Acid	25->100[10] (>45)	79[9]	0.57[11]

1 Chronic Values are NOEC unless otherwise noted
2 Geometric mean excluding 2,4-D 2-EHE product and extreme outlier and "greater thans" = 18.0 (limits 6.4 to 50.5 ± one standard deviation)
3 2,4-D 2-EHE is not usually used for the control of fully aquatic weeds
4 2,4-D K Salt
5 Values in parenthesis are geometric means of all valid individual values
6 2,4-D Acid
7 Neskovick, 1994
8 *Cyprinus carpio* (related species that may not be a separate species)
9 All work conducted under current Pesticide Assessment Guidelines according to current GLP Guidelines
10 Data exhibits extreme variability between laboratories
11 Extreme outlier

Table 15: Water Conditions, Plankton, Heterotrophic Bacterial Floral Counts and Sediment Bacterial Floral Counts in an Indian Pond Treated for the Control of *Euryale Ferox* (Thorny Lily) (Patnaik and Das, 1991)

Parameters	Before Treatment	Immediately After Treatment	8-Weeks After Treatment
Water pH	8.0	8.0	8.2
Total alkalinity (mg/L)	176	156	168
Dissolved Oxygen (mg/L)	2.5	0.94	4.26
Free Ammonia (mg/L)	0.25	0.65	0.08
Nitrate (mg/L)	0.06	0.06	0.15
Phosphate (mg/L)	0.02	0.02	0.04
Phytoplankton (no./L)	324	480	924
Zooplankton (no./L)	432	624	1260
Heterotrophic bacterial counts in water (no./mL)	360	942	298
Bacterial counts in sediment (no/g) dry wt x 10^4	15.87	4.67	9.21

Table 16: Percent Cover of Aquatic Macrophytes in Fritz and Mueller Coves, Beulah Lake, Wisconsin in August 1994 Two Growing Seasons After Treatment with 112 Kg product/ha (100 lbs product/acre) of 2.4-D BEE

Plant Species	2,4-D BEE in Fritz Cove Pre-Treat/Post-Treat	2,4-D BEE in Mueller Cove Pre-treat/Post-Treat	Bottom Fabric Dunn Cove Pre-treat/Post-treat
Total Plant Cover	100/100	100/70	100/100
Eurasian Watermilfoil	~55.0/5	~60.2/3	~85/60
Water Celery	NS[1]/35	NS[1]/0	NS[1]/0
Elodea canadensis	~10/20	~5/10	~10/0
Coontail	~35/10	~25/5	~5/10
Other Species	~10/30	~15/20	~0/30

[1] Not specified. Lumped with other species.

Table 17: Acute Toxicity of 2,4-D to Algae and Aquatic Macrophytes

Test Substance	%A.I. or Formulation	Species	Test Type	Water Type	Growth Stage/ Age	Time (Hours)	LC50 mg a.i./L	NOEC mg a.i./L	Status	Reference	MRID Number
2,4-D, BEE	96	*Selenastrum capricornutum (*Green algae)	Static	AAP[1]	Log	120	24.90	12.50	C[2]	Hughes, 1989	NS[3]
2,4-D, BEE	70	*Dunaliella tertiolecta* (Green algae)	Static	ESW[4]	Log	240	75.00	NS[1]	S[5]	EPA, 1986[B99]	40228401
2,4-D, BEE	70	*Chlorococcum sp.* (Green algae)	Static	AAP	Log	240	75.00	NS	S	EPA, 1986[B99]	40228401
2,4-D, BEE	70	*Isochrysis galbana* (Marine haptophyte)	Static	ESW	Log	240	75.00	NS	S	EPA, 1986[B99]	40228401
2,4-D, BEE	96	*Anabaena flos-aquae* Blue-green algae	Static	AAP	Log	120	6.37	3.14	C	Hughes, 1990i	42068401
2,4-D, BEE	96	*Skeletonema costatum* (Marine diatom)	Static	MANM[6]	Log	120	1.66	0.79	C	Hughes, 1990c	42068404
2,4-D, BEE	70	*Phaeodactylum tricornutum* (Marine diatom)	Static	SiESW[7]	Log	240	150.00	NS	S	EPA, 1986[B99]	40228401
2,4-D, BEE	96	*Naviculla Pelliculosa* (diatom)	Static	SiAAP[8]	Log	120	1.86	1.76	C	Hughes, 1990h	42068403

Table 17: Acute Toxicity of 2,4-D to Algae and Aquatic Macrophytes (Continued)

Test Substance	%A.I. or Formulation	Species	Test Type	Water Type	Growth Stage/ Age	Time (Hours)	LC50 mg a.i./L	NOEC mg a.i./L	Status	Reference	MRID Number
2,4-D, BEE	96	*Lemna gibba* (Duckweed)	Static	Hoagland's[9]	3-4 fronds	336	0.58	0.20	C	Hughes, 1990e	42068402
2,4-D, 2-EHE	94.7	*Selenastrum capricornutum* (Green algae)	Static	AAP	Log	120	>30	15.00	C	Hughes, 1990n	41735203
2,4-D, 2-EHE	94.7	*Anabaena flos-aquae* Blue-green algae	Static	AAP	Log	120	>30 (>0.32)[10]	>30 (>0.32)[10]	C	Hughes, 1990b	41735202
2,4-D, 2-EHE	94.7	*Skeletonema costatum* (Marine diatom)	Static	MANM	Log	120	0.23	0.19	C	Hughes 1990a	41735204
2,4-D, 2-EHE	94.7	*Naviculla Pelliculosa* (diatom)	Static	SiAAP	Log	120	4.10	3.75	C	Hughes, 1990g	41735205
2,4-D, 2-EHE	94.7	*Lemna gibba* (Duckweed)	Static	Hoagland's	3-4 fronds	336	0.50	0.19	C	Hughes, 1990d	41735203
2,4-D, DMA	66.7	*Selenastrum capricornutum* (Green algae)	Static	AAP	Log	120	66.50	19.20	C	Hughes, 1990o	41420002
2,4-D, DMA	EC Form	*Dunaliella tertiolecta*	Static	20mg/mL SW	Log	20 days	185.00	NS	NS	Okay & Gaines, 1995	NS
2,4-D, DMA	66.7	*Anabaena flos-aquae* Blue-green algae	Static	AAP	Log	120	153.00	67.86	C	Hughes, 1990j	41505902

Table 17: Acute Toxicity of 2,4-D to Algae and Aquatic Macrophytes (Continued)

Test Substance	%A.I. or Formulation	Species	Test Type	Water Type	Growth Stage/ Age	Time (Hours)	LC50 mg a.i./L	NOEC mg a.i./L	Status	Reference	MRID Number
2,4-D, DMA	66.7	*Skeletonema costatum* (Marine diatom)	Static	MANM	Log	120	36.60	96.25	C	Hughes, 1990f	41505901
2,4-D, DMA	EC Form	*Phaeodactylum tricornutum*	Static	20 mg/mL SW	Log	20 days	362.00	NS	NS	Okay & Gaines, 1995	NS
2,4-D, DMA	66.7	*Naviculla Pelliculosa* (diatom)	Static	SiAAP	Log	120	5.28	1.70	C	Hughes, 1990d	51505903
2,4-D, DMA	66.7	*Lemna gibba* (Duckweed)	Static	Hoagland's	Log	336	0.58	0.27	C	Hughes, 1990l	41505904
2,4-D, Acid	96.1	*Selenastrum capricornutum (*Green algae)	Static	AAP	Log	120	51.20	19.20	C	MPI, 1990[b99] Brian, 1999	41420002
2,4-D, Acid	96.1	*Selenastrum capricornutum (*Green algae)	Static	AAP	Log	120	33.20	26.20	S	Hughes, 1990n[J97]	41420001
2,4-D, Acid	96.1	*Selenastrum capricornutum (*Green algae)	Static	AAP	Log	96	25.90	24.20	C	St. Laurent, 1992 JMPR, 1997	NS
2,4-D, Acid	NS	*Scenedesmus quadricauda* (Green algae)	Static	AAP	Log	20 days	98.00	NS	NS	Fargasova, 1994	NS
2,4-D, Acid	87	*Chlorococcum sp.* (Green algae)	Static	AAP	Log	240	50.00	NS	S	EPA, 1986	40228401
2,4-D, Acid	Tech	*Chlorella fusca* (Green algae)	Static	AAP	Log	120	88.90	NS	C	Faust et al., 1994[J97]	

Table 17: Acute Toxicity of 2,4-D to Algae and Aquatic Macrophytes (Continued)

Test Substance	%A.I. or Formulation	Species	Test Type	Water Type	Growth Stage/ Age	Time (Hours)	LC50 mg a.i./L	NOEC mg a.i./L	Status	Reference	MRID Number
2,4-D, Acid	97	*Dunaliella tertiolecta* (Green algae)	Static	ESW	Log	240	75.00	NS	S	EPA, 1986[B99]	40228401
2,4-D, Acid	97	*Isochrysis galbana* (Marine haptophyte)	Static	ESW	Log	240	50.00	NS	S	EPA, 1986[B99]	40228401
2,4-D, Acid	NS	*Nostoc linkia* (Blue-green algae)	Static	NS	Log	12 Days	~500	<100	NS	Mishra & Pandey, 1989	
2,4-D, Acid	NS	*Nostoc calcicola* Blue-green algae	Static	NS	Log	12 Days	~500	<100	NS	Mishra & Pandey, 1989	
2,4-D, Acid	NS	*Nostoc sp.* (Blue-green algae)	Static	NS	Log	12 Days	~500	<100	NS	Mishra & Pandey, 1989	
2,4-D, Acid	NS	*Anabaena doliolum* (Blue-green algae)	Static	NS	Log	12 Days	~500	<100	NS	Mishra & Pandey, 1989	
2,4-D, Acid	49%	*Chladymonas reinhardtii* Blue-green algae	Static	MCM[11]	Log	192	>40	NS	NS	Wong & Chang, 1988	
2,4-D, Acid	97%	*Anabaena flos-aquae* (Blue-green algae)	Static	AAP	Log	120	>2.02	<2.02	C	Hughes et al. 1994	43307902

Table 17: Acute Toxicity of 2,4-D to Algae and Aquatic Macrophytes (Continued)

Test Substance	%A.I. or Formulation	Species	Test Type	Water Type	Growth Stage/ Age	Time (Hours)	LC50 mg a.i./L	NOEC mg a.i./L	Status	Reference	MRID Number
2,4-D, Acid	NS	*Nostoc linkia* Blue-green algae)	Static	NS	Log	12 Days	~500	<100	NS	Mishra & Pandey, 1989	
2,4-D, Acid	NS	*Nostoc calcicola* (Blue-green algae)	Static	NS	Log	12 Days	~500	<100	NS	Mishra & Pandey, 1989	
2,4-D, Acid	NS	*Nostoc sp.* (Blue-green algae)	Static	NS	Log	12 Days	~500	<100	NS	Mishra & Pandey, 1989	
2,4-D, Acid	NS	*Anabaena doliolum* (Blue-green algae)	Static	NS	Log	12 Days	~500	<100	NS	Mishra & Pandey, 1989	
2,4-D, Acid	96.9	*Skeletonema costatum* (Marine diatom)	Static	SiESW	Log	120	2.08	<2.08	C	Hughes et al, 1994	43307903
2,4-D, Acid	97	*Phaeodactylum Tricornutum* (Green algae)	Static	MANM	Log	240	50.00	NS	C	EPA 1986, Brian, 1999	40228401
2,4-D, Acid	96.9	*Navicula pelliculosa* (diatom)	Static	SiAAP	Log	120	2.02	>2.02		Hughes et al, 1994	
2,4-D, Acid	NS	*Sinapsis alba* Germination	Static	NS	Log	72	166	NS	NS	Fargasova, 1994	
2,4-D, Acid	NS	*Sinapsis alba* Root elongation	Static	NS	Log	72	1.17	NS	NS	Fargasova, 1994	

Table 17: Acute Toxicity of 2,4-D to Algae and Aquatic Macrophytes (Continued)

Test Substance	%A.I. or Formulation	Species	Test Type	Water Type	Growth Stage/ Age	Time (Hours)	LC50 mg a.i./L	NOEC mg a.i./L	Status	Reference	MRID Number
2,4-D, Acid	96.9	*Lemna gibba* (Duckweed)	Static	Hoagland's	Log	240	0.695	0.0581	C	Hughes et al, 1994	43307903

[1] AAP = Algal Assay Procedure Nutrient Medium (Miller, 1978 in Hughes, 1990)
[2] C = Core study with which an EPA Risk Assessment was conducted
[3] NS = Not specified for this parameter
[4] ESW = Enriched Seawater (EPA, 1986 in Brian 1999)
[5] S = Supplemental study from which useful information can be obtained but may not be suitable for EPA Risk Assessment
[6] MANM = Marine Algal Nutrient Media (Walsh and Alexander, 1980 in Hughes, 1990)
[7] SiESW =Enriched Seawater with silica added (EPA, 1986 in Brian, 1999)
[8] SiAAP = Algal Assay Procedure Nutrient Medium with silica added (Miller, 1978 in Hughes, 1990)
[9] Hoagland's = Standard Hoagland's Hydroponic Nutrient Medium
[10] Value given in parenthesis is the geometric mean of all acceptable values
[11] MPM = Modified Complete Medium
[J97] JMPR, 1997
[B99] Brian Database, 1999

Table 18: Potential Hazard Ratios[1] for Eleven Species of Non-Target Aquatic Green Algae, Blue-Green Algae and Duckweed (*Lemna minor*) (Petersen et al, 1994)[2]

Hazard Rating and Percent Inhibition of Growth when Exposed to EEC (2.9 mg a.e./L of 2,4-D Acid		
	Very High (RQ >1.0; EEC Causes >50% Reduction in Growth)	**Potentially Low (RQ <0.1; EEC Causes <5% Reduction in Growth)**
Lemna minor	34 (5)[3]	
Green Algae		
Cyclotella meneghiana		0 (5)
Nitzschia sp.,F110-D		1 (10)
Scenecdesmus quadricauda, F11		-1[2](12)
Selenastrum capricornutum, U1648		-1 (9)
Blue-Green Algae		
Microcystis aeruginosa, PCC7820		9 (8)
Microcystis aeruginosa, U2063)		11 (13)
Oscillatoria sp.,T129		4 (9)
Pseudoanabaena, F63		-7 (6)
Anabaena inaequalis, U381		-14 (8)
Aphanizomenon flos-aquae, F107		0 (1)

[1] Peterson's Hazard Ratings: Very High if EEC causes >50% reduction in growth and RQ = >1.0; High if EEC causes 25 to 50% reduction in growth and RQ = >>>0.1; Moderate if EEC causes 5 to 25% reduction in growth and RQ = >0.1; Potentially Low if EEC causes <5% reduction in growth and RQ = <0.1.

[2] Negative values indicate an increase in growth over the control.

[3] Values in parenthesis are standard deviations.

Table 19: Laboratory Effects of 2,4-D Formulations on Phytoplankton

2.4-D Formulation	Results	References[1]
2,4-D BEE	0.001 to 1.0 mg/L – no effects on growth. 4.0 mg/L - 10% increase in number of culture vessels showing poor growth	Butler et al.,[1] 1975
2,4-D BEE	Depending on algal strain 36 to 87% of the 2,4-D was apparently metabolized to non-2,4-D compounds in 2 weeks.	Butler et al. 1975[1]
2,4-D BEE	No adverse effect on growth at 10-50 mg/L. Stimulation of growth at 10 mg/L. Growth inhibited by 25% at day 7 by 100 mg/L. Cell pathology noted at 100 mg/L but returned to normal after 7 days in clean water.	Poorman, 1973[1]
2,4-D DMA	2,4-D acid inhibits growth more strongly than 2,4-D DMA or 2,4-D amine. After adaptation to the presence of the herbicide, 2,4-D amine salts up to concentrations of 100 mg/L increase the rate of growth and assimilation of carbon dioxide of *Phaeodactylum tricornutum* (marine diatom) and *Dunaliella tertiolecta* (green algae) if the growth medium is Nitrogen- deficient After a lag-period of approximately 10 days, this species of green algae can adapt and exhibit significant growth to concentrations as high as 500 mg/L.	Okay and Gaines, 1996
2,4-D Sodium Salt	No effects on algal growth at 100 to 300 mg/L. Progressive decreases in growth at 400 to 1,000 mg/L	Sing, 1974[1]
2,4-D Sodium Salt	Cell division synchronization, cell weight, and cell diameter unaffected at all exposure levels. Respiration and photosynthesis inhibited by 244 mg/L	Bertagnoli and Nadakavukaren, 1974[1]
2,4-D Sodium Salt	No effects on survival or growth at concentrations up to 200 mg/L.	Morre, 1974[1]
2,4-D Sodium Salt	Growth limited above 5 mg/L in 1 strain of *Nostoc* sp.; growth limited above 50 mg/L in 1 strain of *Nostoc sp*.; growth limited above 100 mg/L in 17 algal strains of *Nostoc* spp.; growth limited above 500 mg/L in all 3 algal strains of *Nostoc Spp*.	Venkatraman of Rajyalakshmi, 1972[1]
2,4-D Sodium Salt	No toxic effects noted at concentrations up to 200 mg/L.	Vance and Smith, 1969[1]
2,4-D Acid	Growth drastically decreased at 110 to 220 mg/L treatment levels in three different species.	Lembi and Coleridge, 1975[1]

Table 19:Laboratory Effects of 2,4-D Formulations on Phytoplankton (Continued)

2.4-D Formulation	Results	References[1]
2,4-D Acid	*C. microsporum*: Good growth at 5-50 mg/L but none at 60 to 100 mg/L. *B. cinnibarinus*: Fair growth at 10 to 40 mg/L, but poor at 41 to 70 mg/L. *A. nidulans*: Good growth at 10 to 70 mg/L, but poor growth at 100 to 500 mg/L. *S. marcescens*: Good growth at 50 to 700 mg/L, but poor growth at 900 to 1000 mg/L. Uptake of 14C-2,4-D found to be low, with a 10-fold reduction in residue observed after washing algal cells with water.	Voight and Lynch, 1974[1]
2.4-D Acid	2,4-D acid increased the growth rate of *Chlamydomonas reinhardtii* at 1 mg/L but decreased the growth rate at all higher concentrations. The photosynthetic rate was increased at concentrations of 2,4-D acid up to 5 mg/L but was decreased at all higher concentrations. Growth and photosynthetic rate were inhibited by 40 to 50% at concentrations of 40 mg/L	Wong and Chang, 1988
2.4-D Acid	2,4-D acid slightly stimulates the growth of *Scenedesmus quadricuada* at concentrations of 5 mg/L but causes a 50% inhibition of growth at ~100 mg/L and a nearly a complete inhibition of growth at 120 mg/L.	Fargasova, 1994
2.4-D Acid	Up to 100 mg/L increased the growth and nitrogen fixation of *Anabaena* and *Nostoc* species. The EC50s with 2,4-D for these species was ~500 mg/L but the lethal dosage for the entire culture typically ranged from 1,500 to 2,000 mg/L.	Mishra and Padney, 1989
2,4-D Acid	2,4-D at concentrations up to 10 mg/L stimulated the growth of *Anabaenopsis raciboskii* in N -deficient medium. 2,4-D at concentrations of 10 to 100 mg/L slightly increased the rate of nitrogen fixation over the controls. Concentrations higher than 100 mg/L adversely affected growth rate and at concentrations of 1,200 to 1,500 mg/L, growth was completely inhibited.	Das and Singh, 1977.
2,4-D Commercial Grade (unspecified)	Commercial grade 2,4-D was sub-lethal to *Microcystis aeurginosa* at concentrations of 1,000 to1,500 mg/L. Higher concentrations of 2,4-D were lethal to this species. The EC50 for this formulation of 2,4-D was ~200 mg/L to *Microcystis aeurginosa*.	Swain and Adhikary, 1994
2,4-D unspecified Formulation	2,4-D at concentrations of increased the commercial production of various *Anabaena* spp. when exposed to concentrations of up to 0.05 mg/L by 914% over the control. Exact yields depended on the culture methods utilized.	Wang et al, 1991

[1] Listing of original reference which were compiled for 2,4-D in laboratory studies with 2,4-D in Ebasco (1993).

Table 20: Field-Effects of 2,4-D Products on Phytoplankton

2,4-D Formulation	Results	References
2,4-D BEE	Algal (*Chlorella sp.)* growth stimulated after milfoil mortality.	Robinson as Cited by Capp, 1978[1]
2,4-D BEE	Field observations: No difference as a result of herbicide treatment, based on 19-plankton tows.	Whitney et al, 1973[1]
2,4-D BEE	Treatment with 2,4-D DMA at 11.25 to 45 Kg a.e. /ha (2 mg/L) caused an algal bloom on the first day after treatment and an increase in chlorophyll a concentration on the first and 8th day after treatment. Corresponding increases in nitrate concentration and phosphate concentrations were observed. Results indicated that indirect effects of water temperature and increased nutrient concentration due to lysis of milfoil plants might be more important in the field community dominated by Chlorophyta, Pyrrhophyta and Bacilariophyta.	Kobraei and White, 1996
2,4-D DMA	2 mg/L 2,4-D DMA increased algal production. 1 mg/L did not. Water chemistry changes were also noted.	Scott et al., 1978[1]
2,4-D DMA	At 2 mg/L a heavy algal bloom was noted. Not present in control pool.	Stallings and Huckins, 1978[1]
2,4-D DMA	20-40 lbs. active ingredient per acre. 6 of 12 genera not present 24 hours post treatment but present at 2 weeks. 4 of 12 genera more abundant at 24-hours. Differences in concentrations of chlorophyll a levels is observed at 24 hours but not different at 2 weeks. 14C-incorporation lower for 2 weeks. However, differences in chlorophyll a levels and 14C-incorporation may not be significantly different from controls. Plankton retained 2,4-D residues for 6 months post treatment.	Wojtalik et al, 1971[1]
2,4-D sodium salt	Increases in phytoplankton were observed on the day of treatment and 8 weeks after treatment. Concentrations of nutrient nitrates increased on the day of treatment and continued to increase for 8 days after treatment. Phosphate concentrations also doubled from the day before treatment until the 8 days after treatment.	Patnaik and Das, 1991
2,4-D Commercial Formulation	Plankton levels decreased during the course of a year's treatment with 2,4-D at levels varying from 0.5 to 10.5 Kg/ha/year (0.45 to 9.4 lbs/ha/year).	Sarkar, 1991

[1] Listing of original reference which were compiled for 2,4-D in field studies with 2,4-D in Ebasco (1993).

Table 21: Risk Assessment for Blue-green Algae, Green Algae, Diatoms and Macrophytes with 2,4-D Products

Species Name	Common Name	Test Chemicals – 2,4-D Products EC50 (mg a.i../L and mg a.e./L) & [Risk Quotient (RQ[1] = EEC[2]/EC50)] and {Risk Level (Petersen et al, 1994)			
		BBE	**2-EHE**	**DMA**	**Acid**
Anabaena flos-aquae	Blue-green algae	6.37 ai 4.38 ae [0.67] {High}	>30 ai[3] >20 ae [ND] {ND}[4]	153 ai 127 ae [0.02] {Low}	>2.02 ae [<1.4] {ND; Probably Low}[4]
Anabaena doliolum	Blue-green algae				~500 ae [0.006] {Low}
Nostoc linkia	Blue-green algae				~500 ae [0.006] {Low}
Nostoc calcicola	Blue-green algae				~500 ae [0.006] {Low}
Nostoc spp.	Blue-green algae				~500 ae [0.006] {Low}
Chladymonas reinhardtii	Green algae				>40 ae [<0.07] {Low}
Selenastrum capricornutum	Green algae	24.9 ai 17.1 ae [0.17] {Moderate}	>30 ai >20 ae [0.15] {Moderate}	67 ai 56 ae [0.05] {Low}	41 ae [0.07] {Low}
Chlorococcum spp.	Green algae	75 ai 54 ae [0.05] {Low}			50 ae [0.06] {Low}
Chlorella fusca	Green algae				89 ae [0.03] {Low}
Dunaliella tertiolecta	Green algae	75 ai 54 ae [0.05] {Low}		185 ai 153 ae [0.02] {Low}	75 ae [0.04] {Low}
Scenedesmus quadricauda	Green algae				98 ae [0.03] {Low}

Table 21: Risk Assessment for Blue-green Algae, Green Algae, Diatoms and Macrophytes with 2,4-D Products (Continued)

Species Name	Common Name	Test Chemicals – 2,4-D Products EC50 (mg a.i../L and mg a.e./L) & [Risk Quotient (RQ[1] = EEC[2]/EC50)] and {Risk Level (Petersen et al, 1994)			
		BBE	**2-EHE**	**DMA**	**Acid**
Navicula pelliculosa	Freshwater diatom	1.86 ai 1.28 ae [2.28] {Very High)	4.1 ai 2.7 ae [1.08] {Very High}	5.28 ai 4.38 ae [0.67] {High}	2.02 ae [1.4] {Very High}
Skeletonema capricornutum	Marine diatom	1.66 ai 1.14 ae [2.56] {Very High}	0.23 ai 0.15 ae [19] {Very High}	37 ai 31 ae [0.09] {Low}	2.08 ae [1.4] {Very High}
Phaeodactylum tricornutum	Marine diatom	150 ai 103 ae [0.03] {Low}		362 ai 301 ae [0.01] {Low}	50 ae [0.06] {Low}
Isochrysis galbana	Marine Haptophyte	75 ai 54 ae [0.05] {Low}	4.1 ai 2.7 ae [1.08] {Very High}		50 ae [0.06] {Low}
Sinapsis alba Root elongation	Macrophyte				1.17 ae [2.5] {Very High}
Sinapsis alba Germination	Macrophyte				166 ae [0.02] {Low}
Lemna gibba	duckweed	0.58 ai 0.40 ae [7.3] {Very High}	0.50 ai 0.33 ae [8.8] {Very High}	0.58 ai 0.48 ae [6.1] {Very High}	0.695 ae [4.2] {Very High}

[1] RQ = Risk Quotient (unitless)
[2] EEC concentration as reported by Petersen = 2.917 mg a.e./L.
[3] Solubility problem cannot measure analytical concentrations higher than 0.32 mg a.i./L (0.21 mg a.e./L).
[4] ND = Not determined.

Table 22: Acute Toxicity of 2,4-D Products to Fish

Test Formulation	Species	Test Type	Size Class/ Age	Time (Hours)	LC50 (mg a.i./L)	NOEC (mg a.i./L)	Reference
2,4-D, BEE	*Oncorhynchus mykiss* (Rainbow trout)	Flow-through	Juvenile	96	2.00	NS[1]	Alexander et al., 1983[J97]
2,4-D, BEE	*Oncorhynchus mykiss* (Rainbow trout)	Flow-through	Fry	96	0.52	NS	Finlayson & Verrue, 1985[J97]
2,4-D, BEE	*Oncorhynchus mykiss* (Rainbow trout)	Flow-through	Smolts	96	0.47	NS	Finlayson & Verrue, 1985[J97]
2,4-D, BEE	*Oncorhynchus mykiss* (Rainbow trout)	Renewal	Fry	96	0.3	NS	Martens et al., 1980[W89]
2,4-D, BEE	*Oncorhynchus mykiss* (Rainbow trout)	Static	Juvenile	96	0.65-2.2 (1.30)[2]	<0.5-1.7 (<0.85)	Wan et al, 1990;DOW, 1987[B99];ARC, 1974[B99]
2,4-D, BEE	*Oncorhynchus mykiss* (Rainbow trout)	Static	Smolts	96	1.21-3.67 (2.1)	NS	Finlayson & Verrue, 1985[J97]
2,4-D, BEE	*Oncorhynchus gorbuscha* (Pink salmon)	Static	Juvenile	96	0.4-1.1 (0.73)	NS	Wan et al., 1990
2,4-D, BEE	*Oncorhynchus gorbuscha* (Pink salmon)	Static	Fry	96	0.45	NS	Martens et al., 1980[W89]
2,4-D, BEE	*Oncorhynchus kisutch* (Coho salmon]	Static	Juvenile	96	1.1-4.3 (1.92)	NS	Wan et al., 1990
2,4-D, BEE	*Oncorhynchus kisutch* (Coho salmon]	Renewal	Fry	96	0.45	NS	Martens et al., 1980[W89]
2,4-D, BEE	*Oncorhynchus nerka* (Sockeye salmon)	Renewal	Fry	96	0.45	NS	Martens et al., 1980[W89]
2,4-D, BEE	*Oncorhynchus tshawytscha* (Chinook salmon)	Flow-through	Fry	96	0.32	NS	Finlayson & Verrue, 1985[J97]
2,4-D, BEE	*Oncorhynchus tshawytscha* (Chinook salmon)	Flow-through	Smolts	96	0.38	NS	Finlayson & Verrue, 1985[J97]
2,4-D, BEE	*Lepomis macrochirus* (Bluegill sunfish)	Flow-through	20mm	96	0.61	NS	Alexander et al., 1983d[J97]

Table 22: Acute Toxicity of 2,4-D Products to Fish (Continued)

Test Formulation	Species	Test Type	Size Class/ Age	Time (Hours)	LC50 (mg a.i./L)	NOEC (mg a.i./L)	Reference
2,4-D, BEE	*Lepomis macrochirus* (Bluegill sunfish)	Static	0.6-1.2g	96	0.76-1.2 (0.68)	NS	Johnson & Finley, 1980, FWS, 1980[B99]
2,4-D, BEE	*Pimephales promelas* (Fathead minnow)	Static	Finger-lings	96	3.25-5.6 (4.3)	NS	Johnson & Finley, 1980; Mount and Stephan, 1967[W89]
2,4-D, BEE	*Pimephales promelas* (Fathead minnow)	Static	16mm	96	2.50	1.7	Alexander et al., 1983d[B99,J97]
2,4-D BEE	*Ictalurus punctatus* (Channel catfish)	Static	0.4g	96	0.78-1.35 (1.02)	NS	FWS, 1986[B99]
2,4-D, BEE	*Alburnus alburnus* (Bleak)	Static	NS	96	3.2-3.7 (3.4)	NS	Linden et al., 1979[J97]
2,4-D, 2-EHE	*Oncorhynchus mykiss* (Rainbow trout)	Flow-through	0.21g	96	7.20	<2.1	Mayes et al, 1990c[J97]
2,4-D, 2-EHE	*Oncorhynchus mykiss* (Rainbow trout)	Static	Juvenile	96	22-167 (76)	12-32 (19)	Wan et al., 1991; EPA, 1974[B99], UNI, 1976[B99]
2,4-D, 2-EHE	*Oncorhynchus gorbuscha* (Pink salmon)	Static	Fry	96	21-70 (31)	NS	Wan et al., 1991
2,4-D, 2-EHE	*Oncorhynchus kisutch* (Coho salmon]	Static	Fingerling	96	63-158 (116)	NS	Wan et al., 1991
2,4-D, 2-EHE	*Oncorhynchus clarkii* (Cutthroat trout)	Static	Juvenile	96	>50	NS	Woodward, 1982[J97]
2,4-D, 2-EHE	*Lepomis macrochirus* (Bluegill sunfish)	Static	Juvenile	96	18-20	10	EPA, 1977[B99]; UNI, 1976[B99]; Alexander, 1983a[J97]
2,4-D, 2-EHE	*Pimephales promelas* (Fathead minnow)	Static	NS	96	>5	10	Alexander, 1983e[J97]
2,4-D, 2-EHE	*Menidia beryllina* (Inland silvered)	Flow-through	Juvenile	96	0.24->3.0 (>0.85)	0.24-1.1 (0.51)	Ward & Boeri, 1991c Ward & Boeri, 1991d

Table 22: Acute Toxicity of 2,4-D Products to Fish (Continued)

Test Formulation	Species	Test Type	Size Class/ Age	Time (Hours)	LC50 (mg a.i./L)	NOEC (mg a.i./L)	Reference
2,4-D DMA Salt	*Oncorhynchus mykiss* (Rainbow trout)	Static	Mixed Fry, Juvenile & NS	96	100-377 (218)	120-320 (159)	Bentley, 1974[J97]; Alexander et al., 1983b[J97]; Bogers & Enninger, 1990a[J97]; Johnson & Finley, 1980; USACOE, 1978[W80]
2,4-D DMA Salt	*Oncorhynchus tshawytscha* (Chinook salmon)	Static	1.0g	96	>100	NS	Johnson & Finley, 1980
2,4-D DMA Salt	*Lepomis macrochirus* (Bluegill sunfish)	Static/NS	Juvenile	96	106-524 (145)	<87-124 (<103)	DOW, 1983[B89]; BIO, 1991[b99]
2,4-D DMA Salt	*Lepomis macrochirus* (Bluegill sunfish)	Static	Sub-adults	96	177	NS	Schultz, 1973[E93]
2,4-D DMA Salt	*Micropterus dolomieu* (Smallmouth bass)	Static	0.4g	96	236	NS	Johnson & Finley, 1980
2,4-D DMA Salt	*Pimephales promelas* (Fathead minnow)	Static/NS	Fry/Juvenile/NR	96	266-344 (314)	NS	Alexander et al., 1983a[J97]; Schultz, 1973[E93] Johnson & Finley, 1980; DOW,1983[B99]; FWS, 1986[B99]
2,4-D DMA Salt	*Cyprinus carpio* (Common carp)	Static	NS	96	>560-<1000	NS	Bogers & Enninger, 1990b[J97]
2,4-D DMA Salt	*Cirrhina mrigala hamilton* (Hamilton's carp)	NS	Fingerlings	96	>100	NS	Sing & Yadv, 1978[J97]
2,4-D DMA Salt	*Ictalurus punctatus* (Channel catfish)	Static	Sub-adults	96	193	NS	Schultz, 1973[W89,E93]
2,4-D DMA Salt	*Ictalurus punctatus* (Channel catfish)	Static	Fry, Fingerling/ NS	96	119-155 (132)	NS	Johnson & Finley, 1980; U.S.A.C.O.E, 1978[W80]; FWS, 1986[B99]
2,4-D DMA Salt	*Menidia beryllina* (Inland silverside)	Flow-through	0.17g	96	469	<224	Ward, 1991b[J97]

Table 22: Acute Toxicity of 2,4-D Products to Fish (Continued)

Test Formulation	Species	Test Type	Size Class/ Age	Time (Hours)	LC50 (mg a.i./L)	NOEC (mg a.i./L)	Reference
2,4-D Sodium Salt	*Oncorhynchus mykiss* (Rainbow trout)	Static	0.28g	96	>100	NS	EPA, 1973[B99]
2,4-D, Sodium Salt	*Mugil cephalus* (Mullet)	Static	NS	96	32	NS	Tag El Din et al., 1981[J97]
2,4-D, Sodium Salt	*Alburnus alburnus* (Bleak)	Static	Embryo	48	13	NS	Biro, 1979[W89]
2,4-D, Sodium Salt	*Oryzias latipes* (Medaka)	NS	NS	48	>40	NS	Hashimoto & Nishiuchi, 1978[J97]
2,4-D Potassium Salt	*Micropterus salmoides* (Largemouth bass)	Flow-through	Eggs	84	165	NS	Birge, et al., 1979[J97]
2,4-D Potassium Salt	*Micropterus salmoides* (Largemouth bass)	Flow-through	Sac-fry	84	161	NS	Birge, et al., 1979[J97]
2,4-D Potassium Salt	*Carassius auratus* (Goldfish)	Flow-through	Eggs	96	>187	NS	Birge, et al., 1979[J97]
2,4-D Potassium Salt	*Carassius auratus* (Goldfish)	Flow-through	Sac-fry	96	>201	NS	Birge, et al., 1979[J97]
2,4-D Acid	*Oncorhynchus mykiss* (Rainbow trout)	Static	NS	96	358.0	NS	Bentley, 1974[J97]
2,4-D Acid	*Oncorhynchus mykiss* (Rainbow trout)	Static	Fingerlings	96	<100->1000 (~316)	NS	Doe et al., 1988[J97]
2,4-D Acid	*Oncorhynchus mykiss* (Rainbow trout)	Static	0.3-0.34g	96	110-358 (163)	320	DOW, 1983[B99]; FWS, 1986[B99]

Table 22: Acute Toxicity of 2,4-D Products to Fish (Continued)

Test Formulation	Species	Test Type	Size Class/ Age	Time (Hours)	LC50 (mg a.i./L)	NOEC (mg a.i./L)	Reference
2,4-D Acid	*Salvelinus namaycush.* (Lake trout)	Static	0.3g	96	45	NS	Johnson & Finley, 1980
2,4-D Acid	*Oncorhynchus clarkii* (Cutthroat trout)	Static	Fingerlings	96	25-64 (40)	NS	Johnson & Finley, 1980; FWS, 1986[B99]
2,4-D Acid	*Lepomis macrochirus* (Bluegill sunfish)	Static	0.5g/NS	96	180-263 (217)	<204	Alexander, 1983b[w97]; FWS, 1986[B99]
2,4-D Free Acid	*Lepomis gibbosus* (Pumpkin-seed sunfish)	Static	NS	96	95	NS	Rewoldt et al., 1977[W89, J97]
2,4-D Free Acid	*Roccus Americanus* (White Perch)	Static	NS	96	40	NS	Rewoldt et al., 1977[W89, J97]
2,4-D Free Acid	*Morone saxatilis* (Striped bass)	Static	NS	96	70	NS	Rewoldt et al., 1977[W89, J97]
2,4-D Acid	*Pimephales promelas* (Fathead minnow)	Static	0.14-0.9G	96	133-320 (190)	256	Alexander, 1983b[J97] ; FWS, 1986[B99]
2,4-D Acid	*Cyprinus carpio* (Common carp)	Renewal	NS	96	20	NS	Vardia & Durve, 1981[W89]
2,4-D Acid	*Cyprinus carpio* (Common carp)	Static	NS	96	97-270 (152)	NS	Neskovic et al. 1997[J97] Neskovic et al, 1994; Sarkar, 1990; Rewoldt et al., 1977[W89, J97]
2,4-D Free Acid	*Fundulus diaphanus* (Banded killifish)	Static	NS	96	27	NS	Rewoldt et al., 1977[W89, J97]
2,4-D Free Acid	*Anguilla rostrata* (American eel)	Static	NS	96	301	NS	Rewoldt et al., 1977[W89, J97]
2,4-D Acid	*Rasbora nielgeriensis* (Rasbora)	Static	NS	96	5.6	NS	Vardia & Durve, 1981[W89]
2,4-D-Acid	*Labeo boga*	Static	NS	96	3.8	NS	Vardia & Durve, 1981[W89]
2,40D Acid	*Menidia beryllina* (Inland silverside)	Static	NS	96	175	<111	ESE, 1991 Brian Database, 1999

Table 22: Acute Toxicity of 2,4-D Products to Fish (Continued)

Test Formulation	Species	Test Type	Size Class/ Age	Time (Hours)	LC50 (mg a.i./L)	NOEC (mg a.i./L)	Reference
2,4-D Free Acid	*Lepistes reticulata* (Guppy)	Static	NS	96	71	NS	Rewoldt et al., 1977[W89, J97]
2.4-D Acid	*Oryzias latipes* (Medaka)	Static	Sac-fry	96	2780	NS	Holcomb et al., 1995
2.4-D Acid	*Trichogaster pectoralis* (Sepat Siam)	Static	4000-6000 mm	96	153	NS	Ooi & Lo, 1988
2.4-D Acid	*Clarias batrachus* (Keli)	Static	4000-6000 mm	96	60	NS	Ooi & Lo, 1988

[1] NS = Parameter not specified.
[2] Values in parenthesis are geometric means of all acceptable values
[J97] JMPR, 1997
[B99] Brian Database, 1999
[W89] Ecology, 1989
[W80] Ecology, 1980
[E93] Ebara, 1993 Many of the items that can be found in Ebasco can also be found in other reference. Usually only one compendium reference is cited.

Table 23: Acute Toxicity of 2,4-D Products to Invertebrates

Test Formulation	Species	Test Type	Water Type	Size Class/ Age	Time (hrs)	EC50 (mg a.i./L)	NOEC (mg a.i./L)	Reference
2,4-D, BEE	*Daphnia magna* (Daphnid)	Flow	FW[1]	<24 hrs	48	7.2	<3.4	Alexander et al., 1983e[B99]
2,4-D,BEE	*Daphnia magna* (Daphnid)	Static/NS[2]	FW	1st Instar/NS[2]	NS[2]	1.7-5.6 (4.0)[3]	NS[2]	Sanders, 1970[E93] ; FWS, 1986[B99]; Johnson & Finley, 1980; FWS, 1986[B99]
2,4-D, BEE	*Crassostrea virginica* (Eastern oyster)	Flow	SW[4]	Larvae	96	2.6	NS	EPA, 1995[B99]
2,4-D,BEE	*Gammarus fasciatus* (Lined scud)	NS	FW	Juvenile	96	0.44	NS	FWS, 1986[B99] Sanders, 1970[E93]
2,4-D,BEE	*Gammarus fasciatus* (Lined scud)	NS	FW	Mature	96	3.11-11 (5.8)	NS	Johnson & Finley, 1980; Sanders, 1970[E93]
2,4-D,BEE	*Gammarus lacustris* (Bright scud)	NS	FW	NS	48	0.76	NS	FWS, 1969[B99]
2,4-D,BEE	*Gammarus lacustris* (Bright scud)	NS	FW	NS	96	0.44	NS	Sanders, 1970[e93]
2,4-D, BEE	*Nitocra spinepes* (Copepod)	NS	SW	NS	96	3.1	NS	Linden et al., 1979[997,E93]
2,4-D, BEE	*Panaeus aztecus* ((Brown shrimp))	Static	SW	Juvenile	48	5.6	NS	Mayer, 1987[J97]
2,4-D, Iso-BEE	*Chasmagnathus granulata* (Estuarine crab)	Static	SW	1st Zoel	96	0.30	NS	Rodriguez & Amin, 1991
2,4-D, Iso-BEE	*Chasmagnathus granulata* (Estuarine crab)	Static	SW	Juvenile/ Adult	96	2890-3370 (3120)	NS	Rodriguez & Amin, 1991
2,4-D, Iso-BEE	*Uca uruguayensis* (Estuarine crab)	Static	SW	Adult	96	130	NS	Rodriguez & Lombardo, 1991
2,4-D,BEE	*Pteronarcys californica* (Stonefly)	NS	FW	Nymph	96	1.6	NS	Sanders & Cope, 1968[E93]

Table 23: Acute Toxicity of 2,4-D Products to Invertebrates (Continued)

Test Formulation	1.1 Species	Test Type	Water Type	Size Class/ Age	Time (hrs)	EC50 (mg a.i./L)	NOEC (mg a.i./L)	Reference
2,4-D,BEE	*Pteronarcys californica* (Stonefly)	NS	FW	Adult	96	>1000	NS	Pimentel, 1971[E93]
2,4-D BEE	*Aselus brevicaudus* (Aquatic sowbug)	Static	FW	Juvenile /NS	96	1.8-3.6 (2.46)	NS	Johnson & Finley, 1980; Sanders, 1970[E93]
2,4-D BEE	*Cypridopsis vidua* (Seed shrimp)	Static	SW	Juvenile/NS	48	1.8-2.2 (1.99)	NS	Sanders, 1970[E93]; Johnson & Finley, 1980[E93]
2,4-D BEE	*Chironomus plumosus* (Chironomid)	NS	FW	NS	NS	0.39-0.79 (0.56)	NS	EPA, 1988[e93]
2,4-D BEE	*Palaemonetes kadiakensis* (Glass shrimp)	NS	FW	NS	NS	<1.0-1.4 (1.18)	NS	Sanders, 1970[e93]; FWS, 1986[b99]
2,4-D BEE	*Orconectes nous* (Crayfish)	NS	FW	NS	NS	100	NS	Sanders, 1970[E93]
2,4-D, 2-EHE	*Daphnia magna* (Daphnid)	Static	FW	<24 hrs	48	>5.0-5.2[5] (>5.1)	0.6	DOW, 1983[B99]; Alexander et al. 1983a[J97]
2,4-D, 2-EHE	*Daphnia magna* (Daphnid)	Static	FW	<24 hrs	48	5.2	NS	Alexander et al. 1983a[J97]
2,4-D, 2-EHE	*Gammarus fasciatus* (Lined scud)	Static	FW	Juvenile	96	2.4	NS	Johnson & Finley, 1980
2,4-D, 2-EHE	*Crassostrea virginica* (Eastern oyster)	Flow	SW	Juvenile	96	0.21-1.00 (>0.53)	0,21-0.71 (0.39)	Mayer, 1987[J97]; Ward & Boeri, 1991c[J97]
2,4-D, 2-EHE	*Palaemonetes pugio* (Grass shrimp)	Flow	SW	Juvenile	96	>0.003->1.4 (>0.083)	0.003->1.4 (>0.083)	EVS, 1991[B99]; Ward & Boeri, 1991g[J97]; Ward & Boeri, 1991h[J97]
2,4-D, 2-EHE	*Panaeus aztecus* (Brown shrimp)	Flow	SW	Adult	48	0.48	NS	Mayer, 1987[J97]
2,4-D, DMA Salt	*Daphnia magna* (Daphnid)	Static	FW	1st Instar/NS	NS	>100-184[6] (>135)	NS	Alexander et al., 1983c[J97]; Mayer & Ellersieck, 1986[J97]

Table 23: Acute Toxicity of 2,4-D Products to Invertebrates (Continued)

Test Formulation	1.2 Species	Test Type	Water Type	Size Class/ Age	Time (hrs)	EC50 (mg a.i./L)	NOEC (mg a.i./L)	Reference
2,4-D, DMA Salt	*Gammarus fasciatus* (Lined scud)	Static	FW	Mature/NS	96	>100	NS	Johnson & Finley, 1980 Mayer & Ellersieck, 1986[J97]; EPA, 1988[E93]
2,4-D, DMA Salt	*Chaoborus punctipennis* (Glass worm)	NS	FW	Larvae	24	890	NS	Bunting & Robertson, 1975[J97]
2,4-D, DMA Salt	*Crassostrea virginica* (Eastern oyster)	Static	SW	Larvae	48	136-<320 (~209)	NS	Heitmuller, 1975[J97]; Ward, 1991c[J97]
2,4-D, DMA Salt	*Uca pugilator* (Fiddler crab)	Static	SW	NS	96	NS	>1000	Heitmuller, 1975[J97]
2,4-D, DMA Salt	*Panaeus duorarum* (Pink shrimp)	Flow	SW	NS	96	181	65	Ward, 1991d[J97]
2,4-D, DMA Salt	*Cypridopsis vidua* (Seed shrimp)	Static	SW	Mature	NS	8.00	NS	Johnson & Finley, 1980
2,4-D, DMA Salt	*Chironomus plumosus* (Chironomid)	Static	FW	3rd-Instar	48	>100	NS	FWS, 1986[B99]
2,4, DMA Salt	*Palaemonetes pugio* (Grass shrimp)	Flow	SW	Juvenile	96	>0.14	0.14	EVS, 1991[B99]
2,4-D, DMA Salt	*Palaemonetes kadiakensis* (Glass shrimp)	Static	SW	Mature	NS	0.15[7]	NS	Johnson & Finley, 1980
2,4-D DMA Salt	*Aselus brevicaudus* (Aquatic sowbug)	Static	FW	NS	48hr	>100	NS	REF, 1970[B99]
2,4-D, Sodium Salt	*Daphnia magna* (Daphnid)	Static	FW	<24hr	48	932	NS	Presing, 1981[J97]
2,4-D, Sodium Salt	*Macrobranchium lamerrei* (Freshwater prawn)	Static	FW	NS	96	2224	NS	Omar & Shukla, 1984[J97]
2,4-D, Sodium Salt	*Macrobranchium naso* (Freshwater prawn)	Static	FW	NS	96	2397	NS	Omar & Shukla, 1984[J97]

Table 23: Acute Toxicity of 2,4-D Products to Invertebrates (Continued)

Test Formulation	1.3 Species	Test Type	Water Type	Size Class/ Age	Time (hrs)	EC50 (mg a.i./L)	NOEC (mg a.i./L)	Reference
2,4-D, Sodium Salt	*Macrobranchium dayanum* (Freshwater prawn)	Static	FW	NS	96	2275	NS	Omar & Shukla, 1984[J97]
2,4-D, Free Acid	*Tubifex tubifex* (Tubifex worm)	Flow	FW	NS	96	122	87	Bailey & Liu, 1981[J97]
2,4-D, Acid		Static	FW	20mm	96	161	NS	Fargasova, 1994
2,4-D, Acid	*Daphnia magna* (Daphnid)	Static/NS	FW	<24 hrs	48	25-418 (209)	<12	Alexander et al., 1983b[J97]; DOW, 1983[B99]; McCarty & Batchelder, 1977[J97]; Fargasova, 1994; Presing, 1981[J97]; Bunting & Robertson, 1975[W89,E93];FWS, 1970[B99]
2,4-D Acid	*Ceriodaphnia dubia* (Daphnid)	Flow	FW	<24 hrs	24	236	NS	Orius et al., 1991[J97]
2,4-D Acid	*Cyclops vernalis* (Cyclops)	NS	FW	NS	NS	37	NS	Bunting & Robertson, 1985[E93]
2,4-D, Acid	*Gammarus fasciatus* (Lined scud)	NS	FW	NS	NS	3.2	NS	Sanders, 1970[E93]
2,4-D, Free Acid	*Mytilus edulis* (Bay mussel)	NS	SW	NS	96 Mortality	259	NS	Liu & Lee, 1975[J97]
2,4-D, Free Acid	*Mytilus edulis* (Bay mussel)	NS	SW	NS	96 Attachment	262	NS	Liu & Lee, 1975[J97]
2,4-D, Free Acid	*Mytilus edulis* (Bay mussel)	NS	SW	Tocophore	48 Norm. Develop.	212	NS	Liu & Lee, 1975[J97]
2,4-D, Acid	*Crassostrea virginica* (Eastern oyster)	Flow	SW	Juvenile	96	57-467 (123)	30-<135 (<63)	Wade & Overman, 1991[B99]; Ward et al., 1993[J97,B99]; ESE, 1991[B99]
2,4-D, Acid	*Crassostrea virginica* (Eastern oyster)	Flow	SW	Larvae	96 Sell Deposition	57	30	Ward et al., 1993[J97,B99]

Table 23: Acute Toxicity of 2,4-D Products to Invertebrates (Continued)

Test Formulation	1.4 Species	Test Type	Water Type	Size Class/ Age	Time (hrs)	EC50 (mg a.i./L)	NOEC (mg a.i./L)	Reference
2,4-D Acid	*Panaeus duorarum* (Pink shrimp)	Flow	SW	NS	96	554	NS	Vashinav et al., 1990b[J97]
2,4-D Acid	*Cancer magister* (Dungeness crab)	Static	SW	1st Zoel	96	>10	NS	Caldwell, 1977[J97]
2,4-D Acid	*Cancer magister* (Dungeness crab)	Static	SW	Adult	96	>100	NS	Caldwell, 1977[J97]

[1] FW = Freshwater
[2] NS = Parameter not specified.
[3] Number in parenthesis is the geometric mean of all accepted values.
[4] SW = Saltwater
[5] LC50s that appear to be outliers are 0.019, 0.054 and 0.5 mg a.i./L by EPA, 1975[B99]; ARC, 1976[B99]; UNI, [1977b99]. Tests were conducted prior to institution of EPA Pesticide Assessment Guidelines and have been discounted.
[6] LC50s conducted by EPA, 1988 ranged from 4.0 to 100 mg.a.i./L. This data was discounted due to its wide range.
[7] LC50 that appears to be an outliers (>100 mg a.i./L) by REF, 1970[B99]. Test was conducted prior to institution of EPA Pesticide Assessment Guidelines and have been discounted.

[J97] JMPR, 1997
[B99] Brian Database, 1999
[W89] Ecology, 1989
[W80] Ecology, 1980
[E93] Ebasco, 1993 Many of the items that can be found in Ebasco can also be found in other reference. Usually only one compendium reference is cited

Table 24: Chronic Toxicity of 2,4-D Products to Fish

Test Formulation	Species	Test Type	Water Type	Size Class/ Age	Time (Days)	NOEC (mg a.i./L)	MATC (mg a.i./L)	LOEC (mg a.i./L)	Reference
2.4-D, BEE	*Oncorhynchus tshawytscha* (Chinook salmon)	Flow-through	FW[3]	Egg-Juvenile	86	0.040	NS[1]	NS[1]	Finlayson & Verrue, 1985[W89]
2,4-D, BEE	*Pimephales promelas* (Fathead minnow)	Flow-through	FW	Egg-Juvenile	32	0.0805	0.0962	0.115	DOW, 1989[B99]
2,4-D, BEE	*Pimephales promelas* (Fathead minnow)	Flow-through	FW	Life-cycle	10 months	0.3	NS	NS	Mount & Stephan, 1967[W89]
2,4-D, 2-EHE	*Pimephales promelas* (Fathead minnow)	Flow-through	FW	Egg-Juvenile	32	0.12	0.16	0.22	DOW, 1990 [B99]
2,4-D, 2-EHE	*Lepomis macrochirus* (Bluegill sunfish)	Flow-through	FW	Sac-Fry	12	>50	NS	NS	Hiltibran, 1967
2,4-D, 2-EHE	*Lerimyzon sucetta* (Lake chubsucker)	Flow-through	FW	Sac-Fry	12	>25	NS	NS	Hiltibran, 1967
2,4-D, 2-EHE	*Campostoma anomalum* (Stoneroller)	Static	FW	Egg-Sac-Fry	8	>25	NS	NS	Hiltibran,1967
2,4-D, DMA Salt	*Pimephales promelas* (Fathead minnow)	Flow-through	FW	Egg-Juvenile	31	17.1	22	28.4	Dill et al., 1990[J97]
2,4-D, DMA Salt	*Lepomis macrochirus* (Bluegill sunfish)	Static	FW	Sac-Fry	12	>40	NS	NS	Hiltibran,1967
2,4-D, DMA Salt	*Lepomis macrochirus* (Bluegill sunfish)	Static	FW	Sac-Fry	12	>25	NS	NS	Hiltibran,1967
2,4-D, DMA Salt	*Lepomis cyanacellus* (Green sunfish)	Static	FW	Sac-Fry	12	>25	NS	NS	Hiltibran,1967
2,4-D, DMA Salt	*Lerimyzon sucetta* (Lake chubsucker)	Static	FW	Sac-Fry	12	>25	NS	NS	Hiltibran,1967
2,4-D, DMA Salt	*Micropterus dolomieu* (Smallmouth bass)	Static	FW	Sac-Fry	12	>25	NS	NS	Hiltibran,1967

Table 24: Chronic Toxicity of 2,4-D Products to Fish (Continued)

Test Formulation	Species	Test Type	Water Type	Size Class/ Age	Time (Days)	NOEC (mg a.i./L)	MATC (mg a.i./L)	LOEC (mg a.i./L)	Reference
2,4-D Sodium Salt	*Lepomis macrochirus* (Bluegill sunfish)	Static	FW	Sac-Fry	12	>100	NS	NS	Hiltibran,1967
2,4-D, Potassium Salt	*Oncorhynchus mykiss* (Rainbow trout)	Flow-through	FW	Eggs	23-27	NS	LC50= 4.2-11 (6.8)[2] LC1= 0.022-0.032 (0.027)	NS	Birge et al, 1979[J97]
2,4-D, Potassium Salt	*Carrusius auratus* (Goldfish)	Flow-through	Inter To Hard[4]	Eggs-Sac-Fry	8	NS	LC1= 8.9-18.2 (12.7) LC50 = 119-133 (126)	NS	Birge et al, 1979[J97]
2,4-D, Potassium Salt	*Micropterus salmoides* (Largemouth bass)	Flow-through	Inter to Hard[4]	Eggs-Sac-fry	7.5	NS	LC1= 3.2-13.1 (6.47) LC50 = 82-109 (95)	NS	Birge et al, 1979[J97]
2,4-D Acid	*Pimephales promelas* (Fathead minnow)	Flow-through	FW	Eggs-Juvenile	32	63.4	80.4	102	Mayes et al., 1990b
2,4-D Acid	*Oryzias latipes* (Medaka)	Flow-through	FW	Sac-fry	28	27.2-30 (29)	39.2-42.5 (41)	56-60.2 (58)[2]	Holcomb et al., 1995

[1] NS = Not specified for this parameter.
[2] Value given in parenthesis is the mean of all acceptable values.
[3] FW = Freshwater
[4] Intermediate to hard water
[J97] JMPR, 1997
[B99] Brian Database, 1999
[W89] Ecology, 1989
[W80] Ecology, 1980
[E93] Ebasco, 1993. Many of the items that can be found in Ebasco can also be found in other reference. Usually only one compendium reference is cited

Table 25: Chronic Toxicity of 2,4-D Products to Invertebrates (Daphnid)

Test Substance	Species	Type	Water	Age	Time (days)	EC50 (mg (a.i./L)	NOEC (mg a.i./L)	MATC (mg a.i./L)	LOEC (mg a.i./L)	Reference
2,4-D, BEE	*Daphnia magna* (Daphnid)	Flow-through	FW[1]	Life-cycle	21	NS[2]	0.29	0.45	0.7	DO, 1989[B99]
2,4-D, Iso-BEE	*Chasmagnathus granulata* (Estuarine crab)	Static	SW[3]	Chronic Adult	28	>50	NS	NS	NS	Rodriguez et al, 1992
2,4-D, Iso-BEE	*Uca uruguayensis* (Estuarine crab)	Static	SW	Chronic Adult	28	>30	NS	NS	NS	Rodriguez et al, 1992
2,4-D, 2-EHE	*Daphnia magna* (Daphnid)	Flow-through	FW	Life-cycle	21	0.13	0.015	0.020	0.027	Ward & Boeri, 1991b
2,4-D, DMA	*Daphnia magna* (Daphnid)	Flow-through	FW	Life-cycle	21	3-day LC50= 130-243	27.5	40.5	59.6	Ward, 1991a[J97]
2,4-D Acid	*Daphnia magna* (Daphnid)	Flow-through	FW	Life-Cycle	21	235	79	109	151	Ward & Boeri, 1991a
2,4-D Acid	*Ceriodaphnia dubia* (Daphnid)	Renewal	FW	Life-Cycle	4	81.8	~35	49	~70	Oris, et al. 1991
2,4-D Acid	*Ceriodaphnia dubia* (Daphnid)	Renewal	FW	Life-Cycle	7	72.5	20-35 (26)	48.8	35-70 (49.5)	Oris, et al. 1991

[1] FW = Freshwater
[2] NS = Not specified
[3] SW = Saltwater
[4] J97 = in JMPR , 1997

Table 26: Effects of 2,4-D on Survival of Four Species of Fish Reared in Indian Fishponds

	Fish Species				
2,4-D Treatment (Kg/ha/year)	**Water Column Feeders (Free-Swimming Species)**		**Bottom Feeders (Benthic Species)**		**Totals**
	Labeo rohita (Rohu)	*Gibelion catla* (Catla)	*Cirrhinus mrigala* (Mrigal)	*Cyprinus carpio* (Common Carp)	
% Survival (% Change from Control)					
Control	71.0 (0)	62.5 (0)	61.0 (0)	55.0 (0)	
0.5	71.0 (0)	64.7 (3.5)	60.5 (-0.1)	58.5 (3.5)	
2.5	70.8 (-0.3)	64.0 (4.0)	62.7 (2.8)	60.1 (9.3)	
4.5	81.0 (14.1)	73.4 (17.4)	69.8 (14.4)	71.5 (30)	
6.5	61.5 (-13.4)	56.0 (-6.5)	74.1 (21.5)	73.8 (34.2)	
8.5	50 (-29.6)	54.4 (-13.0)	75.8 (14.8)	75.1 (20.1)	
10.5	46.8 (-34.1)	50.5 (-19.2)	78.7 (29.0)	78.9 (23.9)	
Kg/ha Yield (% Change from Control)					
Control	321.8 (0)	215.78 (0)	495.11 (0)	285.67 (0)	1318.36 (0)
0.5	338.71 (5.3)	200.71 (-7.0)	497.65 (0.5)	387.49 (35.6)	1454.56 (10.3)
2.5	334.53 (4.0)	197.4 (-8.5)	520.75 (5.2)	403.87 (41.4)	1456.55 (10.5)
4.5	362.31 (12.6)	206.62 (-4.2)	642.90 (29.8)	588.71 (106.1)	1840.54 (39.6)
6.5	262.61 (-18.40)	185.39 (-14.1)	722.81 (46.0)	640.07 (124.1)	1810.88 (37.4)
8.5	210.00 (-34.7)	170.55 (-21.0)	751.33 (51.8)	676.84 (236.9)	1808.72 (37.2)
10.5	197.95 (-38.5)	159.23 (-56.55)	793.30 (60.2)	690.57 (141.7)	1841.05 (39.6)

Table 27: Effects of Water Characteristics on 2,4-D Product Toxicity to Salmonids

		96-Hour LC50 (mg/L)					
Fish Species	**Dilution Water**	**2,4-D BEE[1]**	**2,4-DP BEE[2]**	**Weedone®[3]**	**90% Weedone® + 10% Diesel Oil**	**Diesel Oil**	**Carrier A[4]**
Oncorhynchus kisutch (Coho salmon]	Soft Alkalinity 1.6 mg/L Hardness = 4.2 mg/L pH = 6.1	1.1	1.5	10	2.2	10,299	19.8
Oncorhynchus kisutch (Coho salmon]	Intermediate Alkalinity 30.2 mg/L Hardness = 43.4 mg/L pH = 7.5	1.5	2.2	8.3	6.6	33,216	20.7
Oncorhynchus kisutch (Coho salmon]	Hard Alkalinity = 63.9 mg/L Hardness = 86 mg/L pH = 8.1	4.3	1.8	9.0	5.6	3,333	18.5
Oncorhynchus gorbuscha (Pink salmon)	Soft Alkalinity 1.6 mg/L Hardness = 4.2 mg/L pH = 6.1	0.4	0.8	1.7	1.2	74	19.8
Oncorhynchus gorbuscha (Pink salmon)	Intermediate Alkalinity 30.2 mg/L Hardness = 43.4 mg/L pH = 7.5	0.9	0.8	1.9	2.4	123	19.7
Oncorhynchus gorbuscha (Pink salmon)	Hard Alkalinity = 63.9 mg/L Hardness = 86 mg/L pH = 8.1	1.1	0.6	2.1	2.2	32	17.7

Table 27: Effects of Water Characteristics on 2,4-D Product Toxicity to Salmonids (Continued)

		96-Hour LC50 (mg/L)					
Fish Species	**Dilution Water**	**2,4-D BEE[1]**	**2,4-DP BEE[2]**	**Weedone®[3]**	**90% Weedone® + 10% Diesel Oil**	**Diesel Oil**	**Carrier A[4]**
Oncorhynchus mykiss (Rainbow trout)	Soft Alkalinity 1.6 mg/L Hardness = 4.2 mg/L pH = 6.1	0.8	0.9	5.0	2.0	3,017	14.8
Oncorhynchus mykiss (Rainbow trout)	Intermediate Alkalinity 30.2 mg/L Hardness = 43.4 mg/L pH = 7.5	1.3	1.4	3.7	5.8	2,186	20.1
Oncorhynchus mykiss (Rainbow trout)	Hard Alkalinity = 63.9 mg/L Hardness = 86 mg/L pH = 8.1	2.2	1.8	6.0	5.5	2,447	22.2

Source: Wan et al, 1990

1 2,4-D BEE = 95% 2,4-dichlorophenyl acetic acid, butoxyethyl ester

2 2,4-DP BEE = 95% 2,4-dichlorophenyl propionic acid, butoxyethyl ester

3 Weedone® = 11.9% 2,4-D BEE; 11.7% 2,4-DP BEE; 76.4% Carrier A

4 Carrier A = Unspecified "Inert Material

Table 28: Acute and Chronic Risk Assessment for 2,4-D DMA, 2,4-D BEE and 2,4-D Acid

2,4-D Formulation	Test Type	Exposure Medium	Species	EEC[1] (mg a.i./L)	Acute LC50[2] (mg a.i./L)	Estimated or Measured Chronic NOEC[3] (mg a.i./L)	Risk Quotient (RQ)[4] (unitless)	Level of Concern (LOQ)[5] (unitless)	RQ exceeds LOQ
2,4-D DMA	Acute	Water	*Oncorhynchus mykiss* (Rainbow trout)	1.36[7]	100	NA	0.014	0.1[A,C]	No
2,4-D DMA	Acute	Water	*Oncorhynchus mykiss* (Rainbow trout)	4.8[8]	100	NA	0.048	0.1[A,C]	No
2,4-D DMA	Acute	Water	*Daphnia magna* (Daphnid)	1.36[7]	>137	NA	0.0099	0.1[A,C]	No
2,4-D DMA	Acute	Water	*Daphnia magna* (Daphnid)	4.8[8]	>137	NA	0.035	0.1[A,C]	No
2,4-D DMA	Acute	Water	*Palaemonetes kadiakensis* (glass shrimp)	1.36[7]	0.15	NA	9.1	0.1[A,C]	Yes
2,4-D DMA	Acute	Water	*Palaemonetes kadiakensis* (glass shrimp)	4.8[8]	0.15	NA	32	0.1[A,C]	Yes
2,4-D DMA	Acute	Sediment	*Palaemonetes kadiakensis* (glass shrimp)	0.1- 0.45 (0.21)[6]	0.15	NA	0.67 - 3.0 (1.4)[6]	0.1[A,C]	Yes
2,4-D DMA	Acute	Water	*Cypridopsis vidua* (Seed shrimp)	1.36[7]	8.0	NA	0.17	0.1[A,C]	Yes
2,4-D DMA	Acute	Water	*Cypridopsis vidua* (Seed shrimp)	4.8[8]	8.0	NA	0.6	0.1[A,C]	Yes
2,4-D DMA	Acute	Sediment	*Cypridopsis vidua* (Seed shrimp)	0.1- 0.45 (0.21)[6]	8.0	NA	0.012 - 0.056 (0.026)[6]	0.1[A,C]	No
2,4-D DMA	Acute	Water	*Chironomus plumosus* (Chironomid)	1.36[7]	>100	NA	<0.0136	0.1[A,C]	No

Table 28: Acute and Chronic Risk Assessment for 2,4-D DMA, 2,4-D BEE and 2,4-D Acid (Continued)

2,4-D Formulation	Test Type	Exposure Medium	Species	EEC[1] (mg a.i./L)	Acute LC50[2] (mg a.i./L)	Estimated or Measured Chronic NOEC[3] (mg a.i./L)	Risk Quotient (RQ)[4] (unitless)	Level of Concern (LOQ)[5] (unitless)	RQ exceeds LOQ
2,4-D DMA	Acute	Sediment	*Chironomus plumosus* (Chironomid)	4.8[8]	>100	NA	<0.048	0.1[A,C]	No
2,4-D DMA	Acute	Sediment	*Chironomus plumosus* (Chironomid)	0.1-0.45 (0.21)[6]	>100	NA	<0.001-0.0045 (<0.0021)[6]	0.1[A,C]	No
2,4-D DMA	Chronic	Water	*Oncorhynchus mykiss* (Rainbow trout)	0.091	100	5.56	0.016	1.0[B,D]	No
2,4-D DMA	Chronic	Water	*Daphnia magna* (Daphnid)	0.091	NA	27.5 (measured)	0.0035	1.0[B,D]	No
2,4-D DMA	Chronic	Water	*Palaemonetes kadiakensis* (glass shrimp)	0.091	0.15	0.0083	11.0	1.0[B,D]	Yes
2,4-D DMA	Chronic	Sediment	*Palaemonetes kadiakensis* (glass shrimp)	0.1– 0.45 (0.21)[6]	0.15	0.0083	25.3	1.0[B,D]	Yes
2,4-D DMA	Chronic	Water	*Cypridopsis vidua* (Seed shrimp)	0.091	8.0	0.44	0.21	1.0[B,D]	No
2,4-D DMA	Chronic	Sediment	*Cypridopsis vidua* (Seed shrimp)	0.1-0.45 (0.21)[6]	8.0	0.44	0.47	1.0[B,D]	No
2,4-D DMA	Chronic	Water	*Chironomus plumosus* (Chironomid)	0.091	>100	>5.56	<0.016	1.0[B,D]	No
2,4-D DMA	Chronic	Sediment	*Chironomus plumosus* (Chironomid)	0.1 –0.45 (0.21)[6]	>100	>5.56	<0.038	1.0[B,D]	No
2,4-D BEE	Acute	Water	*Oncorhynchus mykiss* (Rainbow trout)	0.19[10]	0.3	NA	0.63	0.1[A,C]	Yes

Table 28: Acute and Chronic Risk Assessment for 2,4-D DMA, 2,4-D BEE and 2,4-D Acid (Continued)

2,4-D Formulation	Test Type	Exposure Medium	Species	EEC[1] (mg a.i./L)	Acute LC50[2] (mg a.i./L)	Estimated or Measured Chronic NOEC[3] (mg a.i./L)	Risk Quotient (RQ)[4] (unitless)	Level of Concern (LOQ)[5] (unitless)	RQ exceeds LOQ
2,4-D BEE	Acute	Water	*Oncorhynchus mykiss* (Rainbow trout)	0.100[11]	0.3	NA	0.33	0.1[A,C]	Yes
2,4-D BEE	Acute	Water	*Daphnia magna* (Daphnid)	0.19[10]	4.0	NA	0.048	0.1[A,C]	No
2,4-D BEE	Acute	Water	*Daphnia magna* (Daphnid)	0.100[11]	4.0	NA	0.025	0.1[A,C]	No
2,4-D BEE	Acute	Water	*Chasmagnathus granulata* (1st Zoel Estuarine crab)	3.25[9]	0.3	NA	0.33	0.1[A,C]	Yes
2,4-D BEE	Acute	Water	*Chasmagnathus granulata* (1st Zoel Estuarine crab)	0.19[10]	0.3	NA	0.33	0.1[A,C]	Yes
2,4-D BEE	Acute	Water	*Chasmagnathus granulata* (1st Zoel Estuarine crab)	0.100[11]	0.3	NA	0.33	0.1[A,C]	Yes
2,4-D BEE	Acute	Water	*Gammarus lacustris* (Bright scud)	0.100[11]	0.44	NA	0.23	0.1[A]	Yes
2,4-D BEE	Acute	Sediment	*Gammarus lacustris* (Bright scud)	0.05 to 0.46 (0.15)[6]	0.44	NA	0.23-1.04 (0.34)[6]	0.1[A,C]	Yes
2,4-D acid	Acute	Water	*Cyprinus carpio* (Common carp)	3.25[9]	20	NA	0.163	0.1[A,C]	Yes
2,4-D acid	Acute	Water	*Cyprinus carpio* (Common carp)	0.19[10]	20	NA	0.0095	0.1[A,C]	No

Table 28: Acute and Chronic Risk Assessment for 2,4-D DMA, 2,4-D BEE and 2,4-D Acid (Continued)

2,4-D Formulation	Test Type	Exposure Medium	Species	EEC[1] (mg a.i./L)	Acute LC50[2] (mg a.i./L)	Estimated or Measured Chronic NOEC[3] (mg a.i./L)	Risk Quotient (RQ)[4] (unitless)	Level of Concern (LOQ)[5] (unitless)	RQ exceeds LOQ
2,4-D acid	Acute	Water	*Cyprinus carpio* (Common carp)	0.100[11]	20	NA	0.05	0.1[A,C]	No
2,4-D acid	Acute	Water	*Gammarus fasciatus* (Lined scud)	3.25[9]	3.2	NA	1.02	0.1[A,C]	Yes
2,4-D acid	Acute	Water	*Gammarus fasciatus* (Lined scud)	0.19[10]	3.2	NA	0.059	0.1[A,C]	No
2,4-D acid	Acute	Water	*Gammarus fasciatus* (Lined scud)	0.100[11]	3.2	NA	0.0313	0.1[A,C]	No
2,4-D acid	Acute	Sediment	*Gammarus fasciatus* (Lined scud)	0.05 – 0.46 (0.15)[6]	3.2	NA	0.015-0.144 (0.047)[6]	0.1[A,C]	Probably No
2,4-D acid	Acute	Water	*Cyclops vernalis* (Cyclops)	0.19[10]	37	NA	0.0051	0.1[A,C]	No
2,4-D acid	Acute	Water	*Cyclops vernalis* (Cyclops)	0.100[11]	37	NA	0.0027	0.1[A,C]	No
2,4-D BEE	Chronic	Water	*Daphnia magna* (Daphnid)	0.01	NA	0.29 (measured)	0.034	1.0[B,D]	No
2,4-D BEE	Chronic	Water	*Oncorhynchus mykiss* (Rainbow trout)	0.01[12]	0.3	0.017	0.59	1.0[B,D]	No
2,4-D BEE	Chronic	Water	*Chasmagnathus granulata* (1st Zoel Estuarine crab)	0.01[12]	0.3	0.017	0.59	1.0[B,D]	No

Table 28: Acute and Chronic Risk Assessment for 2,4-D DMA, 2,4-D BEE and 2,4-D Acid (Continued)

2,4-D Formulation	Test Type	Exposure Medium	Species	EEC[1] (mg a.i./L)	Acute LC50[2] (mg a.i./L)	Estimated or Measured Chronic NOEC[3] (mg a.i./L)	Risk Quotient (RQ)[4] (unitless)	Level of Concern (LOQ)[5] (unitless)	RQ exceeds LOQ
2,4-D BEE	Chronic	Water	*Gammarus* spp. (Scuds)	0.01	0.44	0.024	0.41	1.0[B,D]	No
2,4-D BEE	Chronic	Sediment	*Gammarus* spp. (Scuds)	0.020-0.18 (0.06)[6]	0.44	0.024	0.83-7.5 (2.5)[6]	1.0[B,D]	Probably Yes
2,4-D acid	Chronic	Water	*Cyprinus carpio* (Common carp)	0.01[12]	20	1.11	0.0091	1.0[B,D]	No
2,4-D acid	Chronic	Water	*Daphnia magna* (Daphnid)	0.01	NA	27 (measured)	0.00037	1.0[B,D]	No
2,4-D acid	Chronic	Water	*Gammarus fasciatus* (Line scud)	0.01[12]	3.2	0.18	0.056	1.0[B,D]	No
2,4-D acid	Chronic	Sediment	*Gammarus fasciatus* (Lined scud)	0.020-0.18 (0.06)[6]	3.2	0.18	0.11-1.0	1.0[B,D]	No

[1] EEC = Expected environmental concentration.
[2] Acute LC50 = Concentration of 2,4-D that kills or immobilized 50% of the test animals in 96 hours.
[3] Estimated Chronic NOEC = (acute LC50/(acute/chronic toxicity ratio).
[4] RQ = Risk Quotient

LOC = Level of Concern = Value (EEC/toxicity) which should not be exceeded as an indicator of the safety of a particular pesticide application to the biota.
Values in parenthesis are geometric means of range presented.
1.36 mg a.i. /L is the typical United States use rate for 2,4-D DMA.
4.8 mg a.i./L. is the maximum labeled use rate in the United States for 2,4-D DMA products
3.25 mg/L is the concentration of 2,4-D found in bottom-waters of Northwest (Canadian) open water lakes after application of 2,4-D BEE.
0.19 mg/L is the concentration of 2,4-D found in surface of Northwest (Canadian) open water lakes after application of 2,4-D BEE.
0.100 mg./ is the concentration of 2,4-D found 2 to 6 days after application of 2,4-D BEE to Northwest (Canadian) open water lakes.
0.010 mg/L is the geometric mean of the concentration of 2,4-D found 2 to 6 days after application (0.100 mg/L) and 5 to 22 days after application (0.001 mg/L) of 2,4-D BEE to Northwestern (Canadian) lakes.
A = Acute RQ = (Short term EEC)/(acute LC50)
B = Chronic RQ = (Long term EEC)/(estimated chronic EEC)
C = Acute LOC = 0.1 =(short term (EEC)/ (96 hour LC50)
D = Chronic LOC = 1.0 = (long term EEC)/(≥28 day NOEC

Table 29. Toxicity (96-hr LC50) of 2, 4-D to Amphibia (tadpoles)

Test Species	2, 4-D Formulation	Test Results (LD50)	Toxicity Ranking	Reference
Leopard frog (*Rana Pipiens*)	Acid	359 mg a.i./L	Practically non-toxic	Palmer and Krueger, 1997
	DMA	337 mg a.i./L	Practically non-toxic	Palmer and Krueger, 1997
Indian toad (*Bufo melanostictus*)	Acid	8.05 mg a.i./L	Moderate	Vardia et al., 1984
Frog (*Limnodynastes peroni*)	DMA	287 mg a.i./L	Practically non-toxic	Johnson, 1976
Toad (*Bufo marinus*)	DMA	288 mg a.i./L	Practically non-toxic	Johnson, 1976

Table 30: Toxicity of 2, 4-D to Birds

2,4-D Formulation	Organism	LD50 (ppm)	Toxicity Ranking	Reference/Date Reported
BEE	Bobwhite quail	>5,000 (5-day)	Practically nontoxic	Hill et al, 1975
	Japanese quail	>5,000 (5-day)	Practically nontoxic	Hill et al, 1975
	Ring-necked pheasant	>5,000 (5-day)	Practically nontoxic	Hill et al, 1975
	Mallard duck	>5,000 (5-day)	Practically nontoxic	Hill et al, 1975
	Mallard duck	>5,620 (Acute LC50)	Practically nontoxic	Grimes, 1990b
	Mallard duck	>5,000 (5-day)	Practically nontoxic	Hill et al, 1975
	Bobwhite quail	>2,000 (acute LD50)	Practically nontoxic	Lloyd, 1990
	Bobwhite quail	>5620 (Acute LC50)	Practically nontoxic	Grimes, 1990a
	Bobwhite quail	>5000 (5-day LC50)	Practically nontoxic	Hill et al, 1975
DMA	Bobwhite quail	>5,000 (5-day)	Practically nontoxic	Hill et al, 1975
	Japanese quail	>5,000 (5-day)	Practically nontoxic	Hill et al, 1975
	Ring-necked pheasant	>5,000 (5-day)	Practically nontoxic	Hill et al, 1975
	Mallard duck	>5,000 (5-day)	Practically nontoxic	Hill et al, 1975
	Mallard duck	>4640 (8-day)		1997 WHO/1974
	Mallard duck	>5620 (Acute LC50)		1997 WHO/1990
	Mallard duck	>5620 (Acute LC50)		1997 WHO/1991
	Bobwhite quail	500 (Acute LD50)		1997 WHO/1990
	Bobwhite quail	>4640 (8-day)		1997 WHO/1974
	Bobwhite quail	>5620 (Acute LC50)		1997 WHO/1990

Table 30: Toxicity of 2, 4-D to Birds (Continued)

2,4-D Formulation	Organism	LD50 (ppm)	Toxicity Ranking	Reference/Date Reported
Acid	Mallard duck	>1,000	Practically nontoxic	1993, SEIS/1970
	Pheasant	472 (340-654)	Moderate	Hudson et al, 1984
	Coturnix quail	668 (530-842)	Slight	Hudson et al, 1984
	Pigeon	668 (530-842)	Slight	Hudson et al, 1984
	Chukar	200-400	Moderate	Hudson et al, 1984
	Chicken	358-817	Slight	1993, SEIS/1954
	Japanese quail	>5,000	Practically nontoxic	Hill et al, 1975
	Mallard duck	>5,620 (Acute LC50)		Culotta, 1990a
	Mallard duck	>2,000		Hudson et al, 1984
	Bobwhite quail	>5,620 (Acute LC50)		Culotta, 1990b

Table 31: Acute Oral 2, 4-D Laboratory Mammal Toxicity Data

Test Species	Formulation Used	Test Results (LD50)	Toxicity Ranking	Reference
Rat	BEE	866 mg/kg	Slight	Jeffrey, 1987
Rat	2, 4-D Acid	375 ppm	Moderate	Rowe & Hymas, 1954
Rat	2, 4-D Acid	>500 ppm	Slight	McLaughlin, 1951
Rat	2, 4-D Acid	666 ppm	Slight	Hill & Carlisle, 1947
Mice	2, 4-D Acid	375 ppm	Moderate	Hill & Carlisle, 1947
Mice	2, 4-D Acid	368 ppm	Moderate	Rowe & Hymas, 1954
Guinea pig	2, 4-D Acid	469 ppm	Moderate	Rowe & Hymas, 1954
Guinea pig	2, 4-D Acid	1,000 ppm	Slight	Hill & Carlisle, 1947
Guinea pig	2, 4-D Acid	<325 ppm	Moderate	McLaughlin, 1951
Rabbits	2, 4-D Acid	800 ppm	Slight	Hill & Carlisle, 1947
Dog	2, 4-D Acid	100 ppm	Moderate	Drill & Hiratzka, 1953
Mule Deer	2, 4-D Acid	400-800 ppm	Moderate-Slight	Tucker & Crabtree, 1970

Table 32: Terrestrial Plant, Bird and Mammal Federally Endangered Species found in the State of Washington

Terrestrial Plants	Common Name	Scientific Name
	Ute Ladies'-Tresses	*Spiranthes diluvialis*
	Golden Paintbrush	*Castilleja levisecta*
	Nelson's Checker- Mallow	*Sidalcea nelsoniana*
Birds		
	Aleutian Canada Goose	*Branta Canadensis Leucopareia*
	American Peregrine Falcon	*Falco peregrinus anatum*
	Bald Eagle	*Haliaeetus leucocephalus*
	Brown Pelican	*Pelecanus occidentalis*
	Marbled Murrelet	*Brachyramphus marmoratus*
	Northern Spotted Owl	*Strix occidentalis caurina*
	Western Snowy Plover	*Charadrius alexandinus nivosus*
Mammals		
	Gray Wolf	*Canis lupis*
	Grizzly Bear	*Ursus arctos horribilis*
	Woodland Caribou	*Rangifer tarandus caribou*
	Columbian White-Tailed Deer	*Odocoileus virginianus leucurus*

LIST OF APPENDICES

Appendix 1: Acute Toxicity of 2,4-D Products to Fish

Test Substance	%A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hours)	LC50 (mg a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D, BEE	97.4	*Oncorhynchus mykiss* (Rainbow trout)	Flow-through	FW[1]	27mm	96	2.00	1.7	C[2]	Alexander et al., 1983[J97]	41353801
2,4-D, BEE	Form	*Oncorhynchus mykiss* (Rainbow trout)	Flow-through	FW	Fry	96	0.52	NS[3]	Y[4]	Finlayson & Verrue, 1985[J97]	
2,4-D, BEE	Form	*Oncorhynchus mykiss* (Rainbow trout)	Flow-through	FW	Smolts	96	0.47	NS	NS	Finlayson & Verrue, 1985[J97]	
2,4-D, BEE	95	*Oncorhynchus mykiss* (Rainbow trout)	Static	Soft	Juvenile	96	0.8	NS	NS	Wan et al., 1990	
2,4-D, BEE	95	*Oncorhynchus mykiss* (Rainbow trout)	Static	Inter[5]	Juvenile	96	1.3	NS	NS	Wan et al., 1990	
2,4-D, BEE	95	*Oncorhynchus mykiss* (Rainbow trout)	Static	Hard	Juvenile	96	2.2	NS	NS	Wan et al., 1990	
2,4-D, BEE	97.4	*Oncorhynchus mykiss* (Rainbow trout)	Static	FW	27mm	96	2.09	1.7	C	DOW, 1983[B99]	41353801
2,4-D, BEE	77.5	*Oncorhynchus mykiss* (Rainbow trout)	Static	FW	0.69g	96	0.65	<0.5	Y	ARC, 1974[B99]	00050647
2,4-D, BEE	Form	*Oncorhynchus mykiss* (Rainbow trout)	Static	FW	Smolts	96	1.21	NS	NS	Finlayson & Verrue, 1985[J97]	

Appendix 1: Acute Toxicity of 2,4-D Products to Fish (Continued)

Test Substance	%A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hours)	LC50 (mg a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D, BEE	Form	*Oncorhynchus mykiss* (Rainbow trout)	Static	FW	Smolts	96	3.67	NS	NS	Finlayson & Verrue, 1985[J97]	
2,4-D, BEE	NN[6]	*Oncorhynchus mykiss* (Rainbow trout)	Renewal	FW	Fry	96	0.3	NS	Y	Martens et al., 1980[W89]	
2,4-D, BEE	95	*Oncorhynchus gorbuscha* (Pink salmon)	Static	Soft	Juvenile	96	0.4	NS	NS	Wan et al., 1990	
2,4-D, BEE	95	*Oncorhynchus gorbuscha* (Pink salmon)	Static	Inter	Juvenile	96	0.9	NS	NS	Wan et al., 1990	
2,4-D, BEE	95	*Oncorhynchus gorbuscha* (Pink salmon)	Static	Hard	Juvenile	96	1.1	NS	NS	Wan et al., 1990	
2,4-D, BEE	NN	*Oncorhynchus gorbuscha* (Pink salmon)	Static	FW	Fry	96	0.45	NS	Y	Martens et al., 1980[W89]	
2,4-D, BEE	95	*Oncorhynchus kisutch* (Coho salmon)	Static	Soft	Juvenile	96	1.1	NS	NS	Wan et al., 1990	
2,4-D, BEE	95	*Oncorhynchus kisutch* (Coho salmon)	Static	Inter	Juvenile	96	1.5	NS	NS	Wan et al., 1990	

Appendix 1: Acute Toxicity of 2,4-D Products to Fish (Continued)

Test Substance	%A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hours)	LC50 (mg a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D, BEE	95	*Oncorhynchus kisutch* (Coho salmon)	Static	Hard	Juvenile	96	4.3	NS	NS	Wan et al., 1990	
2,4-D, BEE	NN	*Oncorhynchus kisutch* (Coho salmon)	Renewal	FW	Fry	96	0.45	NS	Y	Martens et al., 1980[W89]	
2,4-D, BEE	NN	*Oncorhynchus nerka* (Sockeye salmon)	Renewal	FW	Fry	96	0.45	NS	Y	Martens et al., 1980[W89]	
2,4-D, BEE	Form	*Oncorhynchus tshawytscha* (Chinook salmon)	Flow-through	FW	Fry/ Juvenile	96	0.32	NS	NS	Finlayson & Verrue, 1985[J97]	
2,4-D, BEE	Form	*Oncorhynchus tshawytscha* (Chinook salmon)	Flow-through	FW	Smolts	96	0.38	NS	NS	Finlayson & Verrue, 1985[J97]	
2,4-D, BEE	29G	*Lepomis macrochirus* (Bluegill sunfish)	Static	FW	1.52g	24	>50	NS	S[7]	ARC, 1969[B99]	00053988
2,4-D, BEE	NN	*Lepomis macrochirus* (Bluegill sunfish)	NS	FW	NS	24	2.1	NS	NS	Hughes & Davis, 1963[W80]	
2,4-D, BEE	NN	*Lepomis macrochirus* (Bluegill sunfish)	NS	FW	NS	48	2.1	NS	NS	Hughes & Davis, 1963[W80]	

Appendix 1: Acute Toxicity of 2,4-D Products to Fish (Continued)

Test Substance	%A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hours)	LC50 (mg a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D, BEE	62.5	*Lepomis macrochirus* (Bluegill sunfish)	Static	FW	1.4g	96	1.2	NS	Y	Johnson & Finley, 1980	40098001
2,4-D BEE	29G	*Lepomis macrochirus* (Bluegill sunfish)	Static	FW	0.6g	96	0.76	NS	C	FWS, 1980 Brian, 1999	40098001
2,4-D, BEE	97.4	*Lepomis macrochirus* (Bluegill sunfish)	Flow-through	FW	20mm	96	0.61	0.34	NS	Alexander et al., 1983d[J97]	41353801
2,4-D, BEE	NN	*Pimephales promelas* (Fathead minnow)	Static	FW	Finger-lings	96	5.6	NS	Y	Mount & Stephan, 1967[W89]	
2,4-D, BEE	62.5	*Pimephales promelas* (Fathead minnow)	Static	FW	9g	96	3.25	NS	C	Johnson & Finley, 1980	40098001
2,4-D, BEE	97.4	*Pimephales promelas* (Fathead minnow)	Static	FW	16mm	96	2.50	1.7	NS	Alexander et al., 1983d[B99,J97]	41353801
2,4-D, BEE	70	*Fundulus similis* (longnose killifish)	Static	FW	Juvenile	48	5	NS	S	FWS, 1986[B99]	40228401
2,4-D BEE	60.8	*Ictalurus punctatus* (Channel catfish)	Static	FW	0.4g	96	0.78	NS	C	FWS, 1986[B99]	40098001
2,4-D BEE	29G	*Ictalurus punctatus* (Channel catfish)	Static	FW	0.4g	96	1.35	NS	C	FWS, 1986[B99]	40098001

Appendix 1: Acute Toxicity of 2,4-D Products to Fish (Continued)

Test Substance	%A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hours)	LC50 (mg a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D, BEE	NN	*Alburnus alburnus* (Bleak)	Static	SW	NS	96	3.2-3.7	NS	NS	Linden et al., 1979[J97]	
2,4-D, 2-EHE	60	*Oncorhynchus mykiss* (Rainbow trout)	Static	Soft	NS	96	167	NS	NS	Wan et al., 1991	
2,4-D, 2-EHE	60	*Oncorhynchus mykiss* (Rainbow trout)	Static	Inter	NS	96	164	NS	NS	Wan et al., 1991	
2,4-D, 2-EHE	60	*Oncorhynchus mykiss* (Rainbow trout)	Static	Hard	NS	96	79	NS	NS	Wan et al., 1991	
2,4-D, 2-EHE	94.2	*Oncorhynchus mykiss* (Rainbow trout)	Static	FW	0.26g	96	96	NS	C	EPA, 1977[B99]	LEWDOO0
2,4-D, 2-EHE	39.6	*Oncorhynchus mykiss* (Rainbow trout)	Static	FW	0.35g	96	64	32	C	EPA, 1974[B99]	00050699
2,4-D, 2-EHE	85.9	*Oncorhynchus mykiss* (Rainbow trout)	Static	FW	37mm	96	51	18	C	EPA, 1974[B99]	00050700
2,4-D, 2-EHE	92	*Oncorhynchus mykiss* (Rainbow trout)	Static	FW	Juvenile	96	22	12	C	UNI, 1976[B99]	00045068
2,4-D, 2-EHE	66.9	*Oncorhynchus mykiss* (Rainbow trout)	Flow-through	FW	0.21g	96	7.20	<2.1	C	Mayes et al, 1990c[J97]	41737303

Appendix 1: Acute Toxicity of 2,4-D Products to Fish (Continued)

Test Substance	%A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hours)	LC50 (mg a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D, 2-EHE	NN	*Oncorhynchus mykiss* (Rainbow trout)	Static	FW	NS	96	>5	NS	C	Alexander et al., 1983a[J97]	
2,4-D, 2-EHE	60	*Oncorhynchus gorbuscha* (Pink salmon)	Static	Soft	Fry	96	30	NS	NS	Wan et al., 1991	
2,4-D, 2-EHE	60	*Oncorhynchus gorbuscha* (Pink salmon)	Static	Inter	Fry	96	70	NS	NS	Wan et al., 1991	
2,4-D, 2-EHE	60	*Oncorhynchus gorbuscha* (Pink salmon)	Static	Hard	Fry	96	21	NS	NS	Wan et al., 1991	
2,4-D, 2-EHE	60	*Oncorhynchus kisutch* (Coho salmon)	Static	Soft	Fingerling	96	156	NS	NS	Wan et al., 1991	
2,4-D, 2-EHE	60	*Oncorhynchus kisutch* (Coho salmon)	Static	Inter	Fingerling	96	158	NS	NS	Wan et al., 1991	
2,4-D, 2-EHE	60	*Oncorhynchus kisutch* (Coho salmon)	Static	Hard	Fingerling	96	63	NS	NS	Wan et al., 1991	
2,4-D, 2-EHE	NN	*Oncorhynchus clarkii* (Cutthroat trout)	Static	FW	Juvenile	96	>50	NS	NS	Woodward, 1982[J97]	
2,4-D, 2-EHE	NN	*Lepomis macrochirus* (Bluegill sunfish)	Static	FW	NS	96	>5.0	NS	C	Alexander et al., 1983a[J97]	

Appendix 1: Acute Toxicity of 2,4-D Products to Fish (Continued)

Test Substance	%A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hours)	LC50 (mg a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D, 2-EHE	94.2	*Lepomis macrochirus* (Bluegill sunfish)	Static	FW	0.36g	96	20	NS	C	EPA, 1977[B99]	LEWOO0
2,4-D, 2-EHE	92	*Lepomis macrochirus* (Bluegill sunfish)	Static	FW	Juvenile	96	18	10	C	UNI, 1976[B99]	00045068
2,4-D, 2-EHE	NN	*Pimephales promelas* (Fathead minnow)	Static	FW	NS	96	>5	NS	NS	Alexander et al., 1983e[J97]	
2,4-D, 2-EHE	66.6	*Menidia beryllina* (Inland silverside)	Flow-through	SW	Juvenile	96	>3.0	1.1	C	Ward & Boeri, 1991c	41835202
2,4-D, 2-EHE	95.4	*Menidia beryllina* (Inland silverside)	Flow-through	SW	0.46g	96	>0.24	0.24	C	Ward & Boeri, 199d	41835205
2,4-D DMA Salt	Form	*Oncorhynchus mykiss* (Rainbow trout)	Static	FW	NS	96	377	210	NS	Bentley, 1974[J97]	
2,4-D DMA Salt	67.3	*Oncorhynchus mykiss* (Rainbow trout)	Static	FW	0.23g	96	250	120	C	Alexander et al., 1983b[J97]	41158311
2,4-D DMA Salt	NN	*Oncorhynchus mykiss* (Rainbow trout)	Static	FW	NS	48	240	NS	NS	Bogers & Enninger, 1990a[J97]	

Appendix 1: Acute Toxicity of 2,4-D Products to Fish (Continued)

Test Substance	%A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hours)	LC50 (mg a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D DMA Salt	NN	*Oncorhynchus mykiss* (Rainbow trout)	Static	FW	NS	72	240	NS	NS	Bogers & Enninger, 1990a[J97]	
2,4-D DMA Salt	NN	*Oncorhynchus mykiss* (Rainbow trout)	Flow-through	FW	NS	96	240	NS	C	Bogers & Enninger, 1990a[J97]	
2,4-D DMA Salt	49.6	*Oncorhynchus mykiss* (Rainbow trout)	Static	FW	1.4g	96	>100	NS	Y	Johnson & Finley, 1980	40098001
2,4-D DMA Salt	NN	*Oncorhynchus mykiss* (Rainbow trout)	NS	FW	NS	96	100	NS	NS	USACOE, 1978[W80]	
2,4-D DMA Salt	49.6	*Oncorhynchus tshawytscha* (Chinook salmon)	Static	FW	1.0g	96	>100	NS	C	Johnson & Finley, 1980	40098001
2,4-D DMA Salt	67.3	*Lepomis macrochirus* (Bluegill sunfish)	Static	FW	0.28g	96	524	124	C	DOW, 1983[B89]	41158311
2,4-D DMA Salt	Form	*Lepomis macrochirus* (Bluegill sunfish)	NS	FW	NS	24	220-258	NS	NS	Hughes & Davies, 1963[W80]	
2,4-D DMA Salt	Form	*Lepomis macrochirus* (Bluegill sunfish)	NS	FW	NS	48	220-458	NS	NS	Hughes & Davies, 1963[W80]	
2,4-D DMA Salt	NN	*Lepomis macrochirus* (Bluegill sunfish)	Static	FW	Subadults	96	177	NS	Y	Schultz, 1973[E93]	

Appendix 1: Acute Toxicity of 2,4-D Products to Fish (Continued)

Test Substance	%A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hours)	LC50 (mg a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D DMA Salt	49.6	*Lepomis macrochirus* (Bluegill sunfish)	Static	FW	1.1g	96	168	NS	C	Johnson & Finley, 1980	40098001
2,4-D DMA Salt	NN	*Lepomis macrochirus* (Bluegill sunfish)	Static	FW	0.23	96	106	<87	S	BIO, 1971[B99]	0073091
2,4-D DMA Salt	49.6	*Micropterus dolomieu* (Small-mouth Bass)	Static	FW	0.4g	96	236	NS	C	Johnson & Finley, 1980	40094602
2,4-D DMA Salt	NN	*Pimephales promelas* (Fathead minnow)	NS	FW	NS	48	350	NS	NS	USACOE, 1978[W80]	
2,4-D DMA Salt	NN	*Pimephales promelas* (Fathead minnow)	Static	FW	NS	96	344	NS	C	Alexander et al., 1983a[J97]	
2,4-D DMA Salt	49.6	*Pimephales promelas* (Fathead minnow)	NS	FW	NS	96	335	NS	NS	Schultz, 1973[E93] Johnson & Finley, 1980	
2,4-D DMA Salt	67.3	*Pimephales promelas* (Fathead minnow)	Static	FW	0.104g	96	318	NS	C	DOW,1983[B99]	41158311
2,4-D DMA Salt	49.6	*Pimephales promelas* (Fathead minnow)	Static	FW	0.8g	96	266	NS	C	FWS, 1986[B99]	40098001

Appendix 1: Acute Toxicity of 2,4-D Products to Fish (Continued)

Test Substance	%A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hours)	LC50 (mg a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D DMA Salt	NN	*Cyprinus carpio* (Common carp)	Static	FW	NS	48	>560-<1000	NS	NS	Bogers & Enninger, 1990b[J97]	
2,4-D DMA Salt	NN	*Cyprinus carpio* (Common carp)	Static	FW	NS	72	>560-<1000	NS	NS	Bogers & Enninger, 1990b[J97]	
2,4-D DMA Salt	NN	*Cyprinus carpio* (Common carp)	Static	FW	NS	96	>560-<1000	NS	NS	Bogers & Enninger, 1990b[J97]	
2,4-D DMA Salt	NN	*Cirrhina mrigla hamilton* (Hamilton's carp)	NS	FW	Finger-lings	96	>100	NS	NS	Sing & Yadv, 1978[J97]	
2,4-D DMA Salt	NN	*Ictalurus punctatus* (Channel catfish)	Static	FW	Subadults	96	193	NS	Y	Schultz, 1973[W89,E93]	
2,4-D DMA Salt	NN	*Ictalurus punctatus* (Channel catfish)	Static	FW	Fingerlings	96	155	NS	Y	Johnson & Finley, 1980	
2,4-D DMA Salt	NN	*Ictalurus punctatus* (Channel catfish)	NS	FW	NS	96	125	NS	NS	U.S.A.C.O.E, 1978[W80]	
2,4-D DMA Salt	49.6	*Ictalurus punctatus* (Channel catfish)	Static	FW	0.4g	96	119	NS	C	FWS, 1986[B99]	40098001
2,4-D DMA Salt	66.8	*Menidia beryllina* (Inland silverside)	Flow-through	SW	0.17g	96	469	<224	C	Ward, 1991b[J97]	41835209

Appendix 1: Acute Toxicity of 2,4-D Products to Fish (Continued)

Test Substance	%A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hours)	LC50 (mg a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D Sodium Salt	80	*Oncorhynchus mykiss* (Rainbow trout)	Static	FW	0.28g	96	>100	NS	S	EPA, 1973[B99]	00053986
2,4-D, Sodium Salt	NN	*Mugil cephalus* (Mullet)	Static	FW	NS	24	68	NS	NS	Tag El Din et al., 1981[J97]	
2,4-D, Sodium Salt	NN	*Mugil cephalus* (Mullet)	Static	FW	NS	96	32	NS	NS	Tag El Din et al., 1981[J97]	
2,4-D, Sodium Salt	NN	*Alburnus alburnus* (Bleak)	Static	FW	Embryo	48	13	NS	NS	Biro, 1979[W89]	
2,4-D, Sodium Salt	NN	*Oryzias latipes* (Medaka)	NS	FW	NS	48	>40	NS	NS	Hashimoto & Nishiuchi, 1978[J97]	
2,4-D Potassium Salt	NN	*Micropterus salmoides* (Largemouth bass)	Flow-through	Inter	Eggs	84	165	NS	NS	Birge, et al., 1979[J97]	
2,4-D Potassium Salt	NN	*Micropterus salmoides* (Largemouth bass)	Flow-through	Inter	Sac-fry	84	161	NS	NS	Birge, et al., 1979[J97]	
2,4-D Potassium Salt	NN	*Carassius auratus* (Goldfish)	Flow-through	Inter	Eggs	96	>187	NS	NS	Birge, et al., 1979[J97]	
2,4-D Potassium Salt	NN	*Carassius auratus* (Goldfish)	Flow-through	Inter	Sac-fry	96	>201	NS	NS	Birge, et al., 1979[J97]	

Appendix 1: Acute Toxicity of 2,4-D Products to Fish (Continued)

Test Substance	%A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hours)	LC50 (mg a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D Acid	NN	*Oncorhynchus mykiss* (Rainbow trout)	Static	FW	NS	96	358.0	NS	NS	Bentley, 1974[J97]	
2,4-D Acid	NN	*Oncorhynchus mykiss* (Rainbow trout)	Static	pH 4.54	Finger-lings	96	<100	NS	NS	Doe et al., 1988[J97]	
2,4-D Acid	NN	*Oncorhynchus mykiss* (Rainbow trout)	Static	pH 5.6	Finger-lings	96	<400	NS	NS	Doe et al., 1988[J97]	
2,4-D Acid	NN	*Oncorhynchus mykiss* (Rainbow trout)	Static	pH 6.8	Finger-lings	96	<1000	NS	NS	Doe et al., 1988[J97]	
2,4-D Acid	NN	*Oncorhynchus mykiss* (Rainbow trout)	Static	pH 8.48	Finger-lings	96	>1000	NS	NS	Doe et al., 1988[J97]	
2,4-D Acid	98.7	*Oncorhynchus mykiss* (Rainbow trout)	Static	FW	0.34g	96	358	320	C	DOW, 1983[B99]	41158301
2,4-D Acid	98.7	*Oncorhynchus mykiss* (Rainbow trout)	Static	FW	0.3g	96	110	NS	C	FWS, 1986[B99]	40098001
2,4-D Acid	100	*Salvelinus namaycush* (Lake trout)	Static	FW	0.3g	96	45	NS	C	Johnson & Finley, 1980	40098001
2,4-D Acid	~100	*Oncorhynchus clarkii* (Cutthroat trout)	Static	FW	Finger-lings	96	64	NS	Y	Johnson & Finley, 1980	

Appendix 1: Acute Toxicity of 2,4-D Products to Fish (Continued)

Test Substance	%A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hours)	LC50 (mg a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D Acid	100	*Oncorhynchus clarkii* (Cutthroat trout)	Static	FW	0.5g	96	25	NS	C	FWS, 1986[B99]	40098001
2,4-D Acid	98.7	*Lepomis macrochirus* (Bluegill sunfish)	Static	FW	NS	96	263	<204	C	Alexander, 1983b[w97]	41158301
2,4-D Acid	98.7	*Lepomis macrochirus* (Bluegill sunfish)	Static	FW	0.5g	96	180	NS	C	FWS, 1986[B99]	40098001
2,4-D Free Acid	NN	*Lepomis gibbosus* (Pumpkin-seed sunfish)	Static	FW	NS	24	120	NS	NS	Rewoldt et al., 1977[W89, J97]	
2,4-D Free Acid	NN	*Lepomis gibbosus* (Pumpkin-seed sunfish)	Static	FW	NS	96	95	NS	Y	Rewoldt et al., 1977[W89, J97]	
2,4-D Free Acid	NN	*Roccus Americanus* (White perch)	Static	FW	NS	24	56	NS	NS	Rewoldt et al., 1977[W89, J97]	
2,4-D Free Acid	NN	*Roccus Americanus* (White perch)	Static	FW	NS	96	40	NS	Y	Rewoldt et al., 1977[W89, J97]	
2,4-D Free Acid	NN	*Morone saxatilis* (Striped bass)	Static	FW	NS	24	86	NS	NS	Rewoldt et al., 1977[W89, J97]	

Appendix 1: Acute Toxicity of 2,4-D Products to Fish (Continued)

Test Substance	%A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hours)	LC50 (mg a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D Free Acid	NN	*Morone saxatilis* (Striped bass)	Static	FW	NS	96	70	NS	Y	Rewoldt et al., 1977[W89, J97]	
2,4-D Acid	98.7	*Pimephales promelas* (Fathead minnow)	Static	FW	0.14g	96	320	256	C	Alexander, 1983b[J97]	41158301
2,4-D Acid	98.7	*Pimephales promelas* (Fathead minnow)	Static	FW	0.9g	96	133	NS	C	FWS, 1986[B99]	40098001
2,4-D Acid	98	*Cyprinus carpio* (Common carp)	Static	FW	NS	24	310	NS	NS	Neskovic et al. 1997[J97] Neskovic et al, 1994	
2,4-D Acid	98	*Cyprinus carpio* (Common carp)	Static	FW	NS	48	295	NS	NS	Neskovic et al. 1997[J97]	
2,4-D Acid	98	*Cyprinus carpio* (Common carp)	Static	FW	NS	96	270	NS	NS	Neskovic et al. 1997[J97]	
2,4-D Acid	NN	*Cyprinus carpio* (Common carp)	Static	FW	NS	24	209	NS	NS	Sarkar, 1990	
2,4-D Acid	NN	*Cyprinus carpio* (Common carp)	Static	FW	NS	48	182	NS	NS	Sarkar, 1990	
2,4-D Acid	NN	*Cyprinus carpio* (Common carp)	Static	FW	NS	96	134	NS	NS	Sarkar, 1990	
2,4-D Free Acid	NN	*Cyprinus carpio* (Common carp)	Static	FW	NS	24	175	NS	NS	Rewoldt et al., 1977[W89, J97]	
2,4-D Free Acid	NN	*Cyprinus carpio* (Common carp)	Static	FW	NS	96	97	NS	Y	Rewoldt et al., 1977[W89, J97]	
2,4-D Acid	NN	*Cyprinus carpio* (Common carp)	Renewal	FW	NS	96	20	NS	Y	Vardia & Durve, 1981[W89]	

Appendix 1: Acute Toxicity of 2,4-D Products to Fish (Continued)

Test Substance	%A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hours)	LC50 (mg a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D Free Acid	NN	*Fundulus diaphanus* (Banded killifish)	Static	FW	NS	24	306	NS	NS	Rewoldt et al., 1977[W89, J97]	
2,4-D Free Acid	NN	*Fundulus diaphanus* (Banded killifish)	Static	FW	NS	96	27	NS	Y	Rewoldt et al., 1977[W89, J97]	
2,4-D Free Acid	NN	*Anguilla rostrata* (American eel)	Static	FW	NS	24	427	NS	NS	Rewoldt et al., 1977[W89, J97]	
2,4-D Free Acid	NN	*Anguilla rostrata* (American eel)	Static	FW	NS	96	301	NS	Y	Rewoldt et al., 1977[W89, J97]	
2,4-D Acid	NN	*Rasbora nielgeriensis*	Static	FW	NS	96	5.6	NS	N	Vardia & Durve, 1981[W89]	
2,4-D-Acid	NN	*Labeo boga*	Static	FW	NS	96	3.8	NS	N	Vardia & Durve, 1981[W89]	
2,4-D Acid	96.1	*Menidia beryllina* (Inland silverside)	Static	FW	NS	96	175	<111	C	ESE, 1991, Brian Database, 1999	41737307
2,4-D Free Acid	NS	*Lepistes reticulata* (Guppy)	Static	FW	NS	96	71	NS	Y	Rewoldt et al., 1977[W89, J97]	
2.4-D Acid	99	*Oryzias latipes* (Medaka)	Static	FW	Sac-fry	96	2780	NS	NS	Holcomb et al., 1995	

Appendix 1: Acute Toxicity of 2,4-D Products to Fish (Continued)

Test Substance	%A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hours)	LC50 (mg a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2.4-D Acid	99	*Trichogaster pectoralis* (Sepat siam)	Static	FW	4000-6000 mm	96	153	NS	NS	Ooi & Lo, 1988	
2.4-D Acid	99	*Clarias batrachus* (Keli)	Static	FW	4000-6000 mm	96	60	NS	NS	Ooi & Lo, 1988	

[1] FW =Fresh water
[2] C = Core data for EPA Risk Analysis
[3] NS = Parameter not specified.
[4] Y = Used for risk analysis even though study does not meet guideline requirements for a core study
[5] Inter = Intermediately hard water
[6] NN = Not noted. Probably technical material
[7] S = Supplemental data. Does not meet core requirements for EPA Risk Analysis.
[8] SW = Salt water

[J97] JMPR, 1997
[B99] Brian Database, 1999
[W89] Ecology, 1989
[W80] Ecology, 1980
[E93] Ebasco, 1993. Many of the items that can be found in Ebasco can also be found in other reference. Usually only one compendium reference is cited.

Appendix 2: Acute Toxicity of 2,4-D Products to Invertebrates

Test Substance	% A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hrs)	EC50 (ma a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D, BEE	97.4	*Daphnia magna* (Daphnid)	Flow-through	FW[1]	<24 hrs	48	7.2	<3.4	C[2]	Alexander et al., 1983e[B99]	41353801
2,4-D,BEE	Form	*Daphnia magna* (Daphnid)	NS[3]	FW	NS[3]	NS[3]	5.6	NS[3]	NS[3]	Sanders, 1970[E93]	
2,4-D,BEE	60.8	*Daphnia magna* (Daphnid)	Static	FW	1st-Instar	48	1.7	NS	C	FWS, 1986 Brian, 1999	40098001
2,4-D,BEE	62.5	*Daphnia magna* (Daphnid)	Static	FW	1st-Instar	48	6.4	NS	C	Johnson & Finley, 1980	40098001
2,4-D,BEE	29G	*Daphnia magna* (Daphnid)	Static	FW	1st-Instar	48	4.3	NS	S[4]	FWS, 1986[B99]	40098001
2,4-D, BEE	Form	*Crassostrea virginica* (Eastern oyster)	Flow-through	SW[5]	Larvae	96	2.6	NS	NS	EPA, 1995[B99]	ECOTOX 344
2,4-D,BEE	62.5	*Gammarus fasciatus* (Lined scud)	NS	FW	Juvenile	96	0.44	NS	C	FWS, 1986[B99] Sanders, 1970[E93]	40098001
2,4-D,BEE	62.5	*Gammarus fasciatus* (Lined scud)	NS	FW	Mature	96	6.1	NS	Y[6]	Johnson & Finley, 1980	
2,4-D,BEE	NS	*Gammarus fasciatus* (Lined scud)	NS	FW	NS	24	1.08-8.6	NS	NS	Sanders, 1970[E93]	
2,4-D,BEE	62.5	*Gammarus fasciatus* (Lined scud)	NS	FW	Juvenile	96	3.1-11	NS	NS	Sanders, 1970[E93]	
2,4-D,BEE	Tech	*Gammarus lacustris* (Bright scud)	NS	FW	NS	48	0.76	NS	S	FWS, 1969[B99]	05009242

Appendix 2: Acute Toxicity of 2,4-D Products to Invertebrates (Continued)

Test Substance	% A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hrs)	EC50 (ma a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D,BEE	Form	*Gammarus lacustris* (Bright scud)	NS	FW	NS	96	0.44	NS	S	Sanders, 1970[E93]	
2,4-D, BEE	Tech	*Nitocra spinepes (*Copepod)	NS	SW[7]	NS	96	3.1	NS	NS	Linden et al., 1979[997,E93]	
2,4-D, BEE	70	*Panaeus aztecus* (Brown Shrimp)	Static	SW	Juvenile	48	5.6	NS	C	Mayer, 1987[J97]	40228401
2,4-D, Iso-BEE	Tech	*Chasmagnathus granulata* (Estuarine crab)	Static	SW	1st Zoel	24	4.5-13.5	NS	NS	Rodriguez & Amin, 1991	
2,4-D, Iso-BEE	Tech	*Chasmagnathus granulata* (Estuarine crab)	Static	SW	1st Zoel	48	1.06	NS	NS	Rodriguez & Amin, 1991	
2,4-D, Iso-BEE	Tech	*Chasmagnathus granulata* (Estuarine crab)	Static	SW	1st Zoel	72	0.43	NS	NS	Rodriguez & Amin, 1991	
2,4-D, Iso-BEE	Tech	*Chasmagnathus granulata* (Estuarine crab)	Static	SW	1st Zoel	96	0.30	NS	NS	Rodriguez & Amin, 1991	
2,4-D, Iso-BEE	Tech	*Chasmagnathus granulata* (Estuarine crab)	Static	SW	Juvenile	24	>6400	NS	NS	Rodriguez & Amin, 1991	
2,4-D, Iso-BEE	Tech	*Chasmagnathus granulata* (Estuarine crab)	Static	SW	Juvenile	48	>6400	NS	NS	Rodriguez & Amin, 1991	
2,4-D, Iso-BEE	Tech	*Chasmagnathus granulata* (Estuarine crab)	Static	SW	Juvenile	72	5550	NS	NS	Rodriguez & Amin, 1991	

Appendix 2: Acute Toxicity of 2,4-D Products to Invertebrates (Continued)

Test Substance	% A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hrs)	EC50 (ma a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D, Iso-BEE	Tech	*Chasmagnathus granulata* (Estuarine crab)	Static	SW	Juvenile	96	2890	NS	NS	Rodriguez & Amin, 1991	
2,4-D, Iso-BEE	Tech	*Chasmagnathus granulata* (Estuarine crab)	Static	SW	Adult	24	>10000	NS	NS	Rodriguez & Lombardo, 1991	
2,4-D, Iso-BEE	Tech	*Chasmagnathus granulata* (Estuarine crab)	Static	SW	Adult	48	>10000	NS	NS	Rodriguez & Lombardo, 1991	
2,4-D, Iso-BEE	Tech	*Chasmagnathus granulata* (Estuarine crab)	Static	SW	Adult	72	6670	NS	NS	Rodriguez & Lombardo, 1991	
2,4-D, Iso-BEE	Tech	*Chasmagnathus granulata* (Estuarine crab)	Static	SW	Adult	96	3370	NS	NS	Rodriguez & Lombardo, 1991	
2,4-D, Iso-BEE	Tech	*Chasmagnathus granulata* (Estuarine crab)	Static	SW	Juvenile	672	30	NS	NS	Rodriguez et al., 1992[9]	
2,4-D, Iso-BEE	Tech	*Chasmagnathus granulata* (Estuarine crab)	Static	SW	Adult	672	>50	NS	NS	Rodriguez et al., 1992[9]	
2,4-D, Iso-BEE	Tech	*Uca uruguayensis* (Estuarine crab)	Static	SW	Adult	24	>400	NS	NS	Rodriguez & Lombardo, 1991	
2,4-D, Iso-BEE	Tech	*Uca uruguayensis* (Estuarine crab)	Static	SW	Adult	48	>400	NS	NS	Rodriguez & Lombardo, 1991	
2,4-D, Iso-BEE	Tech	*Uca uruguayensis* (Estuarine crab)	Static	SW	Adult	72	213	NS	NS	Rodriguez & Lombardo, 1991	
2,4-D, Iso-BEE	Tech	*Uca uruguayensis* (Estuarine crab)	Static	SW	Adult	96	130	NS	NS	Rodriguez & Lombardo, 1991	

Appendix 2: Acute Toxicity of 2,4-D Products to Invertebrates (Continued)

Test Substance	% A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hrs)	EC50 (ma a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D, Iso-BEE	Tech	*Uca uruguayensis* (Estuarine crab)	Static	SW	Adult	672	>30	NS	NS	Rodriguez et al., 1992[J97]	
2,4-D,BEE	NS	*Pteronarcys californica* (Stonefly)	NS	FW	Nymph	96	1.6	NS	NS	Sanders & Cope, 1968[E93]	
2,4-D,BEE	NS	*Pteronarcys californica* (Stonefly)	NS	FW	Adult	96	>1000	NS	NS	Pimentel, 1971[E93]	
2,4-D BEE	62.5	*Aselus brevicaudus* (Aquatic sowbug)	Static	FW	Juvenile	96	2.6	NS	S	Johnson & Finley, 1980	40098001
2,4-D BEE	NS	*Aselus brevicaudus* (Aquatic sowbug)	NS	FW	NS	NS	1.8	NS	NS	Sanders, 1970[E93]	
2,4-D BEE	Form	*Aselus brevicaudus* (Aquatic sowbug)	Static	FW	NS	NS	3.2-3.6	NS	NS	Sanders, 1970[E93]	ECOTOX886
2,4-D BEE	NS	*Cypridopsis vidua* (Seed shrimp)	Static	FW	NS	48	1.8	NS	NS	Sanders, 1970[E93]	ECOTOX886
2,4-D BEE	62.5	*Cypridopsis vidua* (Seed shrimp)	Static	FW	Juvenile	48	2.2	NS	S	Johnson & Finley, 1980[E93]	40098001
2,4-D BEE	NS	*Chironomus plumosus* (Chironomid)	NS	FW	NS	NS	0.39-0.79	NS	NS	EPA, 1988[e93]	
2,4-D BEE	NS	*Palaemonetes kadiakensis* (Glass shrimp)	NS	FW	NS	NS	1.4	NS	NS	Sanders, 1970[e93]	

Appendix 2: Acute Toxicity of 2,4-D Products to Invertebrates (Continued)

Test Substance	% A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hrs)	EC50 (ma a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D BEE	62.5	*Palaemonetes kadiakensis* (Glass shrimp)	Static	FW	Juvenile	96	<1.0	NS	S	FWS, 1986[b99]	40098001
2,4-D BEE	NS	*Orconectes nous* (Crayfish)	NS	FW	NS	NS	100	NS	NS	Sanders, 1970[e93]	
2,4-D, 2-EHE	96.2	*Daphnia magna* (Daphnid)	Static	FW	<24 hrs	48	>5.0	0.6	C	DO, 1983[B99]	41158306
2,4-D, 2-EHE	Tech	*Daphnia magna* (Daphnid)	Static	FW	<24 hrs	48	5.2	NS	C	Alexander et al. 1983a[J97]	
2,4-D, 2-EHE	39.6	*Daphnia magna* (Daphnid)	Static	FW	1st Instar	48	0.5	NS	C	EPA, 1975[B99]	LEWDOO 0
2,4-D, 2-EHE	95	*Daphnia magna* (Daphnid)	Static	FW	<24 hrs	48	0.054	NS	C	ARC, 1976[B99]	NS
2,4-D, 2-EHE	92	*Daphnia magna* (Daphnid)	Static	FW	1st Instar	48	0.019	<0.0056	C	UNI, 1977[B99]	00067328
2,4-D, 2-EHE	67L	*Gammarus fasciatus* (Lined scud)	Static	FW	Juvenile	96	2.4	NS	C	Johnson & Finley, 1980	40094602
2,4-D, 2-EHE	Tech	*Crassostrea virginica* (Eastern oyster)	Flow-through	SW	Juvenile	96	1.00	NS	NS	Mayer, 1987[J97]	
2,4-D, 2-EHE	Form	*Crassostrea virginica* (Eastern oyster)	Flow-through	SW	Juvenile	96	>0.71	0.71	S	Ward & Boeri, 1991c[J97]	
2,4-D, 2-EHE	Tech	*Crassostrea virginica* (Eastern oyster)	Flow-through	SW	Juvenile	96	>0.21	0.21	S	Ward & Boeri, 1991c[J97]	
2,4-D, 2-EHE	67	*Palaemonetes pugio* (Grass shrimp)	Flow-through	SW	Juvenile	96	>0.003	0.003	C	EVS, 1991[B99]	41835207

Appendix 2: Acute Toxicity of 2,4-D Products to Invertebrates (Continued)

Test Substance	% A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hrs)	EC50 (ma a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D, 2-EHE	Form	*Palaemonetes pugio* (Grass shrimp)	Flow-through	SW	<24 hrs	96	>1.4	>1.4	C	Ward & Boeri, 1991g[J97]	
2,4-D, 2-EHE	95.4	*Palaemonetes pugio* (Grass shrimp)	Flow-through	SW	0.23g	96	>0.14	0.14	C	Ward & Boeri, 1991h[J97]	41835206
2,4-D, 2-EHE	Form	*Panaeus aztecus* (Brown Shrimp)	Flow-through	SW	Adult	48	0.48	NS	NS	Mayer, 1987[J97]	40228401
2,4-D, DMA Salt	Tech	*Daphnia magna* (Daphnid)	Static	FW	NS	NS	4.0-100	NS	C	EPA, 1988[E93]	
2,4-D, DMA Salt	Tech	*Daphnia magna* (Daphnid)	Static	FW	<24 hrs	48	184	NS	C	Alexander et al., 1983c[J97]	
2,4-D, DMA Salt	Tech	*Daphnia magna* (Daphnid)	Static	FW	<24 hrs	48	>100	NS	NS	Mayer & Ellersieck, 1986[J97]	
2,4-D, DMA Salt	49.6	*Daphnia magna* (Daphnid)	Static	FW	1st Instar	48	4.0	NS	C	Johnson & Finley, 1980	40098001
2,4-D, DMA Salt	NS	*Gammarus fasciatus* (Lined scud)	NS	FW	NS	NS	>100	NS	NS	EPA, 1988[E93]	
2,4-D, DMA Salt	Tech	*Gammarus fasciatus* (Lined scud)	Static	FW	NS	24	>100	NS	NS	Mayer & Ellersieck, 1986[J97]	
2,4-D, DMA Salt	49.6	*Gammarus fasciatus* (Lined scud)	Static	FW	Mature	96	>100	NS	C	Johnson & Finley, 1980 Mayer & Ellersieck, 1986[J97]	40098001
2,4-D, DMA Salt	Tech	*Chaoborus puntipennis* (Glass worm)	NS	FW	Larvae	24	1490	NS	NS	Bunting & Robertson, 1975[J97]	

Appendix 2: Acute Toxicity of 2,4-D Products to Invertebrates (Continued)

Test Substance	% A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hrs)	EC50 (ma a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D, DMA Salt	Tech	*Chaoborus puntipennis* (Glass worm)	NS	FW	Larvae	96	890	NS	NS	Bunting & Robertson, 1975[J97]	
2,4-D, DMA Salt	Form	*Crassostrea virginica* (Eastern oyster)	Static	SW	Juvenile	48	>210-<320	NS	NS	Heitmuller, 1975[J97]	
2,4-D, DMA Salt	Form	*Crassostrea virginica* (Eastern oyster)	Flow-through	SW	Larvae	96	136	40.6	C	Ward, 1991c[J97]	41973401
2,4-D, DMA Salt	Form	*Uca pugilator* (Fiddler crab)	Static	SW	NS	96	NS	>1000	C	Heitmuller, 1975[J97]	
2,4-D, DMA Salt	Form	*Panaeus duorarum* (Pink shrimp)	Flow-through	SW	NS	96	181	65	C	Ward, 1991d[J97]	
2,4-D, DMA Salt	49.6	*Cypridopsis vidua* (Seed shrimp)	Static	SW	Mature	E-Instar	8.00	NS	NS	Johnson & Finley, 1980	40098001
2,4-D, DMA Salt	49.6	*Chironomus plumosus* (Chironomid)	Static	FW	3rd-Instar	48	>100	NS	NS	FWS, 1986[B99]	40098001
2,4, DMA Salt	95.39	*Palaemonetes pugio* (Grass shrimp)	Flow-through	SW	Juvenile	96	>0.14	0.14	S	EVS, 1991[B99]	41835206
2,4-D, DMA Salt	49.6	*Palaemonetes kadiakensis* (Glass shrimp)	Static	SW	NS	NS	>100	NS	S	REF, 1970[B99]	NS
2,4-D, DMA Salt	49.6	*Palaemonetes kadiakensis* (Glass shrimp)	Static	SW	Mature	NS	0.15	NS	C	Johnson & Finley, 1980	40094602

Appendix 2: Acute Toxicity of 2,4-D Products to Invertebrates (Continued)

Test Substance	% A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hrs)	EC50 (ma a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D DMA Salt	49.6	*Aselus brevicaudus* (Aquatic sowbug)	Static	FW	NS	48hr	>100	NS	S	REF 1970[B99]	05001497
2,4-D, Sodium Salt	Tech	*Daphnia magna* (Daphnid)	Static	FW	<24hr	96	932	NS	NS	Presing, 1981[J97]	
2,4-D, Sodium Salt	Tech	*Macrobranchium lamerrei* (Freshwater prawn)	Static	FW	NS	24	2342	NS	NS	Omar & Shukla, 1984[J97]	
2,4-D, Sodium Salt	Tech	*Macrobranchium lamerrei* (Freshwater prawn)	Static	FW	NS	48	2309	NS	NS	Omar & Shukla, 1984[J97]	
2,4-D, Sodium Salt	Tech	*Macrobranchium lamerrei* (Freshwater prawn)	Static	FW	NS	72	2267	NS	NS	Omar & Shukla, 1984[J97]	
2,4-D, Sodium Salt	Tech	*Macrobranchium lamerrei* (Freshwater prawn)	Static	FW	NS	96	2224	NS	NS	Omar & Shukla, 1984[J97]	
2,4-D, Sodium Salt	Tech	*Macrobranchium naso* (Freshwater prawn)	Static	FW	NS	24	2644	NS	NS	Omar & Shukla, 1984[J97]	
2,4-D, Sodium Salt	Tech	*Macrobranchium naso* (Freshwater prawn)	Static	FW	NS	48	2536	NS	NS	Omar & Shukla, 1984[J97]	

Appendix 2: Acute Toxicity of 2,4-D Products to Invertebrates (Continued)

Test Substance	% A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hrs)	EC50 (ma a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D, Sodium Salt	Tech	*Macrobranchium naso* (Freshwater prawn)	Static	FW	NS	72	2435	NS	NS	Omar & Shukla, 1984[J97]	
2,4-D, Sodium Salt	Tech	*Macrobranchium naso* (Freshwater prawn)	Static	FW	NS	96	2397	NS	NS	Omar & Shukla, 1984[J97]	
2,4-D, Sodium Salt	Tech	*Macrobranchium dayanum* (Freshwater prawn)	Static	FW	NS	24	2474	NS	NS	Omar & Shukla, 1984[J97]	
2,4-D, Sodium Salt	Tech	*Macrobranchium dayanum* (Freshwater prawn)	Static	FW	NS	48	2381	NS	NS	Omar & Shukla, 1984[J97]	
2,4-D, Sodium Salt	Tech	*Macrobranchium dayanum* (Freshwater prawn)	Static	FW	NS	72	2333	NS	NS	Omar & Shukla, 1984[J97]	
2,4-D, Sodium Salt	Tech	*Macrobranchium dayanum* (Freshwater prawn)	Static	FW	NS	96	2275	NS	NS	Omar & Shukla, 1984[J97]	
2,4-D, Free Acid	Tech	*Lumbriculus variegatus* (Variegated oligochaete)	Flow-through	FW	NS	48	122	NS	NS	Bailey & Liu, 1981[J97]	

Appendix 2: Acute Toxicity of 2,4-D Products to Invertebrates (Continued)

Test Substance	% A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hrs)	EC50 (ma a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D, Free Acid	Tech	*Lumbricul*us *variegatus* (Variegated oligochaete)	Flow-through	FW	NS	96	122	87	NS	Bailey & Liu, 1981[J97]	
2,4-D, Acid	NS	*Tubifex tubifex* (Tubifex worm)	Static	FW	20mm	96	161	NS	NS	Fargasova, 1994	
2,4-D, Acid	NS	*Daphnia magna* (Daphnid)	NS	FW	<24hrs	48	>100	NS	NS	Bunting & Robertson, 1975[W89]	
2,4-D, Acid	Tech	*Daphnia magna* (Daphnid)	Static	FW	1st-Instar	26	>100	100	S	FWS, 1970 [B99]	05001465
2,4-D, Acid	Tech	*Daphnia magna* (Daphnid)	Static	FW	<24 hrs	48	36	NS	C	Alexander et al., 1983b[J997]	
2,4-D, Acid	98.7	*Daphnia magna* (Daphnid)	Static	FW	1st-Instar	48	25	<12	C	DO, 1983[B99]	41158301
2,4-D, Acid	Tech	*Daphnia magna* (Daphnid)	Static	FW	<24 hrs	48	247	NS	C	McCarty & Batchelder, 1977[J97]	
2,4-D, Acid	NS	*Daphnia magna* (Daphnid)	Static	FW	<24 hrs	48	181	NS	C	Fargasova, 1994	
2,4-D, Free Acid	Tech	*Daphnia magna* (Daphnid)	Static	FW	<24 hrs	48	418	NS	NS	Presing, 1981[J97]	
2,4-D Acid	Tech	*Ceriodaphnia dubia* (Daphnid)	Flow-through	FW	<24 hrs	24	236	NS	NS	Orius et al., 1991[J97]	
2,4-D Acid	NS	*Cyclops vernalis* (Cyclops)	NS	FW	NS	NS	37	NS	NS	Bunting & Robertson, 1985[E93]	

Appendix 2: Acute Toxicity of 2,4-D Products to Invertebrates (Continued)

Test Substance	% A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (hrs)	EC50 (ma a.i./L)	NOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D, Acid	NS	*Gammarus fasciatus* (Lined scud)	NS	FW	NS	NS	3.2	NS	NS	Sanders, 1970[E93]	
2,4-D, Free Acid	Tech	*Mytilus edulis* (Bay mussel)	NS	SW	NS	96 Mortality	259	NS	NS	Liu & Lee, 1975[J97]	
2,4-D, Free Acid	Tech	*Mytilus edulis* (Bay mussel)	NS	SW	NS	96 Attachment	262	NS	NS	Liu & Lee, 1975[J97]	
2,4-D, Free Acid	Tech	*Mytilus edulis* (Bay mussel)	NS	SW	Tocophore	48 Norm. Develop	212	NS	NS	Liu & Lee, 1975[J97]	
2,4-D, Acid	96.1	*Crassostrea virginica* (Eastern oyster)	Flow-through	SW	Juvenile	96	146	<135	C	Wade & Overman, 1991[B99]	41848001
2,4-D, Acid	95.1	*Crassostrea virginica* (Eastern oyster)	Flow-through	SW	Larvae	96	57	30	C	Ward et al., 1993[J97,B99]	42979701
2,4-D Acid	Tech	*Panaeus duorarum* (Pink shrimp)	Flow-through	SW	NS	96	554	NS	C	Vashinav et al., 1990b[J97]	41737306
2,4-D Acid	96.1	*Crassostrea virginica* (Eastern oyster)	Flow-through	SW	NS	96 Deposition	467	187	C	ESE, 1991[B99]	41835211
2,4-D Acid	Tech	*Cancer magister* (Dungeness crab)	Static	SW	1st Zoel	96	>10	NS	NS	Caldwell, 1977[J97]	
2,4-D Acid	Tech	*Cancer magister* (Dungeness crab)	Static	SW	Adult	96	>100	NS	NS	Caldwell, 1977[J97]	

[1] FW = Freshwater
[2] C = Core data for EPA Risk Analysis
[3] NS = Parameter not specified
[4] S = Supplemental data. Does not meet core requirements for EPA Risk Analysis
[5] SW = Salt water

Appendix 2: Acute Toxicity of 2,4-D Products to Invertebrates (Continued)

[6] Y = Used for risk analysis even though study does not meet guideline requirements for a core study
[7] SW = Saltwater
[J97] JMPR, 1997
[B99] Brian Database, 1999
[W89] Ecology, 1989
[W80] Ecology, 1980
[E93] Ebasco, 1993 Many of the items that can be found in Ebasco can also be found in other reference. Usually only one compendium reference is cited

Appendix 3: Chronic Toxicity of 2,4-D Products to Fish

Test Formulation	%A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (Days)	NOEC (mg a.i./L)	MATC (mg a.i./L)	LOEC (mg a.i./L)	Status	Reference	MRID Number
2.4-D, BEE	NS[1]	*Oncorhynchus tshawytscha* (Chinook salmon)	Flow-through	FW[2]	Egg-Fry	86	0.040	NS[1]	NS[1]	Y[3]	Finlayson & Verrue, 1985[W89]	NS
2,4-D, BEE	96	*Pimephales promelas* (Fathead minnow)	Flow-through	FW	Egg-Fry	32	0.0805	0.0962	0.115	C[4]	DOW, 1989[B89] Brian, 1999	41345701
2,4-D, BEE	NS	*Pimephales promelas* (Fathead minnow)	Flow-through	FW	Life-cycle	10 months	0.3	NS	NS	Y	Mount & Stephan, 1967[W89]	NS
2,4-D, 2-EHE	94.7	*Pimephales promelas* (Fathead minnow)	Flow-through	FW	Egg-Fry	32	0.12	0.16	0.22	C	DOW, 1990 [B99]	41737305
2,4-D, 2-EHE	NS	*Lepomis macrochirus* (Bluegill sunfish)	Static	FW	Sac-Fry	12	>50	NS	NS	NS	Hiltibran, 1967	
2,4-D, 2-EHE	NS	*Campostoma anomalum* (Stoneroller)	Static	FW	Sac-Fry	8	>25	NS	NS	NS	Hiltibran, 1967	
2,4-D, DMA Salt	99.3	*Pimephales promelas* (Fathead minnow)	Flow-through	FW	Egg-Fry	31	17.1	22	28.4	C	Dill et al., 1990[J97]	41766701
2,4-D, 2-EHE	NS	*Lepomis macrochirus* (Bluegill sunfish)	Static	FW	Sac-Fry	12	>40	NS	NS	NS	Hiltibran, 1967	
2,4-D, 2-EHE	NS	*Lepomis cyanellus* (Green sunfish)	Static	FW	Sac-Fry	12	>25	NS	NS	NS	Hiltibran, 1967	
2,4-D, 2-EHE	NS	*Lermyzon sucetta* (Lake chubsucker)	Static	FW	Sac-Fry	12	>25	NS	NS	NS	Hiltibran, 1967	

Appendix 3: Chronic Toxicity of 2,4-D Products to Fish (Continued)

Test Formulation	%A.I. or Form	Species	Test Type	Water Type	Size Class/ Age	Time (Days)	NOEC (mg a.i./L)	MATC (mg a.i./L)	LOEC (mg a.i./L)	Status	Reference	MRID Number
2,4-D, 2-EHE	NS	*Micropterus dolomieu* (Smallmouth bass)	Static	FW	Sac-Fry	12	>25	NS	NS	NS	Hiltibran, 1967	
2,4-D, Potassium Salt	NN[5]	*Oncorhynchus mykiss* (Rainbow trout)	Flow-through	Inter	Egg-sac-fry	23-27	NS[1]	LC50= 11 LC1= 0.032	NS	NS	Birge et al, 1979[J97]	NS
2,4-D, Potassium Salt	NN	*Oncorhynchus mykiss* (Rainbow trout)	Flow-through	Hard	Egg-sac-fry	23-27	NS	LC50= 4.2 LC1= 0.022	NS	NS	Birge et al, 1979[J97]	NS
2,4-D, Potassium Salt	NN	*Carassius auratus* (Goldfish)	Flow-through	Inter	Eggs	8	NS	LC1= 18.2 LC50 = 133	NS	NS	Birge et al, 1979[J97]	NS
2,4-D, Potassium Salt	NN	*Carassius auratus* (Goldfish)	Flow-through	Hard	Sac-fry	8	NS	LC1= 8.9 LC50 = 119	NS	NS	Birge et al, 1979[J97]	NS
2,4-D, Potassium Salt	NN	*Micropterus salmoides* (Largemouth bass)	Flow-through	Inter	Eggs	7.5	NS	LC1= 13.1 LC50 = 109	NS	NS	Birge et al, 1979[J97]	NS
2,4-D, Potassium Salt	NN	*Micropterus salmoides* (Largemouth bass)	Flow-through	Hard	Sac-fry	7.5	NS	LC1= 3.2 LC50 = 82	NS	NS	Birge et al, 1979[J97]	NS
2,4-D Acid	96.1	*Pimephales promelas* (Fathead minnow)	Flow-through	FW	Egg-Fry	32	63.4	80.4	102	C	Mayes et al., 1990b	41737304
2,4-D Acid	99	*Oryzias latipes* (Medaka)	Flow-through	FW	Sac-fry	28	27.2-30	39.2-42.5	56-60.2	NS	Holcomb et al., 1995	NS

[1] FW = Freshwater
[2] NS = Not specified for this parameter.
[3] Y = Data does not fulfill EPA core requirements but it was used in an EPA risk assessment
[4] C = EPA Core Data. Adequate for EPA risk assessment
[5] NN = Not noted. Probably technical material

[J97] JMPR, 1997
[B99] Brian Database, 1999
[W89] Ecology, 1989
[W80] Ecology, 1980
[E93] Ebasco, 1993. Many of the items that can be found in Ebasco can also be found in other reference. Usually only one compendium reference is cited.

Appendix 4: Chronic Toxicity of 2,4-D Products to Invertebrates (Daphnid)

Test Substance	% A.I. or Form	Species	Test Type	Water Type	Age	Time (days)	EC50[1] (mg (a.i./L)	NOEC[2] (mg a.i./L)	MATC[3] (mg a.i./L)	LOEC[4] (mg a.i./L)	Status	Reference	MRID Number
2,4-D, BEE	96	*Daphnia magna* (Daphnid)	Flow-through	FW[1]	Life-cycle	21	NR	0.29	0.45	0.7	C[3]	DO, 1989[B99]	41353802
2,4-D, Iso-BEE	Tech	*Chasmagnathus granulata* (Estuarine crab)	Static	SW[2]	Chronic Adult	28	>50	NS[4]	NS[4]	NS[4]	NS[4]	Rodriguez et al, 1992	
2,4-D, Iso-BEE	Tech	*Uca uruguayensis* (Estuarine crab)	Static	SW	Chronic Adult	28	>30	NS	NS	NS	NS	Rodriguez et al, 1992	
2,4-D, 2-EHE	95.4	*Daphnia magna* (Daphnid)	Flow-through	FW	Life-cycle	21	0.13	0.015	0.020	0.027	C	Ward & Boeri, 1991b	41835207
2,4-D, Dimethylamine Salt	Tech	*Daphnia magna* (Daphnid)	Flow-through	FW	Life-cycle	21	3-day LC50= 130-243	27.5	40.5	59.6		Ward, 1991a[9]	
2,4-D Acid	91.3	*Daphnia magna* (Daphnid)	Flow-through	FW	Life-cycle	21	235	79	109	151	C	Ward & Boeri, 1991a	41835211
2,4-D Acid	99	*Ceriodaphnia dubia* (Daphnid)	Renewal	FW	Life-cycle	4	81.8	~35	49	~70	NS	Oris, et al. 1991	
2,4-D Acid	99	*Ceriodaphnia dubia* (Daphnid)	Renewal	FW	Life-cycle	7	72.5	20-35 (26)	48.8	35-70 (49.5)	NS	Oris, et al. 1991	

EC50 = Concentration that effects 50% of the test animals. In these studies, the endpoint for the EC50 effect is immobility or mortality.
NOEC = Statistical No Observed Effect Concentration. Endpoints of interest are, immobility, reduction in live-born young and reduction in Growth.
MATC = Maximum allowable toxic concentration. This concentration is the geometric mean of the NOEC and LOEC.
LOEC = Statistical Lowest observed Effect Concentration. Endpoints of interest are: immobility, reduction in live-born young and reduction in Growth.
FW = Freshwater.
SW = Saltwater.
C = Core study used by EPA for risk assessment.
NS = Parameter not specified.

2,4-D

Volume 3, Section 5

HUMAN HEALTH EFFECTS

119 PAGES

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5.0 HUMAN HEALTH EFFECTS

5.1 OBJECTIVE AND APPROACH

The Washington State Department of Ecology (DOE) contracted with Compliance Services International (CSI) to update their existing document concerning potential human health impacts from aquatic applications of the herbicide 2,4-D. This herbicide is currently being used to control noxious aquatic plants in the State of Washington.

The purpose of this section is to provide the most recent health information to the Washington State Department of Ecology concerning the potential toxicological risks to public health associated with 2,4-D aquatic weed control and to assist the agencies in making decisions regarding herbicide use.

The objectives of this section are to help: (1) develop a public health risk assessment for 2,4-D as it applies to use of noxious plant control; (2) provide an overview of epidemiology and carcinogenicity of 2,4-D; and (3) present the information in a quantitative manner that permits direct comparison of the estimated exposure concentrations with concentrations that are expected to protect public health.

5.1.1 Information Compilation

Information concerning 2,4-D biotransformation, toxicology, carcinogenicity, epidemiology and environmental fate and transport were obtained from computerized searches of the scientific and medical literature, EPA Office of Pesticide Programs (OPP), and the herbicide manufacturer (Dow AgroSciences) and the Industry Task Force II on 2,4-D Research Data.

The computerized Dialogue Information retrieval system was used to search the scientific and medical literature. The first edition of the WDOE 2,4-D document was issued in 1993. This revision includes an extensive review of the scientific and medical literature concerning 2,4-D from 1990 to present. However, many pre-1990 publications listed in the 1993 review are included in this document. The following databases were searched for the original WDOE 2,4-D document and in compiling information for the second edition (WDOE, 1993).

- Biosis Previews
- National Technical Information Services (NTIS)
- Aquatic Sciences and Fisheries Abstracts
- Agricola
- Medline
- Toxline
- CAB Abstracts
- Cancerlit
- Life Sciences Collection
- The New England Journal of Medicine Online
- Registry of Toxic Effects of Chemical Substances (RTECS)
- Dissertation Abstracts Online
- Agris International
- Pascal

Descriptions of these databases are provided at the end of this document.

In addition, the U.S. Department of Agriculture funded an extensive literature review in 1988. This information is summarized in their 1988 publication "Managing Competing and Unwanted Vegetation - Final Environmental Impact Statement." Therefore, this document was also used as an information source.

5.1.2 Risk Assessment Methodology

The risk assessment methodology and 2,4-D exposure scenarios that appeared in the original document have been maintained and utilized in the second edition (WDOE, 1993).

This risk assessment is structured according to guidelines described by the USEPA (1989) and USEPA Region 10 guidance, and is comprised of four basic units: Exposure assessment, Toxicity assessment, Risk Characterization, and Uncertainty analysis.

- **Exposure assessment**

  The exposure assessment involves determination of potentially exposed populations and estimating doses likely to result from potential exposures. The results of contaminant fate and transport analyses are used to evaluate the extent and magnitude of 2,4-D in various aquatic environments. This information is used to determine exposure pathways, such as inhalation or ingestion of groundwater. Some pathways will naturally be ruled out, depending on the fate and transport of the chemical in the environment. In this step, the individual chemical-specific exposure estimates for each exposure route (i.e., dermal, inhalation, ingestion) are developed.

  A determination of potentially exposed populations is also integral to the exposure assessment. This involves the identification, enumeration, and characterization of those population segments likely to be exposed. The goal of this analysis is not only to determine which population groups will potentially be exposed but also to determine how and with what frequency and duration such exposure occurs. Only the general public was evaluated as part of this assessment; applicators and mixers of 2,4-D were not considered in the exposed population.

  In order to account for variability in treatment area size and location, and in human activities associated with these areas in Washington State, several "generic" exposure scenarios were developed (Table 1). These scenarios are intended to be representative of typical activities undertaken within Washington. The amine and ester forms of 2,4-D were evaluated.

  For acute exposure scenarios it was assumed that "worst case" conditions exist for both 2,4-D environmental concentrations and exposure parameters. Chronic exposures were calculated for three different types of environments (small ponds, irrigation ditches, and large lakes) and assumed "worst case" exposures. This is further discussed in Sections 5.13 – 15 and listed in Tables 4-12.

- **Toxicity assessment**

  The purpose of the toxicity assessment is: (1) to weigh the available evidence regarding the potential for contaminants to cause adverse effects in exposed individuals (i.e., hazard identification), and (2) to provide a quantitative estimate of the relationship between the magnitude of exposure and the likelihood or severity of adverse effects (i.e., the

dose-response assessment) (USEPA, 1989). These elements are discussed in the subsections, which follow.

As part of the hazard identification step of the toxicity assessment, information is assembled on the potential for a chemical to cause adverse health effects (e.g., carcinogenic, noncarcinogenic) in humans. A hazard identification is intended to characterize the nature and extent of the health hazards associated with chemical exposures. This step is generally addressed through the development of a toxicological summary for each chemical which discusses parameters such as pharmacokinetics, various critical health effects (e.g., carcinogenic effects, reproductive, developmental, or other systemic effects) and the association of these effects with exposure at different chemical concentrations over varying time periods. The sources for this information include human epidemiological studies and clinical cases, experimental animal studies, and supporting data such as in vitro studies (USEPA, 1989). Generally, the preferred sources of information for dose-response assessment are properly conducted epidemiological studies. Where appropriate human studies are available, they are weighted more heavily, with animal studies used as supporting evidence.

When human data are lacking, as is usually the case, animal studies are used to evaluate potential adverse effects and quantify dose-response. Differences between animals and humans in relation to metabolic processes, behavior, and physiology, etc., result in a high degree of uncertainty in the dose-response values derived from these sources. However, the likelihood of a chemical causing adverse effects in humans increases as similar effects are observed among sexes, species, and exposure routes in well-conducted animal studies (USEPA, 1989).

The dose-response assessment is intended to quantify the relationship between the magnitude of exposure to a chemical and the occurrence of adverse health effects. This step involves an analysis of correlation between the severity or frequency of adverse effects and the levels of exposure at which these effects occur for each chemical. Typically, this entails a review of the toxicological literature to identify chemical-specific dose-response estimates through oral, inhalation and dermal routes of exposure.

Chemicals may elicit two general categories of adverse health impacts in exposed individuals--noncarcinogenic and carcinogenic effects. Noncarcinogenic effects, or any health impact other than cancer, may result from acute, subchronic or chronic exposures. For most noncarcinogenic effects, protective mechanisms within an individual are assumed to exist that must be overcome before an adverse effect is elicited. The level above or below which effects may or may not be elicited is referred to as a threshold level. Examples of noncarcinogenic effects include central nervous system disorders (e.g., neurological damage or impairment), blood disorders (e.g., anemia), organ toxicity (e.g., kidney, liver, heart) and reproductive toxicity (e.g., gametotoxicity, fetal toxicity, etc.).

In developing dose-response values for noncarcinogenic effects (i.e., the reference dose or RfD), the goal is to identify the highest no-observed-adverse-effect-level, NOAEL (i.e., the upperbound of the tolerance range) or the lowest-observed-adverse-effect-level, LOAEL, from well designed human or animal studies. One or more order-of-magnitude uncertainty factors are incorporated to adjust this level based on considerations of the following: (1) the duration of the experimental exposure, (2) effects elicited (if any), (3) extrapolation of the data to other species (i.e., interspecies variability, such as extrapolation to humans), and (4) sensitive subgroups (i.e., intraspecies variability). Additional modifying factors varying

between a value of 1 and 10 may also be incorporated in the derivation of the RfD if additional considerations are necessary. The general formula to derive a RfD is as follows:

$$\text{RfD (mg/kg-day)} = \frac{\text{NOEL or LOEL}}{\text{Uncertainty} * \text{Modification Factors}}$$

RfDs are generally taken from the preferred source of dose-response values-EPA's computerized Integrated Risk Information System (IRIS) - and represent "verified" (interagency reviewed) quantities.

The RfD approach was designed to predict risk to human health from low level, chronic exposures over the course of a lifetime. It is assumed that a person can be exposed to the calculated RfD dose of a chemical for their lifetime and not be expected to develop adverse health effects. Potential risks associated with acute exposures were assessed using the Margin of Safety (MOS) approach, discussed in Section 5.14. The MOS is also a calculated measure of the degree of safety determined by dividing the person's estimated or calculated exposure by the lowest animal toxicology study NOEL. MOS values greater than 100 are indicators that exposure to the chemical decreases the potential for the development of adverse health effects. Conversely, MOSs less than 100 are signals of concern that the person may be in an overexposure situation that could result in signs and/or symptoms of illness.

- **Risk characterization**

  The risk characterization involves comparing the dose estimates for the different exposure pathways with the hazard information and determining the probability that health effects could occur. This is accomplished by comparing expected environmental doses to those doses, which elicit a toxicological response in laboratory animals.

  Human doses are calculated from expected environmental concentrations of 2,4-D based on the herbicide application rates and information regarding chemical fate and transport. The expected environmental concentrations are discussed in Section 5.17.

  Non-carcinogenic risk from single, acute 2,4-D exposures was estimated using the MOS approach. The methodology of Shipp et al. (1986) was used to determine potential reproductive, systemic, and teratogenic effects. In this method 2,4-D doses which are shown to cause toxic effects in laboratory animals are compared to predicted human doses.

  Details of the margin of safety approach and risk characterization methodology are discussed in Section 5.20. MOSs are calculated for each pathway on a daily basis, starting from immediately after 2,4-D application and ending 22 days after application. Once an initial environmental concentration was calculated degradation rates were used to calculate change in concentration over time. Daily doses were then calculated using these EECs.

  For chronic exposures the RfD approach was used. Hazard indices were calculated for each exposure pathway. The hazard index is the ratio of the chronic daily intake to a reference (RfD) intake.

- **Uncertainty analysis**

  In this section of the risk assessment, each step is reviewed to identify the uncertainties involved and to evaluate their impact on the assessment results. For example, uncertainties

may result from the use of default exposure parameter values or the use of simplified estimation procedures in the event of lack of data. The uncertainty analysis is generally presented in a qualitative format.

5.1.3 CHEMICAL FORMULATIONS

The 2,4-D dimethylamine and butoxyethyl ester (2,4-D BEE) formulations are registered for aquatic weed control. The 2,4-D dimethylamine salt has a Federal aquatic registration and is available in liquid formulations containing ~46.8% amine salt (~38.9% 2,4-D acid equivalent) and a granular product of 95% 2,4-D dimethylamine (~78.9% acid equivalent). The State of Washington registers the 2,4-D BEE, that contains 27.6% ester salt (19% 2.4-D acid equivalent), for aquatic use.

2,4-D BEE is hydrolyzed to the 2,4-D acid within a few hours to a day following application (JMPR, 1997). Similarly, it has been demonstrated that fish rapidly hydrolyze absorbed 2,4-D BEE to the acid equivalent and eliminate the chemical from the tissue (Shearer and Halter, 1980). Primarily because of the rapid hydrolyses and excretion of the amine and ester salts to the acid equivalent, most toxicology studies have been conducted with the 2,4-D acid.

5.2 EXPOSURE ASSESSMENT

- **Exposed Population**

The Risk Assessments of the exposed population refers to the general public and does not include people who may be occupationally exposed to 2,4-D e.g., mixing, loading, or applying the chemical. The general public, as considered in this document, includes adults of both sexes and children. All of the 2,4-D water, sediment and fish concentrations used to conduct risk assessments are the same as those listed in the original WDOE 2,4-D 1993 2,4-D document.

- **Potential Routes of Exposure**

The potential routes of exposure, resulting in the greatest 2,4-D exposure to the various population groups, primarily included ingestion of treated water either during swimming or through daily use of potable or treated water as a drinking water source. Other potential routes of exposure included dermal contact with water and sediments and ingestion of sediments and fish taken from treated water. Currently, there does not appear to be any scientific group or governmental agency that has determined an estimated “background” level exposure to 2,4-D due to other uses of chlorophenoxy herbicides, e.g. consumer products, professional pesticide application, municipal use, agriculture or diet exposure. However, based on 2,4-D’s chemical and physical properties, label use-rates, environmental fate, dermal absorption rate, rapid excretion and low toxicity, it does not seem like the “background” exposure is significant.

The issue of ingesting wild berries and game that may be contaminated with 2,4-D is not considered to pose a health risk due to the low degree of exposure and that the chemical is rapidly metabolized and excreted if ingested. The human health impacts due to the ingestion of wild berries has been investigated by the USDA (1988). They calculated that the lifetime cancer risk from the ingestion of wild berries after 2,4-D application is $4.14 \times 10^{-8,}$ or the chance that approximately 4 people out of every 100,000,000 exposed will contract cancer. This risk was calculated using conservative "worst case" conditions and is well below the range of 1×10^{-4} to 1×10^{-7} designated as acceptable by the USEPA. For noncarcinogenic effects the margin of safety (ratio of a "safe" dose derived from a laboratory study to an expected environmental dose) for eating wild berries ranged from 96 to greater than 1 million for various application methods. These results indicate that under absolute worst-case conditions the dose received would be at least 96 times lower than the estimated "safe dose." Given that the cancer risk and margins of safety were calculated using extremely conservative, worst case assumptions and that they indicate little if any potential for harm, berry eating will not be addressed further in this assessment. The conservative nature of the exposure assumption indicates that the assessment applies to sensitive subgroups such as the elderly and ill.

The USDA (1988) has also addressed ingestion of wild game. Results of their "worst case" analysis also indicates that there is little or no risk from ingestion of wild meat after 2,4-D application. However, the USDA risk assessment also modeled exposure and risk to the public from procuring berries and wild meat (i.e., berry picking and hunting) and determined that most of the health risk was due to dermal contact with freshly sprayed vegetation. The main potential 2,4-D exposure pathways considered in this document include ingestion of treated water, sediments, fish and, dermal contact with water and sediments. .

- **Formulations and Application Rates**

  - **Formulations**

    Although approximately 30 2,4-D formulations are registered for use in Washington State, this analysis will focus on two identical formulations: Navigate® and Aqua-Kleen® Aqua-Kleen® and Navigate® are manufactured by Rhone-Poulenc and contain 27.6% 2,4-Dichlorophenoxyacetic acid, butoxyethyl ester (2,4-D BEE). Aqua-Kleen® and Navigate® are granular formulations that are more likely to be used for submergent vegetation than for emergent noxious weeds such as purple loosestrife.

  - **Application Rates**

    The Aqua-Kleen®/Navigate® labels suggest 100 to 200 lbs/acre for susceptible weeds and 150 to 200 lbs/acre for slightly to moderately resistant weeds. Purple loosestrife is not identified on the label. On a 2,4-D BEE basis, these rates correspond to 27 lbs/acre to 55 lbs/acre. On an acid equivalent basis, these rates correspond to 19 lbs/acre to 38 lbs/acre. Guidance on the label states, "Rates of application vary with resistance to weed species to the chemical, density of weed mass at time of treatment, water depth, and rate of water flow through the treated area. Use the higher rate for dense weeds, when water is more than 8 feet deep, and where there is a large volume turnover." Therefore, to be conservative, an application rate of 38 lbs. a.e./acre was used in the calculations.

5.2.1 Ingestion of Surface Water

- **Exposure Data**

  The herbicide 2,4-D may be utilized on lakes or ponds which are used as residential drinking water sources. This assessment assumes that water is drawn directly from lakes, ponds, or irrigation ditches and utilized without any chemical or physical pre-treatments.

- **Estimated Dose**

  Chronic daily water intake is calculated as follows (USEPA, 1989):

$$Intake\ (mg/kg\text{-}day) = \frac{CW \times IR \times EF \times ED}{BW \times AT}$$

Where:
CW = Contaminant concentration in Water (mg/L)
IR = Intake Rate (2 L/day)
EF = Exposure Frequency (365 days/year)
ED = Exposure Duration (30 years)
BW = Body Weight (70 kg)
AT = Averaging Time (30 years x 365 days/year = 10,950 days)

All exposure values are standard USEPA Region 10 guidance default values and represent very conservative assumptions. Estimated intakes are presented in Table 13.

A review of Sections 5.15 – 5.17 and the hazard quotients presented on Table 25, indicate that the greatest degree of 2,4-D exposure from labeled aquatic herbicide control, is through ingestion of treated water either during swimming or use of treated water as a drinking water source.

5.2.2 Dermal Contact with Water (Swimming)

2,4-D formulations used according to label directions for aquatic weed control are applied by means of direct underwater injection, overhead wand surface spraying, aerial application or a drip system. Aquatic dermal exposure to 2,4-D would occur primarily by swimming in recently treated water. Based on the 2,4-D water concentrations listed in Tables 5-7 and the low dermal absorption of the chemical as discussed in Section 5.3.2, the dose of the chemical received from skin contact with treated water is not considered significant. A comparison of the calculated hazard quotients listed on Table 25 reveal that the quotients for dermal 2,4-D exposure while swimming are approximately 18 and 128 times less than exposure received from ingestion of fish and treated water, respectively.

- **Exposure Data**

  The estimated aquatic environmental concentrations of 2,4-D, presented in the original WDOE 2,4-D document, were based on the highest or the maximum 2,4-D use-rate of 38 pounds acid equivalent/acre (WDOE, 1993). This is the prescribed label application rate to eradicate purple loosestrife that is an emergent and not submersed aquatic plant. Nevertheless, it represents a label high use-rate situation and serves as the basis for calculating the 2,4-D water concentrations and risk assessments in both the original and the revised editions of this document. The results of the evaluations do not indicate that at maximum label use-rates does 2,4-D pose a significant health risk as indicated from the data and calculations presented in the Tables of this document.

- **Estimated Dose**

  Acute dermal doses are derived by the formula:

$$\text{Dermal Dose (mg/kg)} = \frac{Cs \times CF \times SA \times AF \times ABS}{BW}$$

  Where:
  Cs = Concentration in sediment (mg/kg)
  CF = Conversion factor (0.000001 kg/mg)
  SA = Surface area exposed (1840cm^2)
  AF = Adherence factor (0.95 mg/cm^3)
  ABS = Absorption factor (10.5%)
  BW = Body weight (70 kg)

  The only body parts assumed to contact sediments are the feet and lower legs of adults and lower body and hands of a child. Acute doses from a single incident of dermal contact with sediment are presented in Table 9.

  Chronic intake from dermal contact with sediments is calculated as follows (USEPA, 1989):

$$\text{Intake (mg/kg-day)} = \frac{Cs \times CF \times SA \times AF \times ABS \times EF \times ED}{BW \times AT}$$

  Where:
  CS = Contaminant concentration in sediment (mg/kg)
  CF = Conversion factor (0.000001 kg/mg)
  SA = Surface area (1,840 cm^2)
  AF = Adherence factor (0.95 mg/cm^2-event)
  ABS = Absorption factor (10.5 %)
  EF = Exposure frequency (22 events/year)
  ED = Exposure duration (30 years)
  BW = Body weight (70 kg)
  AT = Averaging time (30 years x 365 days/year = 10,950 days)

  In this scenario it is assumed that both lower legs and feet contact sediment. Intake values from dermal contact with sediments are presented in Table 10.

5.2.3 Ingestion of Fish

- **Exposure Data**

  The herbicide 2,4-D generally does not bioaccumulate to a great extent, and the small amounts which do accumulate are rapidly eliminated once exposure ceases (Norris, 1982). Reinert and Rodgers (1987) report that bioconcentration factors ratio of 2,4-D concentrations in organisms to that in water range from 1 to 7 in various species of fish.

  These values are for the DMA (dimethylamine salt) formulation of 2,4-D. Shipp et al. (1986) utilized a BCF of 1 for prediction of fish tissue 2,4-D concentration, and the USDA (1988) utilized a BCF of 5. A review of BCFs (USEPA, 1993) indicate that these values are representative of those typical for freshwater fish. A BCF of 5 was utilized to calculate fish

tissue concentrations from water concentrations. Bioaccumulation factors (BAF) describe the ratio of 2,4-D in fish tissue to that in sediment and were not available for fish.

- **Estimated Dose**

The species of fish found in ponds and lakes in Washington State vary widely. Thus, generalizations regarding applicability of the above BCFs to these fish must be made. A BCF of 5 was assumed to be representative of BCFs for resident fish. This low BCF is further supported by negligible and below detection limit concentrations of 2,4-D measured in numerous fish species (Schultz and Harmon, 1973; Gangstad, 1983, Hoeppel and Westerdahl, 1983).

Concentrations of 2,4-D in fish were calculated using a standard methodology (Rand and Petrocelli, 1985) in which concentrations are multiplied by the BCF, so that

Estimated water concentrations are taken from section 5.6.2.1, and resulting fish concentrations and acute human doses are presented in Table 11. Fish 2,4-D concentrations are calculated from water concentrations. The calculated fish concentrations are likely to be overestimates of actual tissue values as bioconcentration is a dynamic process of uptake and depuration, particularly for a hydrophilic chemical such as 2,4-D.

A single acute human dose is calculated by the formula (USEPA, 1989):

$$\text{Dose (mg/kg)} = \frac{c_{fish}\ \text{(mg/kg)} * \text{kg fish consumed}}{\text{human body weight (kg)}}$$

Where:
Kg fish consumed = 0.4 kg
human body weight = 70 kg,

Chronic dietary intake is calculated using the following equation (USEPA, 1989):

$$\textit{Intake (mg/kg-day)} = \frac{CF \times IR \times FI \times EF \times ED}{BW \times AT}$$

Where:
CF = Contaminant concentration in fish (mg/kg)
IR = Intake Rate (0.4 kg/meal)
FI = Fraction Ingested (100%)
EF = Exposure Frequency (52 meals/year)
ED = Exposure Duration (30 years)
BW = Body Weight (70 kg)
AT = Averaging Time (30 years x 365 days/year = 10,950 days)

The fraction ingested describes the percent of a person's total fish intake which is derived from the site of interest. For this conservative analysis it was assumed that 100% of a person's fish diet is taken from a 2,4-D treated water body. A BCF of 5 was used to calculate 2,4-D concentrations in fish. All other values are standard USEPA default values (Region 10 guidance). The chronic intake values are shown in Table 12.

5.2.4 Incidental Ingestion of Sediment

- **Exposure Data**

  Incidental ingestion of sediment may occur during recreational activities such as swimming or wading. The intake equation includes different intake scenarios for children and adults to account for the likelihood that children will ingest more sediment than adults.

- **Estimated Dose**

  Chronic daily intake of incidental ingestion of sediment is estimated as follows (USEPA Region 10 Guidance, 1991):

$$Intake\ (mg/kg-day) = CS\ x\ CF_1\ x \left(\frac{\frac{IR_C\ x\ EF\ x\ ED_C}{BW_C} + \frac{IR_A\ x\ EF\ x\ ED_A}{BW_A}}{AT\ x\ CF_2} \right)$$

  Where:
  CS = Contaminant concentration in Sediment (mg/kg)
  CF_1= Conversion Factor (0.000001 kg/mg)
  CF_2 = Conversion Factor (365 days/year)
  IR_c = Intake Rate, child (200 mg/day)
  IR_A = Intake Rate, adult (100 mg/day)
  EF = Exposure Frequency (22 days/year)
  ED_c = Exposure Duration, child (6 years)
  ED_A = Exposure Duration, adult (24 years)
  BW_c = Body Weight, child (15 kg)
  BW_A = Body Weight, adult (70 kg)
  AT = Averaging Time (30 years x 365 days/year = 10,950 days)

  All exposure parameter values are standard default values from USEPA region 10 guidance with the exception of exposure frequency, taken from the statewide recreation survey. Chronic daily intake values are shown in Table 14.

TOXICITY ASSESSMENT

5.3 PHARMACOKINETICS OF 2,4-D

Results of 2,4-D pharmacokinetic investigations have demonstrated that the chemical is rapidly absorbed, distributed throughout the system, and excreted. Findings from rat metabolism studies indicate that approximately 86-94% of an oral dose was absorbed from the gastrointestinal tract and about 85-94% of the absorbed dose, excreted in the urine unchanged within 48 hours. 2,4-D does not appear to be metabolized and is primarily excreted by the kidneys. Although ingestion of 2,4-D is a minor route of exposure compared to skin contact, the chemical is more toxic through the oral route of exposure because of its rapid and near complete absorption from the gut. Although most of the pharmacokinetic or biotransformation findings concerning 2,4-D have been derived from oral dosing studies, the results can be applied to the doses absorbed following

dermal and inhalation routes of exposure. (WHO 1996, FAO 1996, Timchalk, et al 1990, Munro, et al, 1992; WHO, 1984).

2,4-D can also bind to plasma proteins, thus reducing its potential systemic toxicity. Bound 2,4-D is excreted along with the unbound molecule primarily in the urine. Ingestion of large amounts of 2,4-D, e.g. suicidal cases, could result in saturation of the plasma protein binding sites contributing to an increase of free unbound 2,4-D, thus affecting kidney function, decrease excretion and potentially result in systemic poisoning. (WHO 1996, FAO 1996).

Other investigations have similarly demonstrated that the salts and esters of 2,4-D dissociate and hydrolyze to 2,4-D. This similarity in metabolic fate explains why their toxicities are essentially similar to 2,4-D. It should be further noted that 2,4-D does not accumulate in the body tissues (WHO 1984, WHO 1996; US EPA 1996c).

5.3.1 Oral

Two comprehensive studies (Erne, 1966; Khanna and Fang, 1966) report that absorption, distribution, and elimination of 2,4-D is rapid and complete following oral administration. Khanna and Fang reported 94% to 99% of the 2,4-D dose is excreted unchanged primarily in the urine within 48 hours. The ester form is generally not as readily absorbed from the gut compared to the amine salt, probably due to its low water solubility (Erne, 1966).

Pelletier, et al (1990), conducted an investigation where rats were administered oral doses of 14-C labeled 2,4-D at 1.0 and 0.4 mg/kg and determined that approximately 95% of the dose was absorbed within 6 hours and excreted in the urine by 24 hours. Arnold, et al (1991) reported rapid gut absorption of 2,4-D amine salt in dogs dosed orally. The amine salt of 2,4-D was hydrolyzed to the 2,4-D acid in the stomach, rapidly absorbed and eliminated in the urine. This same finding has been demonstrated in rats dosed with 2,4-D ethyl hexyl ester (Frantz and Kropscott, 1993). A review of the excretion of 2,4-D through biliary excretion appears to be a minor route of elimination based on the toxicological investigations conducted by Griffin, et al (1997).

5.3.2 Dermal

Results of 2,4-D percutaneous absorption studies in man, laboratory animals and *in vitro* investigations indicate that the chemical has a low rate of dermal penetration. An early human study by Feldman and Maibach (1974) involved application of 14-C labeled 2,4-D acid in acetone to the forearms and found that approximately 6 percent of the dose was absorbed over a 5-day observation period (Webster and Maibach, 1985; Harris and Solomon, 1992).

A similar study conducted by Moody et al (1990, 1992) applied 14-C labeled 2,4-D amine in water or 2,4-D isooctyl ester in acetone to the foreheads of human volunteers and demonstrated that the dermal penetration rate over 7 days was 58% and 6%, respectively. The site of application was washed 24 hours post-treatment and urine samples collected at 4, 8, and 12 hours and daily for 7 days post-treatment.

Moody et al (1994) conducted a follow-up study where 14-C labeled 2,4-D amine in either acetone, water or the mosquito repellent N,N-diethyl-m-toluamide (DEET), was applied to the palm and forearm of human volunteers. Treated areas were covered, and washed following 24 hours of contact. Urine samples were collected for 5 days at 24-hour intervals. The results of the investigation revealed that the total cumulative percent absorption of 2,4-D was essentially

similar for the application site and three test solvents. The range of cumulative 2,4-D absorption over the 5-day test period was 7% -14%.

Moody and Nadeau (1994) conducted a 2,4-D *in vitro* human skin study and determined that the dermal penetration rate over 48 hours was approximately 20%. The investigators also found that 2,4-D absorbed through pig skin during a 48-hour *in vitro* permeability rate study was similar to the human finding, while rat skin under the same *in vitro* conditions, had a greater rate of penetration of approximately 47%.

Results of rat *in vivo* 2,4-D dermal absorption investigations have demonstrated that the penetration rates for 2,4-D and 2,4-dimethylammonium salts over 5 days was approximately 10% -15% (Knopp and Schiller, 1992), while Pelletier et al (1990), noted similar rates of dermal absorption in rats following shaving, clipping and washing the skin.

Wester, et al (1996), found that dermal contact with 2,4-D treated soil did not affect the 24-hour percutaneous absorption rate in human in vitro tests. The human *in vitro* results were 1.8±1.7%, 1.7±1.3% and 1.4±1.2% for exposure to 2,4-D soil loads of 5, 10 and 40 mg /cm2 skin, respectively. It appears that dermal absorption over time is not dose or skin contact time dependent for soil treated 2,4-D.

The above clinical and laboratory 2,4-D skin absorption investigations involved the test material being directly applied to the dermal site. This type of skin contact would result in a greater exposure and absorption of the chemical in comparison to the exposure situation of a person swimming in water recently treated with 2,4-D. Under laboratory testing conditions, the chemical remains for an established time period in direct contact with the skin, while a water environment involves significant dilution and diffusion of the chemical, thus reducing the degree of skin exposure and dose absorbed.

Other significant factors that contribute to reduced dermal exposure of 2,4-D would be the low label use rate of 2 parts per million for aquatic weed control and the binding of the applied chemical by aquatic particulate matter, vegetation and soil. In addition, based on the results of the human 2,4-D skin penetration study, only about 6% of the chemical that maintains sufficient contact with the skin may be absorbed over 24 hours. Therefore, any dose a person may receive from swimming or contacting water treated with 2,4-D according to label directions, would not be expected to absorb a toxic amount of the herbicide.

It appears that dermal absorption over time is not dose or skin contact time dependent for soil treated 2,4-D. The dermatokinetics of soil and skin represent a complex situation that makes risk assessments from this type of exposure difficult.

5.4 SYNERGISM WITH OTHER PESTICIDES

A review of the medical and scientific literature failed to provide any evidence that 2,4-D or any of its amine salts or esters have been associated with synergistic activity with other pesticides.

5.5 2,4- IMPURITIES

There are no known impurities present in sufficient quantities in US manufactured 2,4-D considered to be of toxicological concern. The main impurity that may exist at low concentrations is 2,4-dichlorophenol, one of the starting chemicals in the manufacturing process. There has been

concern that 2,4-D products contain low levels of chlorinated dioxins and furans. As discussed in Volume 3, Section 2, 2,4-D Chemical Characteristics of this document, findings from extensive analyses and investigation of the US manufactured 2,4-D has not demonstrated the presence of halogenated dioxins and furans that exceed the limits of quantitation (LOQs) expressed in the EPA Data Call-In Notice for dioxins and furans in 2,4-D products (EPA, 1987). The LOQs vary based on the specific congener, however the main dioxin of concern is 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) having a LOQ of 0.1 parts per billion (ppb). Depending upon the congener, quantitation limits may range upwards to 100 ppb based on toxicological equivalence to 2,3,7,8-TCDD (Hammond, 1999). Although results of 2,4-D batch analyses indicate that occasionally some dioxins and furans are detected, they remain below the LOQs and not considered of toxicological significance (EPA, 1987; Hammond, 1999).

5.6 ACUTE TOXICITY

Numerous laboratory animal acute toxicology studies have been conducted on the various forms of 2,4-D. Some LD50 results are summarized in Table 15 and Appendix II and others described in Table 16. However, as evidenced by the results of the acute toxicology testing of the chlorophenoxy herbicides, it appears that they have moderate to low degrees of oral systemic toxicity, while the routes of dermal and inhalation exposure result in signs of skin and mucosal irritation and questionable systemic toxicity (WHO, 1996; Kennepohl and Munro, 2000).

5.6.1 Oral Toxicity

Signs of toxicity demonstrated in laboratory animals administered an acute oral dose of 600 mg 2,4-D/kg included myotonia effects, e.g. muscular weakness (mainly of the hind quarter), decrease in spontaneous locomotor activity, ataxia, and central nervous system depression and gasping (Paulino et al, 1996). The investigators also noted that the rats displayed significant elevations of blood alanine aminotransferase, aspartate aminotransferase, alkaline phosphates, lactate dehydrogenase and creatinine; while the glucose and total protein were decreased. None of the hematology parameters were affected except for an elevation of packed cell volume. No gross or microscopic lesions were observed in any of the test animals. See Table 15 and Appendix II.

Beasley, et al (1991) detected electromyogram (EMG) subclinical manifestations of myotonia or a muscular rigidity in dogs dosed orally with 2,4-D at doses ranging from 8.8 to 86.7 mg/kg. However, no clinical signs of toxicity or myotonia were evident in any of the dogs in these dose groups. No EMG changes were found in dogs dosed at 1.3 mg/kg. Each dog served as its own control prior to dosing, so that comparative pre- and post-dosing EMG readings were recorded. Based on the study design, the authors were not able to conclusively determine whether the EMG needles or 2,4-D was responsible for the finding of muscle fibrillations. Nevertheless, the results of the investigation indicate that the EMG may have value in diagnosing chlorophenoxy overexposure.

In contrast to the demonstrated sensitivity of the EMG in detecting changes in 2,4-D dosed dogs, Arnold, et al (1991) found that at the same dose levels and species, the electroencephalograph (EEG) was less sensitive. EEG changes occurred only at 2,4-D dose levels of 175 and 220 mg/kg and only when clinical manifestations of myotonia were evident.

5.6.2 Dermal Toxicity

A review of the acute dermal 2,4-D toxicology studies where the dose levels are less than 2000 mg/kg indicates that no signs of systemic toxicity or deaths occurred, Table 15 and Appendix II.

Various chemical formulations have produced skin irritation depending upon the formulation tested (FAO, 1996).

The dermal LD50 of 2,4-D (unspecified formulations) was 1,500 mg/kg in rats and 1,400 mg/kg in rabbits (USDA, 1984). However, application of 3,980 mg/kg 2,4-D butyl ester or diethylamine salt, to shaved skin of rabbits produced no signs of toxicity or deaths.

A review of the rabbit dermal LD50 findings >2,000 mg/kg listed in Table 15 reveals that 2,4-D and its various forms present evidence of a low degree of systemic toxicity from the dermal route of exposure (WHO, 1996).

5.6.3 Dermal Irritation

Results of rabbit 4-hour skin irritation studies indicate that the 2,4-D amine and esters produced mild to no evidence of irritation (Keller et al, 1977; Myer, 1981; Carreon et al, 1983; Jeffrey, 1987; Mizell et al, 1989; Schults et al, 1990b; Berdasco, 1992).

5.6.4 Dermal Sensitization

The sensitization potential of 2,4-D acid, amine salts and esters have been assessed by the Buehler method in guinea pigs and found to produce no evidence of delayed contact hypersensitivity (Gargus, 1986; Jeffrey, 1986; Jeffrey and Rao, 1986; Schultz et al, 1990e), while positive sensitization findings have been claimed by other investigators (USEPA, 1988).

5.6.5 Inhalation Toxicity

Acute inhalation overexposure to 2,4-D in animal studies have demonstrated signs of respiratory tract irritation, e.g. salivation, lacrimation, mucoid nasal discharge, labored breathing, dried red or brown material around the eyes and nose. None of the signs persisted beyond 3-7 days post exposure, nor were there any deaths (FAO, 1996). No signs of systemic toxicity following 2,4-D exposure have been reported.

5.6.6 Eye Irritation

The 2,4-D amine salts and esters have demonstrated severe to moderate eye irritation in rabbit eye irritation studies. The concentrated (undiluted) test material is placed in the conjuctival sac of the eye and not removed for the duration of the 21-day observation period. The effects on the eye are recorded at various time periods throughout the study. Results indicate that the chlorophenoxy chemicals cause inflammation of the conjunctiva, ocular discharge, inflammation of the iris and in some cases corneal opacity (Keller et al, 1977; Kirsh, 1983; Carreon etal, 1983; Carreon and Rao, 1986, Jeffrey, 1987; Berdasco and Mizell, 1989; Schults et al, 1990a).

5.7 SUBCHRONIC TOXICITY

Repeated daily, seasonal or intermittent chemical exposure commonly occurs in the course of our lives, e.g. the work environment, application or spraying of a chemical or through dietary intake of a treated food crop or water. Most human chemical exposures are either acute (one time exposure) or subchronic (exposure to a chemical for several days or weeks). The potential for subchronic exposure to 2,4-D might occur when the chemical is used for aquatic herbicidal weed control. Such exposures would primarily involve dermal contact with the chemical through swimming or contacting recently treated bodies of water, ingesting the water or sediment, or

dermal contact with treated sediments and aquatic weeds. Inhalation exposure to 2,4-D in aquatic herbicidal use situations basically applies to applicators that may inhale the spray mist or dust. Application of 2,4-D or any other pesticide product, in compliance with label directions, is not expected to result in adverse health effects.

The subchronic toxicity of 2,4-D has been studied in numerous animal toxicology investigations and is summarized in Tables 16 and 17. It is important to understand that most subchronic toxicology studies involve groups of animals receiving different doses during the course of the study. The studies are designed so that groups consist of a control (no exposure to the chemical) and test groups receiving a low, mid and high dose of the chemical. Theoretically, the low dose demonstrates no adverse effects or only minimal signs, the mid dose receives a dose that will cause slight observable signs of toxicity and measurable effects while the high dose group will have more severe adverse effects. The results of subchronic studies consist of observed signs of illness, blood chemistry and hematology analyses and gross and histopathological findings. Based on the results, the target organs(s) associated with the toxicity of the chemical can be identified and a no observable adverse effect level (NOAEL) dose can be calculated for the chemical or product. The results are used for determining the degree of toxicity of the chemical, risk assessments, establishment of acceptable exposure levels, dietary and drinking water standards, label precautionary statements and other sources of health information.

The systemic toxicological findings from the 2,4-D subchronic studies are consistent with the various forms of the chemical and among different species tested. It appears that when the threshold of saturation for renal clearance is exceeded there is an affect on the kidney and thyroid gland. A review of the histopathological findings reveal changes in the epithelial cells of the kidney proximal tubule brush border and thyroid follicular cells along with a reduction in serum thyroxin. The changes in the kidney and thyroid gland are consistent for all forms of 2,4-D. A review of the rat subchronic test results in Tables 16 and 17 and Appendix II demonstrate a consistent NOAEL of 15 mg/kg/dy for 2,4-D acid, amine salts and esters (Kennepohl and Munro, 2000; Charles 1996: Paulino 1996).

Charles et al (1996), also conducted a 13-week dog subchronic 2,4-D dietary feeding study and found that the kidney and liver were the target organs in the 3.75 and 7.5 mg/kg/dy dose groups. The animals demonstrated signs of toxicity of decreased body weight gain and food consumption. Clinical chemistry findings included minor elevations in blood urea nitrogen, creatinine and alanine aminotransferase. The NOAEL for the study was 1.0 mg/kg/dy. There was no indication of any immunotoxic or oncogenic response.

Rawlins, et al (1998), dosed female sheep with three forms of 2,4-D at 10 mg/kg/dy 3 times/wk for 7 weeks and found no subchronic effects on the metabolic and reproductive endocrine systems. Their findings were based on the absence of any signs of toxicity or changes in thyroxine, cortisol, insulin, estradiol, lactic dehydrogenase or oviductal intra-epithelial cysts.

A review of the subchronic dermal 2,4D rabbit investigations indicated that when the technical chemical (96.1% pure) was applied to the skin of rabbits no signs or clinical findings of systemic toxicity were observed. The only adverse effect noted was mild skin irritation. The systemic NOEL was the highest dose tested of 1000 mg/kg/dy (Schultze, 1990b). Similar findings were determined when the subchronic dermal toxicity of 2,4-D the butoxyethylhexyl ester and triisopropanolamine were tested in rabbits (Mizell, 1990a,b).

Other rabbit subchronic dermal investigations demonstrated that some of the 2,4-D salts and esters tested produced moderate skin irritation, but no evidence of systemic involvement was

observed during the 21-day test period (Schultze, 1990c,d; Mizell, 1990c). An investigation involving application of 2,4-D diethanolamine at dose levels of 150 and 440 mg/kg/dy to the skin of rabbits for 21-days, resulted in systemic effects as evidenced by elevated liver enzymes, hepatocytes containing hyaline droplets and hepatocyte hypertrophy. Severe skin irritation was also noted in the high dose animals, while 150 mg/kg/dy was the NOEL for skin irritation (Siglin, 1991).

5.7.1 Neurotoxicity

- **Animal Neurotoxicology Investigations**

  As discussed previously, results of some 2,4-D subchronic toxicology studies have revealed changes in neurological function that may be related to a myotonia condition, e.g. hunched posture, ataxia, slight transient, gait and coordination changes, decreased motor activity, altered forelimb grip (Jeffries et al, 1994; Elo and MacDonald, 1989; Arnold et al, 1991; Beasley et al, 1991). It appears that findings of adverse neurological function occur at dose levels that overwhelm the renal mechanism for excreting 2,4-D from the blood. No histopathological lesions of the nervous system have been observed in any of the subchronic or chronic animal investigations (Charles et al, 1996a; Yano et at, 1991 a,b; Szabo and Rachunek, 1991; Schulze, 1991).

  The myotonia demonstrated when animals have received high doses of chlorophenoxy herbicides may be due to effects mediated at the junction of skeletal muscle nerves and muscle tissue, however the biochemical mechanism is not well understood (Buslovich and Pichugin, 1983).

  Mattsson and Eisenbrant (1990) reviewed the literature and found no substantiated reports or scientific evidence that the chlorophenoxy herbicides have produced peripheral or polyneuropathy in humans or animal species tested.

  Several investigators have noted changes in neurobehavioral parameters when conducting toxicology Functional Observational Battery (FOB) testing following acute and chronic exposure to 2,4-D. The FOB tests are designed to measure motor activity and compare the results with the neurohistopathology. Some FOB investigations have revealed decreased activity levels, behavioral and motor skill abnormalities in rats and rabbits when the animals were administered 2,4-D doses greater than 60 and 30 mg/kg/dy, respectively (Rodwell, 1991; Martin, 1991; Breslin et al, 1991; Liberacki et al, 1991; Zablotney et al, 1991; Mattsson et al, 1991; Oliveira-Neto, 1993; Jeffries et al, 1994, Duffard et al, 1995).

  Mattsson, et al (1997) conducted a 2,4-D rat acute and one-year chronic neurological investigation using the FOB motor activity parameters and neurohistopathology to evaluate any potential neurotoxic effects. Based on the results of the studies, the investigators found that the acute and chronic NOAELs were 15 mg/kg and 75 mg/kg/dy, respectively. The lowest effect dose found in the acute study was 75 mg/kg and involved a transient slight change in gait response in one female animal. No FOB changes were observed in chronic studies at 75 mg/kg/dy. The authors noted that rats receiving 2,4-D at 150 mg/kg/dy demonstrated slight increases in forelimb grip strength and retinal degeneration changes in females. Except for the retinal degeneration findings, there were no treatment-related pathological observed in the brain, spinal cord or peripheral nervous systems.

- **Human Neurological Case Reports:**

  There are reports in the early scientific and medical literature claiming that exposure to chlorophenoxy herbicides resulted in the development of a variety of neurological disorders. The publications describe conditions diagnosed as peripheral neuropathy characterized by numbness, aching of arms and legs, muscle spasms, denervation of muscles and decreased nerve conduction velocity. Some authors noted reversible, partial reversibility or incomplete recovery following their patients' alleged exposure to chlorophenoxy compounds (Goldstein et al, 1959; Monarca and DiVito, 1961, Todd, 1962; Berkely and Magee, 1963).

  The cases were reviewed by Mattsson and Eisenbrandt (1990) and the authors concluded that the anecdotal reports were not consistent with known findings from 2,4-D toxicology studies, epidemiology investigations or human experience in using the herbicides. In addition, the reports of neurological disorders associated with use of the herbicides failed to indicate whether the patients were questioned about the specific chemicals used, other chemicals involved, extent of exposure, pre-existing medical conditions, alcohol or medication used. Also, the patients had other signs and symptoms, e.g. fever, respiratory illness and GI disturbances associated with their peripheral neuropathies. These early claims of peripheral neuropathy seem to be unique to the late 50's and early 60's in that the literature appears absent of any other reports of chlorophenoxy-associated peripheral neuropathy disorders. The neurotoxicity of 2,4-D acid, amine salts and esters have been extensively studied during the past 15 years by means of animal toxicology studies and numerous epidemiological investigations, and found to be negative. Initial reports of neuropathy have not been corroborated by the numerous animal toxicology and epidemiology investigations of the past 15 years.

  Mattsson and Eisenbrandt also reviewed the work of Singer et al, 1982, who claimed that the measurements of both motor and sensory nerve conduction velocities of 2,4-D and 2,4,5-T manufacturing workers were significantly slower than compared with nonexposed control subjects. Singer found that there appeared to be a correlation between the duration of employment and the slowing of the sural nerve conduction velocity. The overall findings revealed that approximately 46% of the study group (56 workers) and 5% of the controls (25 subjects) had some type of nerve conduction slowing.

  Mattsson and Eisenbrandt also stated that the Singer study made no mention of the magnitude or quantitation of the decreased conduction velocity, whether the conduction velocities were in the range of normal, no estimation of the degree and duration of chlorophenoxy exposure, type of work done in the manufacturing plant or whether any of the employees had been incapacitated because the alleged decrease in their neurological conduction velocities. Also, there was no explanation or discussion of the control group as to whether they were matched to the test group subjects according to occupation, age, physical size, clothing, shoe type, exposure to chemicals, social habits or medical history. In addition, findings from the Singer investigation are not supported by the data and information provided by 2,4-D toxicology or epidemiology studies or findings regarding the use history of the chemical.

  The National Academy of Sciences, Institute of Medicine, conducted a neurological health evaluation of Vietnam veterans exposed to herbicides and found no association between herbicide exposure and clinical measured neurological disorders. The investigators measured cognitive and neuropsychiatric effects, motor/coordination dysfunction of the central and peripheral nervous systems (Goetz, 1994).

In summary, the neurological effects associated with overexposure to chlorphenoxy herbicides appear to be related to doses that exceed the threshold for normal renal clearance of the chemicals. No evidence of pathological lesions have been observed in either the central or peripheral nervous systems in humans or animals dosed with 2,4-D acid, amine salts or esters.

5.7.2 Immunotoxicity

Results of recent subchronic and chronic toxicology studies have not provided any evidence that 2,4-D is immunotoxic. The investigations examined hematological, clinical chemistry and histopathological evaluations and found no evidence that the chlorophenoxy herbicides induce immune system dysfunction (Charles et al, 1996 a,b,c). Investigations by Blakley (1997 and 1998), indicate that 2,4-D may have demonstrated some potential immunosuppressive effects when tested in mice, using a sheep red blood cell immunoassay technique. The lowest dose of 110 ppm 2,4-D tested during the 26 day exposure period, demonstrated a positive immunosuppressant response. In comparison, Table 8 lists the highest water concentration of 2,4-D following application was 2.3 ppm detected in an irrigation ditch. The amount of 2,4-D listed in Table 4 is nearly 50 times less than the amount tested in the mouse immunotoxicity study (Blakley, 1997). Therefore, based on the degree of exposure a swimmer would receive from contacting 2,4-D treated water, it seems unlikely that a sufficient amount of the chemical could be absorbed into the system to affect the immune system. Assuming the chemical can affect the immune system, then the chance of overexposure appears minimal when 2,4-D is used according label directions for aquatic weed control (since it is applied only 1-2 times/year), undergoes degradation and absorption into vegetation, does not bioconcentrate and any of the chemical that becomes absorbed into the human system is rapidly excreted in the urine.

A review of the subchronic and chronic 2,4-D toxicology studies presented in Volume 2, Section 5, Human Health Effects of this document, demonstrate that when the chemical is administered to test animals at high dose levels, histopathological changes occur in many organ systems including those involved in immunological function, e.g. bone marrow, lymphatic system, spleen and adrenal, thyroid and thymus glands. Repeated daily overexposure to 2,4-D primarily results in histopathological lesions of the kidneys and liver. Once the kidney function has been compromised, then 2,4-D cannot be effectively excreted and the combination of decreased urinary excretion and increasing chemical exposure results in the decline of all body functions. Thus, the findings in the animal toxicology subchronic and chronic studies demonstrate that overexposure to 2,4-D at high dose levels results in initial histopathological changes to the liver and kidneys followed by adverse affects to the other organ systems. Any 2,4-D affects on the immune system would result indirectly from the high dose of the chemical causing renal failure and liver changes.

Earlier investigations have suggested that chlorophenoxy herbicides may be associated with immunosuppression possibly mediated by their dioxin contaminants (Zahm and Vineis, 1988). Blakely (1986a, 1986b) conducted studies with 2,4-D n-butylester on female mice to determine immunosuppression effects and concluded that the chemical was unlikely to have immunotoxicological significance. Although TCDD has demonstrated immunosuppressive findings in some animal studies (Vos et al, 1980), the dioxin has not been found to cause such an effect in highly exposed humans (Evans et al, 1987; Reggiani, 1980). Further, TCDD is not considered to be a contaminant in the manufacture of 2,4-D. Currently, there is no conclusive evidence to associate exposure to chlorophenoxy herbicides with immune suppression and subsequent possible carcinogenic development.

5.7.3 Human Case Reports and Studies

The phenoxy herbicides have demonstrated a low degree of toxicity both in humans and animals (Ahrens, 1994; Burnside, 1993, Munro, 1992). A review of the scientific and medical literature failed to provide any human case reports of systemic toxicity or poisoning following overexposure when using chlorophenoxy herbicide products according to label directions. Aside from cases involving accidental and suicidal ingestion of massive amounts of 2,4-D containing products (Durakovic, 1992), there are numerous anecdotal reports to poison control centers and the Washington State Pesticide Incident Reporting and Tracking database concerning oral, dermal, inhalation and eye exposures to the chemical. A review of these cases reveal minor exposure associated with signs and symptoms of skin, eye and/or respiratory tract irritation. Cases involving possible swallowing of spray mist may result in irritation of the digestive tract as evidenced by nausea, vomiting or diarrhea. There are some claims of allergic reactions following contact with chlorophenoxy herbicides, but no substantiated cases of systemic poisoning from labeled use of the products. Claims of neurological effects e.g. peripheral neuropathy, reduced nerve conduction, carcinogenicity, have not been supported by findings from the animal studies and remain controversial based on a review of epidemiological investigations (Burnside, 1993; Munro, et al 1992).

A review of the 2,4-D animal toxicology investigations indicate that at high doses, the kidneys' ability to effectively excrete the chemical decreases to a threshold where systemic toxicity occurs. The high dose effects observed in the toxicology studies may not be relevant to the typical low dose human exposures resulting from label use of the phenoxy chemical products as discussed in Section 5.2.4, Exposure Parameters and Assessment.

The following anecdotal human case reports describe possible associations of 2,4-D exposure with neurological effects that have not been supported by findings from EPA guideline toxicology studies, quality epidemiological investigations or conclusions from chlorophenoxy toxicology scientific review panels (Patty, 1998; Kennepohl, 2000; Munro, 1992; US EPA, 1996; WHO, 1996).

5.8 CHRONIC TOXICITY

The non-carcinogenic chronic toxicity of 2,4-D has been studied in rats and dogs (Jeffries et al, 1995 and Dalgard, 1993). The results of both investigations indicated that the liver and kidneys are the target organs when 2,4-D is administered in the diets at high dose levels. As previously discussed, 2,4-D is rapidly absorbed from the gut into the blood and excreted primarily in the urine as the parent compound within 48-hours. None of the chlorophenoxy herbicides are accumulated in any tissues (Munro et al, 1992; WHO, 1996; FAO, 1996).

Jeffries et al, (1995), conducted a rat 2,4-D chronic feeding study and found that the animals in high dose male group administered 150 mg/kg/dy demonstrated decreases in body weight, body weight gain and food consumption; increases in liver enzymes, decreases in T4; increases in absolute and relative thyroid weights and increased incidences of histopathological lesions in the eyes, liver, lungs, and mesenteric fat. The NOAEL for male rats was 75 mg/kg/dy. Similar clinical and histological findings were observed in high dose female rats at 75 mg/kg/dy. The NOAEL for female rats was 5 mg/kg/dy (Jeffries et al, 1995; Charles et al, 1996c).

The results of the one-year 2,4-D dog study also demonstrated that the liver and kidneys were the target organs. Both the male and female animals dosed at 5 and 7.5 mg/kg/dy, displayed reductions in body weight gain and food consumption along with minor increases in blood urea

nitrogen, creatinine and alanine aminotransferase. The alterations in serum chemistry were corroborated by the histopathological findings observed by inflammation of the liver and increased pigment in the kidney tubular epithelium. The NOAEL for the dog study was 1 mg/kg/dy (Dalgard, 1993; Charles et al, 1996a). See Tables 16, 19, 20 and Appendix II.

In summary, the Agency determined that the NOELs for the chronic studies were 5 mg/kg/dy for female rats and 75 mg/kg/dy for male rats, and 5 mg/kg/dy for both sexes of mice.

5.9 DEVELOPMENTAL AND REPRODUCTIVE TOXICITY

5.9.1 Animal Toxicology Developmental and Reproductive Studies:

The results of the 2,4-D developmental or teratology and multigenerational reproduction studies indicate that the chemical is not considered to be a teratogen or reproductive hazard when administered below maternally toxic doses. It appears that when 2,4-D exceeds maternally toxic doses it saturates the threshold of renal clearance resulting in signs of systemic toxicity. 2,4-D doses above the renal threshold level demonstrate some developmental effects, e.g. decreased fetal weight gain, increased incidence of lumbar ribs and wavy ribs and delayed ossification (Kennepohl and Munro, 2000). See Tables 18, 20 and Appendix II.

Toxicology tests to evaluate the reproductive and developmental toxicity potential of chemicals are typically conducted with mice, rats or rabbits. Multigenerational studies are designed to provide data on maternal and paternal reproductive capabilities, offspring defects, development and growth.

A review of the 2,4-D acid, amine salts and ester developmental and reproduction studies is presented in Table 18 (Kennepohl and Munro, 2000). The teratogenic potential of several salts and esters of 2,4-D have been studied in rats and rabbits and support the findings of the 2,4-D acid investigations that no adverse developmental findings occurred below maternal toxic doses. These findings also complement the results of 2,4-D pharmacokinetic studies that demonstrated a rapid and complete conversion of the 2,4-D salts and esters to the acid form. Results of the 2,4-D salt and ester developmental studies are consistent with the acid derivative findings when the doses are converted to acid equivalent-doses (Lochry, 1990; Schroeder, 1990a: Schroeder, 1990b; Martin, 1992a and 1992b; Martin, 1991; Breslin, 1991; Liberacki, 1991; Zablothy, 1991).

Rodwell (1985) conducted a 2-generation reproductive toxicity study on 2,4-D in Fischer 344 rats at dose levels of 0,5,20, and 80 mg/kg/dy. The high dose group animals demonstrated severe toxicity in the F1 generation and were deleted from the study. Significant maternal toxicity was observed in decreased body weight in the 20 mg/kg/dy animals. No adverse fertility effects in the 2,4-D treated animals were observed. Based on the results of the investigation the 2,4-D reproduction toxicity NOEL in the rat was 5 mg/kg/dy for both maternal toxicity and reproductive toxicity.

A review of the histopathological sections of various 2,4-D subchronic and chronic studies provides further support that the chemical does not affect the reproductive organs, except in some of the high dose groups. Findings from the rat subchronic feeding study demonstrated that rats fed 300 mg/kg/dy demonstrated a slight decrease in the mean testes/body weight ratio and a histological finding of slight testicular atrophy. The NOAEL for subchronic testicular effects was 100 mg/kg/dy. Other than at the high dose levels observed in the diethanolamine salt of 2,4-D rat subchronic investigation by Serrone et al, (1991), no ovarian or uteran effects have been reported to be associated with exposure to the chemical (Charles et al, 1996a,b; Dalgard 193a,b,c,d;

Gorzinski, et al, 1981; Hansen et al, 1971; Jeffries et al, 1995; Schultze, 1990 and 1991a,b,c,d; Serota, 1983a,b, 1986, 1987; Szabo and Rachunek, 1991; Yano et al, 1991a,b). In addition, findings from the 2,4-D chronic rat study demonstrated that the NOAEL for testicular effects was 75 mg/kg/dy (Charles et al, 1996c).

Results of the dog chronic and subchronic studies involving the 2,4-D acid, amine and ester revealed that decreased testes weights were demonstrated at 13 weeks at the 3.75 and 7.5 mg/kg/dy dose levels. No corroborative histopathological changes were noted or any evidence of decreased body weight gains. No effects on the ovaries were demonstrated. A follow-up at 1 year in the chronic study did not reveal any decrease in testicular weights or ovarian effects (Charles, 1996a). These findings support the overall assessment of 2,4-D not being associated with adverse reproductive performance parameters.

5.9.2 Human Reproductive Case Reports

A study conducted by Lerda and Rizzi (1991) involving 32 male farm workers (sprayers) who claimed exposure to 2,4-D, reported the finding of adverse reproductive effects, e.g. asthenospermia, necrospermia and teratospermia. The workers were monitored and found that the asthenospermi and necrospermia diminished, but the latter effect persisted. A review of this study indicates that there was no information regarding the subjects' levels of exposure to 2,4-D, use of other chemicals, medical condition or history, and use of alcohol or medications. There was also no information on comparison of study and control groups to determine if they were matched by age or other factors. Therefore, no conclusions regarding the male reproductive effects demonstrated by the subjects in this study can be associated with their claimed exposure to 2,4-D.

Contrary to Lerda and Rizzi's report, a study by Wolfe (1995) of male Vietnam War "Operation Ranch Hand" male military defoliant workers, exposed to Agent Orange (50:50 ratio of 2,4-D and 2,4,5-T), revealed no significant decreases in reproductive effects measured in terms of the associated number of spontaneous abortions, still births or birth defects.

Based on the current 2,4-D toxicology and epidemiological findings and the weight of the evidence, it does not appear that exposure to the chemical has been associated with adverse male reproductive effects (Rodwell, 1985; Wolf et al, 1995).

5.10 MUTAGENIC EFFECTS

A review of the *in vitro* and *in vivo* 2,4-D mutagenicity testing indicates that the chemical is not considered to be a mutagen. The EPA SAB panel (USEPA, 1994) concluded, following their review of the mutagenicity studies, that "the currently available evidence suggests that 2,4-D is nongenotoxic." The EPA RfD/Peer Review panel (USEPA, 1996) also concluded that neither 2,4-D, amino salts nor esters were bacterial mutagens and not active in mammalian cell DNA repair assays (Lawlor, 1994; Cifone, 1990; Ivett, 1990). Although there were conflicting results in the *in vitro* mammalian cell cytogenetic assays, the EPA RfD/Peer Review panel concluded that "2,4-D does not pose a mutagenic hazard and there is no concern for mutagenicity at this time."

5.11 CARCINOGENICITY REVIEW

The carcinogenic potential of 2,4-D has been extensively studied via recent rat, mouse and dog chronic toxicity investigations (Serota, 1986; Jeffries, et al, 1995; Charles, et al, 1996a,b,c). Findings from the studies found no evidence of carcinogenicity. The negative findings in the

chronic studies are supported by the negative mutagenicity results evidenced in both 2,4-D *in vivo* and *in vitro* testing (Munro, 1992, Charles et al, 1999a,b). A WHO review of the mutagenesis studies also concluded that 2,4-D and its salts were not genotoxic (WHO, 1996; FAO, 1996). Based on the toxicology test results, the weight-of-the-evidence indicates that 2,4-D is not considered to be a carcinogenic in laboratory animals (Table 19 and Appendix II).

Results of the Serota (1986) 2,4-D rat chronic toxicology test indicated that the high dose (45 mg/kg/dy) male rats had an increased incidence of brain tumors or astrocytomas. Although the finding of astrocytomas had not occurred in previous chronic 2,4-D studies (Innes et al, 1969; Hansen et al, 1971; Arkhipov and Kozlova, 1974), another rat chronic investigation was conducted with the high dose being 150 mg/kg/dy. The results of the latest 2,4-D rat chronic study did not demonstrate any evidence of astrocytomas (Jeffries et al, 1995; Charles et al, 1999c).

The 1987 Serota 2,4-D 18 month mouse oncogenicity study also demonstrated no evidence of carcinogenicity. (Serota, 1987; Charles; 1999c). The Serota chronic mouse study did not provide any evidence of increased tumor incidence even at the high dose of 45 mg/kg/dy. The EPA, in their review of the study, was of the opinion that the maximum tolerated dose had not been achieved and requested that the study be conducted at a higher dose level. The second mouse oncogenicity study included 2,4-D doses of 125 and 300 mg/kg/dy in the male and female mice, respectively. There was no evidence of carcinogenicity in the animals at these dose levels (Charles, 1999c).

Results of the 1-year 2,4-D dog chronic feeding study were also negative with regard to any evidence of carcinogenicity. The dose levels were 0, 1, 5, and 7.5 mg/kg/dy. There were only minor histological findings of alterations in the kidneys and liver similar to those observed in the dog subchronic investigation. The NOAEL was 1.0 mg/kg/dy (Charles, 1999a).

In addition, findings from the epidemiology investigations of workers involved in use of 2,4-D in agricultural, pesticide control, military defoliation operations and manufacturing facilities have been controversial. The association of exposure to 2,4-D and the development of non-Hodgkins lymphoma or other oncogenic findings has not been definitively demonstrated by these studies.

Several recent scientific review panels have analyzed the extensive number of toxicology and epidemiology studies and have concluded that the weight-of-the evidence at this time does not support 2,4-D being a carcinogen (Harvard, 1990; Munro et al, 1992; USEPA, 1994; USEPA, 1997). The EPA Carcinogenicity Peer Review Committee in 1997 concluded that 2,4-D should remain in EPA Carcinogen Class D. A Class D carcinogen rating indicates that there is insufficient evidence of carcinogenicity to place the compound in any of the three higher classes of being a known, probable or possible human carcinogen (EPA CPRC, 1997). In addition, the National Toxicology Program did not include 2,4-D or any of the chlorophenoxy herbicides in its 1998 report listing of: 1) known human carcinogens or; 2) chemicals reasonable anticipated to be human carcinogens (NTP, 1998).

The weight of evidence categories defined by EPA are presented below (USEPA, 1986):

Group A - Human Carcinogen
Group B- Probable Human Carcinogen
Group B1- At least limited evidence of carcinogenicity to humans
Group B2- A combination of sufficient evidence in animals and inadequate evidence in humans

Group C- Possible Human Carcinogen (limited evidence of carcinogenicity in animals in the absence of human data)
Group D- Not classified as to human carcinogenicity
Group E- Evidence of noncarcinogenicity in humans (no evidence in at least two adequate animal tests in different species or in both epidemiological and animal studies)

In summary, based on the recent findings of both the subchronic and chronic rodent studies and the mutagenicity testing reviews, EPA has concluded that "2,4-D acid was not carcinogenic in male or female rats or mice" (US EPA CPRC, 1997). Therefore, 2,4-D has been placed in EPA Carcinogen Group D – insufficient evidence to classify as a human carcinogen.

5.12 EPIDEMIOLOGY REVIEW

Numerous chlorophenoxy herbicide epidemiological studies have been conducted presenting conflicting results and conclusions regarding an association between 2,4-D exposure and the development of non-Hodgkin's lymphoma (NHL) and/or soft tissue sarcoma (STS). However, a review of the literature specifically concerning exposure to 2,4-D appears to indicate that if there is an association, it is with NHL than STS.

As discussed in Appendix I, epidemiology studies are difficult to conduct and interpretation of the results is often controversial primarily because humans lead variable lives and life styles. Laboratory animal toxicology studies on the other hand must be conducted by specific protocols and closely monitored throughout the "in life" portion and during the histopathology review. Epidemiologists are confronted with many design problems in gathering information, e.g. identification of the specific groups or classes of pesticides which the subjects were allegedly exposed; extent and duration of exposure; type of exposure; mixed chemical exposure; lack of investigation of other potential causative factors; medical history of each subject; correct diagnosis or cause of death; small number of subjects; authors reliance on information provided by secondary or tertiary sources; and recall bias. Therefore, the epidemiologist is not always able to have a complete or unbiased quality database that ultimately determines the strength of the investigation and the value of the risk assessment. Appendix 1 discusses the types of epidemiological investigations, sources of error in gathering information and how the results of the studies are expressed.

In summary, epidemiology studies are designed to measure potential human exposures to specific chemicals and through statistical analyses determine any association with a disease condition. Since humans lead different lives and life styles, it is difficult to maintain consistency of the variables in study and control groups. Therefore, at the end of the study the epidemiologist may have to deal with incomplete data and information in order to perform calculations and arrive at a conclusion as to whether the findings support an association between the chemical exposure and the health condition being measured. In the case of 2,4-D many epidemiological studies have been conducted with mixed results and conclusions. Nevertheless, the weight of the evidence from the investigations indicates that the chemical does not appear to be a carcinogen, but the various scientific review groups continue to recommend that further studies be conducted. (Harvard, 1990; Munro, 1992; US EPA, 1994; WHO, 1996).

The following sections discuss some of the 2,4-D case control and cohort epidemiological studies. As mentioned, there are many such investigations and the most significant are presented followed by a review and discussion of the positive and negative aspects of the findings and investigators conclusions.

5.12.1 Case Control Studies

Several studies have been sponsored by the National Cancer Institute (NCI) to determine if there was a link between herbicide use and the development of Hodgkin's disease (HD), soft-tissue sarcoma (STS) and non-Hodgkin's lymphoma (NHL). Cantor et al (1991) conducted a study to determine any association of pesticide exposure and NHL in farmers from Iowa and Minnesota. He used personal interviews in 62 cases and 1,245 controls. Cantor and coworkers found significantly elevated risks of NHL for use of certain insecticides, however no significant increased incidents of NHL was associated with use of 2,4-D (OR1.2, 95% CI 0.9-1.6). There

was no quantification of exposure in the study. The authors also found no increase in the incident rate of NHL with latency or failure to use protective equipment.

A population-based case-control study was conducted among Kansas farmers (Hoar et al, 1986) concerning use of all herbicides. The study information gathering consisted of 10-minute telephone interviews of the patients or next-of-kin. The interviewers never inquired about the frequency of 2,4-D use. The subjects were asked about the frequency of herbicide use. It was assumed that 2,4-D and 2,4,5-T were the most extensively used herbicide compared to other weed control chemicals. Only histologically confirmed cases of NHL were included in this analysis. Herbicide exposure was further defined by type of herbicide, years of use, days of exposure per year and use of protective equipment. Types and amounts of pesticides used by some of the subjects were corroborated by checking records of their pesticide suppliers. Smoking, caffeine intake, non-farming pesticide use and concurrent immunosuppressive disease did not affect the risk.

The authors of the Kansas study reported that farm herbicide use was associated with NHL (OR 1.6, 95% CI 0.9-2.6). Relative risk of NHL increased significantly with number of days of herbicide exposure per year and latency. Farm workers (men only surveyed) exposed to herbicides more than 20 days per year had a NHL OR of 6.0, 95% CI 1.9-19.5. Farmers who began using herbicides prior to 1946 had an OR of 2.2 while those who did not use protective equipment had a higher odds ratio (OR 2.1) than for those who did (OR 1.5). Assuming that 2,4-D was the herbicide primarily used, the investigators claimed an association with NHL. Controlling for concurrent insecticide use did not affect the OR for herbicide use. Neither STS nor HD was associated with herbicide exposure.

The results of the Hoar et al (1986) investigation were difficult to interpret, because the information on exposure was derived from 10 minute telephone interviews with subjects or their next-of-kin. There is reasonable doubt whether spouses or relatives would have a detailed knowledge of the subject's daily herbicidal use or be able to recall such practices over 15-20 years later. In addition, no data were collected specifically on the frequency or duration of 2,4-D use, thus is seems improbable to estimate directly an association between the amount of 2,4-D exposure and the incidence of NHL (Olsen and Bodner, 1996; WHO, 1996).

The results of the Kansas study prompted the authors to undertake a similar population-based case-control study in Nebraska (Zahm et al, 1990). The study specifically evaluated the potential role of 2,4-D in causing NHL among people residing in 66 counties in Nebraska. The study involved 201 white men diagnosed with histologically confirmed NHL between July 1, 1983 and June 30, 1986, and 725 controls. The study design also consisted of telephone interviews of direct informants (farmers) and next-of-kin, or "proxy" respondents.

There was a non-significant 50% excess of NHL among men who mixed or applied 2,4-D (OR 1.5, 95% CI 0.9-2.5). Among those exposed to 2,4-D for 20 or more days per year the risk of NHL was increased (OR 3.3, 95% CI 0.5-22.1). Although the individual OR was not significant, the trend of higher ORs with increasing days of exposure was significant ($p = 0.051$). Risk also increased with degree of exposure, as measured by time spent in contaminated clothing and application method, but not with number of years of use. The lack of association between risk and number of years of use is also consistent with the Kansas study. Risk increased substantially among those men who usually waited to change into clean clothes after handling or using pesticides. Farmers who changed immediately, at the end of the workday, or who wore the clothes for more than one day had ORs of 1.1, 1.5 and 4.7 respectively (p for trend = 0.15). Risk was unaffected by the use or lack of use of personal protective equipment.

The Nebraska study also investigated the histology, tumor grade, degree of maturation and immunologic type of NHL associated with 2,4-D exposure. Exposure to 2,4-D did not appear to be specific to any subgroup of NHL. Adjusting for use of organophosphate insecticides decreased the risk of 2,4-D while adjusting for fungicide use increased the risk. The authors concluded, based on this study and their previous Kansas study (Hoar-Zahm et al, 1986), that the use of 2,4-D in an agricultural setting increases the risk of NHL among persons who frequently handle 2,4-D.

The Nebraska Study was similar in design to the Kansas epidemiology investigation where exposure information was derived from interviews with subjects or their next-of-kin. A review of the case control epidemiology studies concerning 2,4-D indicate the potential significance of the type of subject that provides information regarding pesticide exposure. Subject bias can affect the results of the investigation because of difficulty in recall by subjects or inaccurate information given by a proxy informant. Olsen and Bodner (1996), conducted a respondent reassessment of the Nebraska (Zahm, 1990) and the Iowa (Cantor, 1992) epidemiology studies involving 2,4-D and NHL. They found that when comparing data provided by direct informants vs. proxy sources regarding use of 2,4-D in the studies, that for the highest frequency of use, 10 dys/yr, the odds ratio for an association with NHL was 2.5 (95% CI 0.8-8.0) and 0.7 (95% CI 0.3-1.9) for proxy-derived and direct informants, respectively. The authors concluded that the type of respondent can significantly affect the association of cancer and pesticide exposure.

Hardell and Sandstrom (1979) conducted a case control study of agriculture and farm workers who claimed exposure to chlorophenoxy herbicides. The investigators found an OR of 5.3 for the incidence of STS. The findings were somewhat consistent with those of Hardell (1981) and Eriksson et al (1981). However, the study investigators noted that the effects of the individual chemicals could not be evaluated since nearly all of the exposed subjects were also exposed to some herbicides contaminated with dioxins including 2,3,7,8-TCDD (WHO, 1996).

Two studies were conducted in New Zealand by Smith et al (1984) and Pearce et al (1986) concerning the use of chlorophenoxy herbicides and the incidence of STS and NHL. The Smith study failed to show any statistical significant association between use of chlorophenoxy herbicides and STS. Similar results were obtained by Pearce et al where they were unable to find an association between subjects spraying primarily 2,4,5-T and NHL. The only non-significant association between the parameters were found in workers using the chemicals 10-19 dys/yr (OR 2.2, 95% CI 0.4-13), however the risk was significant in subjects exposed >20 dys/yr.

Vineis et al (1987), studied the incident rate of STS among men and women and use of chlorophenoxy herbicides in northern Italy. The chlorophenoxy herbicides that the workers were exposed to included 2,4-D, MCPA and 2,4,5-T. No excess risk of cancer associated with exposure to the herbicides was found in the male subjects. However, the relative risk among living women was 2.7. When the comparison group was restricted to women alive at the time of interview, less than 75 years of age and exposed to chlorophenoxy herbicides during the 1950-1955 time period, the age adjusted OR was 15.5. The women had been employed as rice weeders beginning in 1950 and were exposed to the herbicides being used experimentally to control weeds. When only those living women who had regular jobs in agriculture were considered the age adjusted OR was 3. The study was limited by lack of information to quantitate the amount and frequency of exposure. Also, the authors could not determine what effect specific chemicals had on the study findings. Use of some chlorophenoxy herbicides would have had trace contamination of dioxins, e.g. 2,3,7,8-TCDD, a chemical suspected of being a carcinogen (WHO, 1996).

A case control study conducted by Woods et al. 1987, in Washington State, investigated the possible association of occupational use of chlorophenoxy herbicides and the incidence rate of STS or NHL. The study involved 128 cases of STS, 575 patients with NHL and 694 controls. All data were obtained by personal interviews as opposed to information obtained by telephone or next-of-kin contacts. There was no excess risk of NHL or STS for subjects with past occupational exposure to phenoxy herbicides. However, there was an elevated risk of NHL among several subgroups. Men who had been farmers had a relative risk of 1.33 (95%, CI 1.03-1.7), while forestry herbicide applicators who applied 2,4-D, 2,4,5-T and other commercial chemicals had a relative risk of 4.8 (95% CI 1.2-19.4). Those potentially exposed to phenoxy herbicides in any occupation for 15 years or more during the period prior to 15 years before their cancer diagnosis, had a relative risk of 1.71. Workers who reported using 2,4-D specifically had an insignificant OR of 0.73 even at high exposure levels. An increased risk of both STS and NHL was observed among those who had previous occurrence of chloracne, a chemically induced skin rash, indicative of potential dioxin 2,3,7,5-TCDD exposure, a contaminant and suspected carcinogen found in 2,4,5-T (WHO, 1996).

A 1990 study by Brown et al focused on farmers in Iowa and Minnesota who had used herbicides and /or insecticides. Analyses showed a small but significant risk for all leukemias (OR = 1.2, 95% CI 1.0-1.5) among persons who lived or worked on a farm as an adult. Significantly elevated risks were also seen for chronic lymphocytic leukemia among farmers (OR = 1.4, 95% CI 1.1-1.9) compared to non-farmers. The authors then analyzed leukemia occurrence among farmers who reported using different classes of herbicides, e.g. 2,4-D and 2,4,5-T. There were no significantly elevated increases in leukemia among the chlorophenoxy group (OR = 1.2, 95% CI 0.9-1.6). There were non-significant excesses for specific leukemia cell types but no evidence of a dose-response effect. Interestingly, there was a significantly increase risk for leukemia (OR = 1.9, 95% CI 1.3-2.9) among farmers who reported no exposure to pesticides. This finding was seen only among the Iowa group.

Wolfe et al (1990) conducted a 20-year case control comprehensive health assessment of Vietnam Air Force veterans who were involved in the mixing, loading and spraying of Agent Orange during Operation Ranch Hand. Agent Orange was a herbicide that contained a 50:50 mixture of 2,4-D and 2,4,5-T along with a contaminant dioxin 2,3,7,8-TCDD. The contaminant was created in the manufacturing process of 2,4,5-T and not in the method for making 2,4-D. The dioxin 2,3,7,8-TCDD is considered to be a carcinogen and the reason that 2,4,5-T is no longer registered by the EPA or sold as a herbicide.

Wolfe et al, adjusted the “Operation Ranch Hand” population into study groups for age, rank and occupation. It was found that no increase in deaths based on the case standardized mortality ratio of 1.0. In addition, there were no significant differences determined regarding accidental, malignant neoplasms and circulatory deaths.

Kogevinas, et al (1995) conducted two nested case-control studies involving chemical exposure to production workers and spray applicators exposed to phenoxy herbicides, chlorphenols and dioxins. The results of the investigation indicated that there was an excess of soft tissue sarcomas among applicators, but not in process workers. Also, there was a weak association with non-Hodgkin’s lymphoma (NHL) associated with the two groups. The authors conclude that the dioxin TCDD was a major contaminant in some of the chemicals and correlated with increased cases of soft tissue sarcoma. The results of the study did not implicate that exposure to 2,4-D was associated with increases in either soft tissue sarcoma or NHL.

Wolfe et al, (1995) conducted a case control study and determined that paternal exposure to Agent Orange during the Vietnam war did not result in any significant increase in spontaneous abortions or stillbirths, birth defects, delays in development or hyperkinetic syndrome.

Ha et al, (1996) investigated the claim of increased gestational trophoblastic disease in Vietnamese women that were allegedly exposed to the spray or residues of Agent Orange used during the Vietnam war. A case control study was conducted in Ho Chi Minh City and the results of the investigation revealed no significant differences between cases and controls. A case control study was also negative for the disease among those exposed to pesticides in agricultural use.

Garry et al (1996) conducted a case control epidemiology linkage study where information was gathered strictly from Minnesota State Registries. The investigators compiled data for the time period of 1989-1992 concerning pounds of herbicide active ingredients (included 2,4-D) applied in each county, birth registry recorded birth anomalies and the number and sex of private state-licensed pesticide appliers. Pounds of herbicide used were obtained, but not for insecticides, fungicides or other types of pesticides. The objective of the study was to determine whether there was an association between pounds of "chlorophenoxy herbicides and/or fungicides" used and the incidence of birth anomalies among appliers vs. the general unexposed population. None of the registry-listed subjects were questioned or interviewed concerning their potential exposure to pesticides or work and medical histories.

The authors divided the State into high and low chlorophenoxy/fungicide use regions. They reported that in all regions there was a significant increase in the number of birth anomalies found in the chlorophenoxy/fungicide applier group. The birth anomalies included circulatory/respiratory, urogenital and musculoskeletal/integumental defects.

Since nearly all of the appliers were male, the authors hypothesized that their alleged chlorophenoxy/fungicide exposure produced chromosomal damage to germ cell resulting in higher birth defects for their offspring. A fundamental principle of reproductive epidemiology is to determine whether anomalies are homogeneous and relevant to the exposure or dysmorphically non-specific, thus questioning the etiological potential of the chemical(s). In the Garry study, a male genotoxic effect was theorized to have occurred months prior to conception which would exclude fetal malformations due to *in utero* exposure or maternal factors such as intrauterine malposition or personal factors, e.g. smoking, drug and alcohol consumption. It is interesting that the different number of birth anomalies span all of the study geographical regions investigated. Further, the areas having a low number of applier group anomalies associated with male genotoxic affects, in the corn/soybean region (26.8 anomolies/1000 live births) and the forest/urban region (23.7/1000) were less than the general nonexposed population in the high chlorophenoxy /fungicide use wheat growing region (26.9/1000). It seems that if the pesticides truly have a genotoxic effect, then the highest rates of anomalies would be among appliers regardless of the region.

Another study weakness appears to be the misclassification of the applier exposure status. There are a number of situations where mistakes in the registry classification could occur, e.g. the father being certified months or years after the time of conception, the father did not apply pesticides even if he was certified, protective equipment and clothing was worn while applying pesticides, birth certificate incorrectly listed the biological father as a pesticide applier and father's name mistakenly placed on the certification list.

Conversely, an exposed child could have been misclassified as unexposed under a number of circumstances. Such situations of misclassification may include: maternal exposure (1% of the

appliers were women) to a pesticide at the time of conception or during early pregnancy and the husband not a certified applier, the father was certified in 1989 and 1990 but not at the time of conception in 1991, neither father nor mother were certified appliers but exposed to pesticides by someone else or they used consumer pesticide products and a mistake made in not entering the father in the certification database.

Based on the study methodology, the substantial margin for error reduces the credibility of the findings. This appears to be a major weakness of epidemiology linkage investigations.

5.12.2 Animal Case-Control Study

A National Cancer Institute study (NCI) (Hayes et al, 1991) reported the results of a case-control study involving dogs diagnosed as having canine malignant lymphoma (CML) (the canine equivalent of NHL) associated with owner use of 2,4-D. The owners were asked by questionnaire or phone interview about use of chemicals in the home, use of lawn chemicals and opportunity for the dog to be exposed to the chemicals. The investigators determined a weak OR of 1.3 (95% CI 1.04-1.67) for dogs having access to yards where the owners had potentially applied 2,4-D at a high frequency/year. The NCI study also proported that the study demonstrated a dose response relationship between increased use of 2,4-D and the increase in CML.

The Hayes canine epidemiology study was reviewed by Carlo (1992), who concluded that the study had definite limitations and did not support an association between use of 2,4-D and canine malignant lymphoma. The major weakness of the investigation was lack of precise data as to the specific chemical and extent of the dogs exposure. In addition, Carlo was of the opinion that the study also suffered from bias or confounders, e.g. recall, use of 2,4-D with other chemicals, other herbicides or pesticides and use of different methods to solicit information from owners. Based on their review, they concluded that the dog's potential 2,4-D exposure was not associated with canine malignant lymphoma.

The US EPA SAP 1994, also reviewed the NCI study and concluded that the investigation needed to be repeated emphasizing some quantitation as to the degree of exposure to specific chemicals in order to substantiate their results and conclusions.

Kaneene & Miller (1999), reviewed copies of the original study data obtained through the Freedom of Information Act, and concluded that they could not confirm a dose-response relationship between the number of 2,4-D applications and CML nor could they find a statistically significant association between CML and 2,4-D use.

Further rebuttal to the Hayes canine epidemiology study are the results of the 2,4-D one-year dog feeding study. Charles et al (1996a) demonstrated that daily dietary intake of 2,4-D at the highest study dose of 7.5 mg/kg/dy/1yr, did not result in any oncogenic finding. The non-carcinogenic NOEL for the 2,4-D dog chronic study was 1 mg/kg/dy/1 yr. Similar findings were also demonstrated following administration of the 2,4-D amine and ester in the dogs' diet for 1 year (Charles et al, 1996a).

5.12.3 Cohort Studies

As in the case-control epidemiology studies, very few study groups are known to have been exposed exclusively to 2,4-D. It is commonly found that exposures to chlorophenoxy herbicides involved 2,4-D and 2,4,5-T (contains the dioxin 2,3,7,8-TCDD contaminant), thus it cannot be

determined what agent or other factor may be responsible for the disorder being studied. Therefore, the cohort epidemiology investigations provide more specific information regarding chemical exposure data since they primarily involve chlorophenoxy manufacturing plant workers.

A study conducted in Denmark (Lynge, 1985), reviewed 3,844 workers in one manufacturing plant and 615 in another that had been employed from 1947 and 1951, respectively, and followed until December 31, 1982. Exposure was largely to 2,4-D, although a small amount of 2,4,5-T was manufactured in the larger plant between 1951 and 1959. This study showed an excess of soft tissue sarcomas, the only cohort study to do so. When only those cases in which the latency periods exceeded 10 years were examined there were 4 observed with 1.09 expected. When the same analysis was completed for malignant lymphomas a non-significant increase was observed (4 observed, 3.04 expected). However, it can not be concluded that either of these increases were due to 2,4-D, as workers were also exposed to 2,4,5-T.

Bond et al. (1988) studied all causes of mortality in a cohort study of 878 Dow Chemical employees who were potentially exposed to 2,4-D between 1945 and 1983. The employees were potentially exposed in any of four separate buildings in which 2,4-D was manufactured, esterified, ammoniated, formulated, or packaged. The study was designed to determine whether cancer or other causes of death had occurred excessively and in relation to exposure. Since a subject may have cancer but die from another condition, a study of cancer mortality would tend to underestimate the risk of developing cancer. Exposures were estimated using historical plant 2,4-D air monitoring data and employee work histories. A total of 111 deaths were identified among the 878 cohort members. This study employed a standardized mortality ratio (SMR) which was calculated as the ratio of observed to expected deaths multiplied by 100.

Bond determined that there were no significant increases for all causes of death. There was a non-significant increase in mortality (SMR=115) from malignant neoplasms. Non-significant excesses were noted for cases of cancer of the large intestine (SMR=212, 95% CI 57-544) and lymphatic and hematopoietic cancer (SMR=202, 95% CI 65-472). An analysis of mortality was also completed allowing for a latency period of 15 years, thus eliminating from the analysis of all persons exposed after 1967. There was no significant effect on the mortality patterns described above. When the analysis was limited to the 2,4-D production area, the SMR for lymphopoietic cancer was 312, which was statistically significant. There was no apparent association between duration of exposure and cumulative dose demonstrating 2,4-D exposure and cause of death. Every other cohort study reported to date has been negative. A small cohort study of Swedish railroad workers exposed to herbicides at least 40 days per year revealed no soft tissue sarcomas or non-Hodgkin's lymphoma in the group (Axelson and Sundell, 1974; Axelson et al., 1980). However, an excess of stomach cancer (2 cases vs. 0.33 expected) was noted among those workers exposed to phenoxy herbicides or phenoxy herbicides plus amitrole. The small number of workers (348) involved in the study renders it lacking in power to clearly indicate the role of herbicides in these cancers.

In Finland 1,971 male herbicide applicators exposed to 2,4-D and 2,4,5-T at least two weeks per year from 1955 to 1971 were followed until 1980. There were no excess cancers, STS, or NHL reported (Riihimaki, et al., 1982). A Canadian study (Green, 1986) focused on Hydro plant workers exposed to phenoxy herbicides, and reported that no cases of STS or NHL were identified.

The results of a very large study in Sweden were published in 1986 by Wiklund and Holm. A cohort of 354,620 men born between 1891 and 1940, identified as agricultural or forestry workers, were studied. A reference cohort of nearly 2 million men having other occupations was

also followed, from 1961 through 1979. The cohort was divided into subgroups based on occupation and presumed herbicide exposure. Although large numbers of soft tissue sarcomas occurred, there was no significant excess in any of the subgroups. Relative risks ranged from 0.9 to 1.0.

A 1991 study by Coggon et al. studied four British cohorts of chemical manufacturers comprising a total of 2,239 men employed during 1963-85. The subjects were traced from December 31, 1987 to 1990. Exposures were to phenoxyherbicides and related chlorophenols, and dioxins. Levels of exposure to specific chemicals could not be estimated. Non-significant increases in lung cancer were observed for all cohorts, as was an overall increase in death from all causes. There were no deaths from Hodgkin's disease or soft tissue sarcoma. A non-significant increase in deaths due to non-Hodgkin's lymphoma was reported.

Saracci, et al, (1991), conducted a mortality cohort study on 18,910 production and spray workers claiming exposure to 2,4-D, 2,4-DB, MCPA, MCPB, 2,4,5-T and related compounds. Exposure to various chemicals was obtained by questionnaires, work records and job histories. No significant differences were found between the chlorophenoxy herbicide workers and the cause-specific national death rates for all-cause mortality, neoplasms, common epithelial cancers or lymphomas. The investigators did note an increase in the excess rate of soft tissue sarcomas among spray workers that included increased cancer of the testicle, thyroid gland other endocrine glands and nose and nasal cavities. The authors conclude that the excess carcinomas were likely due to the TCDD contaminant in some of the herbicides. 2,4-D was not specifically implicated as being associated with an increased incidence of either soft tissue sarcoma or non-Hodgkin's lymphoma.

Tamburro (1992) conducted a retrospective cohort study of army veterans self-reporting exposure to Agent Orange during their service in Vietnam during 1962-1971. Since dioxin (TCDD) exposure has been associated with chloracne, the cohort was divided into two groups; 1) those who experienced rashes and 2) those who did not develop rashes during their service in Vietnam. Following an extensive medical examination, it was found that the rash group had a higher frequency of liver disorders than the non-rash group, 31% vs. 18%, respectively. Abnormal liver function correlated with the herbicide exposure index in both groups, but was more prominent in the rash group. The authors concluded that their review of the data strongly supported the evidence that chronic liver abnormalities among Vietnam veterans in their test groups, claiming Agent Orange exposure, were mainly due to viral or alcohol causality and not to herbicides and TCDD.

Fleming et al (1999a) conducted a data linkage retrospective cohort epidemiological study of pesticide applicators in Florida. The investigators utilized eight State and private databases to collect information in utilizing a standardized incidence ratio (SIR) analysis to determine the cancer incidence rate in Florida pesticide applicators compared to the general unexposed population. Data was obtained from the registries for the years 1975-1993 that included a total of 33,658: licensed male (30,155) and female (3,503) pesticide applicators.

The linkage or registry data collection design of the study did not involve any interviews or questionnaires completed by subjects from either of the two populations. Exposure to pesticides was based on a subject being a licensed applicator and the years of service employed in the specific occupation. There was no determination as to degree or extent of exposure nor identification of any pesticide applied.

The results of the investigation revealed that the overall cancer incident rate was consistently and significantly less than the general population (SRI = 0.76; 95% CI, 0.71-0.82 and 0.34; 95% CI, 0.20-0.54, for years 1975-1979 and 1980-1994, respectively). The authors also found that the applicator cancer incident rate was significantly decreased even in the subpopulations, gender, license type and groups using alcohol and tobacco.

It was also determined that there was no significant increase in the incidence of soft tissue sarcoma or non-Hodgkins lymphoma in any of the subpopulations. In fact, there were no confirmed cases of soft tissue sarcoma in the entire cohort.

The only significant increase found was the incidence of prostate and testicular cancer (SIR = 1.97; 95% CI 1.76-2.20 and 2.37; 95% CI 1.33-3.91). It was interesting that the incidence of prostate cancer was increased inversely with fewer years being licensed as a pesticide applicator. The design and parameters of the study do not permit what chemicals, medical histories or other factors may have been associated with the findings of an increased incidence in prostate cancer.

Fleming et al (1999b) also used the same data and conducted a standardized mortality ratio (SMR) analysis to compare the death rate in the two study groups. The results were similar to the SRI study, only the testicular cancer death rate was significantly increased (SMR = 2.38; 95% CI 1.82-3.04). No other significant increases in cancer mortality were found. Conversely, there were significant decreases in the death rate incidence of malignant neoplasms, mouth/pharynx cancer and other lymphatic cancer.

5.12.4 Reviews of Epidemiology Studies

To date, three scientific review papers have been published in which epidemiology data was critically re-evaluated. Bond et al (1989) reviewed all available cohort and case-control studies published up to 1987. The authors created graphs of the probability densities for the odds ratios from the eight case-control studies of STS, HD or NHL. The results demonstrate gross inconsistencies which are not attributable to chance. The combined results of the cohort studies of workers exposed to phenoxy herbicides provide little or no evidence of carcinogenicity and do not support a conclusion that phenoxyherbicides present a carcinogenic hazard to humans.

A 1990 paper by Johnson et al reviewed epidemiology studies found in the literature between 1979 and 1987 which focused on the association between phenoxy herbicides and chlorophenols and STS. Cohort studies and case control studies were considered separately. The authors concluded that the case-control studies, with the exception of the earliest reports, do not support a chemical-disease association. The cohort studies give inconclusive results, confirming the 1989 review by Bond et al.

A scientific panel, funded by the Industry Task Force II on 2,4-D Research, was convened at the Harvard School of Public Health to review the toxicology and epidemiological findings concerning 2,4-D. It was the consensus of the panel that the weight-of-the-evidence did not provide strong support for predicting that 2,4-D is a human carcinogen. Eleven of the 13 panelists felt it was “possible” that 2,4-D can cause cancer in humans, while 2 were of the opinion that it was unlikely. The panel also concluded that there is little epidemiological evidence for an association between 2,4-D use and STS or HD. However, they stated that the epidemiological evidence for an association between 2,4-D and NHL is suggestive and requires further investigation (Ibrahim et al, 1991).

Another comprehensive review of the 2,4-D literature, sponsored by the Industry Task Force II on 2,4-D Research Data (Munro, 1992), concluded that the weight-of-the-evidence was weak regarding an association between exposure to 2,4-D and the risk of cancer. The task force was of the opinion that the structure of 2,4-D does not suggest carcinogenicity and that the lack of animal evidence made it unlikely that 2,4-D was a human carcinogen. It was also stated that the rigorous EPA standards and product label changes would help reduce exposure to 2,4-D, thus making its public health impact "negligible."

Potential exposure to 2,4-D has been reduced by EPA regulations that are directed at use of the herbicide. EPA requires that the number of 2,4-D applications be limited to two per year. In addition, applicators must wear complete protective clothing, gloves and eye protection while spraying a 2,4-D product. Following spray application of 2,4-D, the label recommends washing skin surfaces immediately after contact, and keeping and washing contaminated clothing separately from other laundry.

5.12.5 Epidemiology Discussion

The literature and number of toxicology and epidemiology studies concerning 2,4-D are extensive. There has been a concern that exposure to 2,4-D may be associated with non-Hodgkin's lymphoma, Hodgkin's disease and soft tissue sarcoma. Munro, 1992, conducted a critical review of over 90 epidemiology studies relating to 2,4-D, other chlorophenoxy herbicides and other herbicidal agents, and determined that the evidence associating 2,4-D with cancer is weak. Other review groups following examination of 2,4-D toxicology and epidemiology data and information have concluded similar findings (Harvard, 1990; USEPA SAB, 1994; Kennepohl and Munro, 2000).

A review of the epidemiology studies from various parts of the world reveals that not only was 2,4-D used in various types of herbicide control, but other chlorophenoxy compounds, some possibly contaminated with the dioxin (2,3,7,8-TCDD, a known potent carcinogen) and other chemicals were applied, thus confounding the question of whether 2,4-D exposure has the etiologically potential to be a carcinogen. Further factors for consideration are the type of occupations being studied, extent of 2,4-D exposure, type of spraying, methodology in conducting exposure assessments and consistency of the dose response relationship result in inconclusive evidence of an association of 2,4-D and cancer (Harvard, 1990; Ibrahim, 1991; EPA SAB, 1994).

The strongest association of 2,4-D with NHL appears to be the findings of increased incidences of the disorder in agricultural workers (Hoar et al, 1986; Bond, 1988; Zahm et al, 1990). However, a review of the case control studies does not provide strong support for the hypothesis of 2,4-D carcinogenicity because of the weaknesses in the study methodologies, information as to specific chemical exposure, extent of exposure, use of other chemicals, virus etiology and immune system modulation (Olsen et at, 1996). Other case control studies provide conflicting findings that no association with 2,4-D or other chlorophenoxy herbicide exposure and cancer was determined (Wolf et al, 1990; Goetz, 1994; Kogevinas et al, 1995).

Recent epidemiological investigations by Fleming et al, 1997; Beacher et al, 1996 and Zahm 1997 involving factory workers and lawn care workers (Zahm, 1997), demonstrated inconsistent findings and provided no conclusive evidence of an increased cancer risk associated with exposure to 2,4-D. The Fleming study involved 33,669 pesticide applicators and found no cases of soft tissue sarcoma or non-Hodgkin's lymphoma.

Aquavella et al (1998) conducted a meta-analyses of 37 epidemiology studies of farm workers that had presented a history of occupational exposure to chlorophenoxy herbicides. A review of the data and information gathered in the studies led the author to conclude that agricultural workers did not have significant increased cancer rates except for the finding of an increased incidence of lip cancer.

In summary, 2,4-D has been used extensively throughout the world for the past 50 years as both an agricultural and consumer herbicide. Based on the wide use of the chemical, 2,4-D has been one of the most toxicologically and epidemiologically studied herbicides. An overview of the results of the numerous 2,4-D investigations, particularly by many scientific review panels, has not provided conclusive evidence that 2,4-D is a carcinogen or human health risk.

5.13 RISK ANALYSIS

Based on the results of the hundreds of 2,4-D toxicology epidemiology and environmental fate studies conducted during the last 50 years, there does not appear to be any expected adverse health effects from its labeled use as an aquatic herbicide.

Table 20 lists the lowest dose no observable effect levels (NOELs) for 2,4-D as determined in the animal toxicology studies. The table includes systemic effects, reproduction and teratology toxicological endpoints. The NOEL is the highest dose administered to a group of laboratory animals, either in a subchronic or chronic study, that does not produce any signs of toxicity, changes in hematology, blood chemistry, urinalysis, or evidence of tissue injury observed in the gross and histopathological examinations.

The lowest NOEL dose for 2,4-D was 1.0 mg/kg/dy for systemic toxicity determined from the results of a dog 90-day subchronic feeding study (Charles et al, 1996a). The NOELs for the 2-generation reproduction and teratology studies were higher as indicated by dose levels of 5 and 25 mg/kg/dy (Rodwell, 1985; USEPA, 1996 and Rodwell, 1983).

Utilizing the lowest 2,4-D NOEL of 1.0 mg/kg/dy, a risk calculation can be determined between aquatic herbicide use and potential exposure to treated water. Table 4 lists initial water concentrations of two 2,4-D products in small lakes, irrigation canals and large lakes. Since 2,4-D BEE is the primary chlorophenoxy herbicide product used in the State of Washington for aquatic weed control, selection of the highest water concentration of 2.3 mg 2,4-D/Liter of treated canal water was used to calculate a health risk assessment based on the 1.0 mg/kg/dy NOEL.

Based on the analytical findings in Table 4, the worst case 2,4-D exposure scenario would be ingestion of the irrigation canal water. Further, assume that all of the 2,4-D ingested in the water is absorbed into the system. The first question is how much water containing 2.3 mg 2,4-D/Liter of treated water must be swallowed to equal the NOEL dose of 1.0 mg 2,4-D/Kg body weight/day? One ml of the treated canal water contains 0.0023 mg of 2,4-D. In order to obtain 1.0 mg of 2,4-D it would require approximately 435 mls of treated water. Therefore, the next question is the amount of treated water various size people would have to drink in order to obtain the 1.0 mg/kg/dy toxicology study NOEL. A review of the table below indicates that a 22 (10 kg) and 154 (70 kg) pound person would have to drink 1 and 8 gallons of treated canal water, respectively each day in order to receive a 2,4-D dose of 1.0 mg/kg/dy. Assuming that the person was swimming while in the process of drinking the large volumes of water, how much additional 2,4-D would be absorbed through the skin? Answer, very little since it has been demonstrated that the human dermal absorption of 2,4-D is approximately 6% over 24 hours. Thus, the skin

exposure contribution would be insignificant to the daily 2,4-D dose (Webster and Maibach, 1985; Harris and Solomon, 1992; Moody, 1992).

2,4-D Exposure to Treated Canal Water Dose and Health Risk Assessment

Weight	**Canal Water Swallowed (mls equivalent to 1 mg 2,4-D)**		**Qts***	**Gals***
#s	**Kg**			
2.2	1	435	0.45	-
22	10	4,350	4.5	1.0
44	20	8,700	9.0	2.25
88	40	17,400	18	4.5
132	60	26,100	27	6.75
154	70	30,450	32	8.0

*number of quarts of canal water containing 0.0023 mg 2,4-D to equal NOEL dose of 1.0 mg/kg/dy
**number of gallons of canal water containing 0.0023 mg 2,4-D to equal NOEL dose of 1.0 mg/kg/dy

The above exposure scenario would also be affected by the following 2,4-D pharmacokinetic factors: First: the chemical, once absorbed into the blood, is quickly excreted into the urine by the kidneys. Second: since the daily water dose of 2,4-D would be equivalent to the NOEL, no adverse toxic effects to the kidneys or other body organ systems would be expected. Third: 2,4-D does not bioaccumulate, so the daily dose of 1.0 mg/kg/dy could be ingested and a cumulative body burden of the chemical would not build up in the system to result in the development of renal damage and decreased 2,4-D excretion.

In summary, no adverse health effects are expected from the labeled use of chlorophenoxy herbicides for aquatic weed control. As discussed above in the health and risk assessment, extraordinary and heroic daily exposures would have to occur in order to obtain a NOEL dose of 2,4-D. Such degrees of exposure would not be expected in the real world of aquatic weed control.

5.14 APPROACH FOR DETERMINING RISKS

The potential risk of non-carcinogenic effects is usually evaluated by comparing an environmental dose to a reference, or "safe" dose. Under the reference dose (RfD) approach uncertainty factors are added to the lowest NOEL dose reported in animal studies, as listed in Appendix IV. An uncertainty factor of 10 is generally used to estimate a safe human exposure level from experimental studies when there is no indication of carcinogenicity and valid human studies are available. A more conservative uncertainty factor of 100 is supplied when there are few or no valid human studies available but there are valid long-term animal studies.

Thus, the RfD represents a lifetime "safe" dose for protection against threshold (non-carcinogenic) health effects. The EPA published RfD for 2,4-D is derived from a study in which liver, kidney, and blood disorders were produced in dogs dosed orally. The oral RfD is converted to a dermal RfD when evaluating dermal exposure according to USEPA guidance (USEPA, 1996).

In the RfD method, hazard quotients are calculated for each exposure pathway by dividing the chronic daily intake by the RfD. Hazard quotients are then summed to obtain a hazard index. A hazard quotient of "1" indicates that the chronic daily intake is the same as the RfD (the level of exposure below which adverse health effects are unlikely to occur for even sensitive populations).

Thus, the greater the value of the chronic daily intake/RfD ratio, the greater the level of concern. Hazard quotients should not be interpreted as statistical probabilities. A hazard quotient of 0.001 does not mean that there is a one in one thousand chance of an effect occurring.

A similar method, the Margin of Safety (MOS) approach was used to evaluate acute exposures. In this approach NOELs from animal toxicity studies for specific toxic effects, such as reproduction, systemic, or teratogenic effects are compared to estimated human doses. This method allows the risk assessor to use a variety of "safe" doses specific to each human route of exposure. The RfD approach was not used for single acute exposures as the RfD is designed to be protective of long term chronic exposures. The MOSs computed in this risk assessment are direct comparisons of NOELs and LELs from animal studies to estimated doses. Thus, the lower the MOSs, the greater the risk of toxic effects occurring (indirect contact to RfD approach). For example, an MOS of 1,000 means the laboratory determined "safe" dose is 1,000 higher than the estimated human dose. The standard margin of safety is 100 (Shipp et al., 1986)). A margin of safety greater than 100 is considered to represent negligible risk, and margin of safety less than 100 is considered to represent a risk of toxic effects. MOSs are meant to be general indicators of potential risk.

NOELs and LELs used to calculate margins of safety are taken from three animal studies. Based on the 2,4-D mammalian toxicology database, an excellent review of available mammalian toxicity, three toxic endpoints were selected to estimate risk: systemic toxicity, reproductive effects and teratogenic effects. Table 20 summarizes the studies and resulting NOELs and LELs.

5.15 PROJECTED NONCARCINOGENIC RISKS

5.15.l Acute exposures

Noncarcinogenic risks for each acute exposure pathway are summarized in Tables 21 through 24. MOSs for every pathway and scenario except dermal contact with vegetation are greater than 100 ranging up to over 1×10^{11} for all pathways, indicating that there is essentially no risk of these effects occurring. The lowest MOS (167) occurred in the high dose, dermal contact with vegetation scenario. If interpreted in light of the fact that the standard default USEPA safety factor for extrapolation from laboratory animal "safe" doses to human "safe" doses is 100, the MOS is acceptable.

Margins of safety are also consistently lower (i.e. higher risk) for all pathways in scenario 2: irrigation ditch, due to the high sediment and water concentrations predicted. All MOSs rapidly increase with increasing days after application, and should be interpreted in light of the uncertainties discussed in Appendix I..

5.15.2 Chronic exposures

Noncarcinogenic effects for chronic exposures are summarized in Table 25. Hazard quotients range from 3 x 10-01 to 8x 10-10 for all exposure pathways and scenarios. Hazard quotients less than “1” indicate that the chronic daily intake is lower than the USEPA reference or “safe” dose (RfD). The cumulative hazard index, in which hazard quotients are summed across pathways are all well below "I" for the three environmental exposure scenarios. The highest cumulative hazard index is 3.5 x 10-01, for amine formulation, irrigation ditch scenario.

Among the hazard quotients (chronic daily intake/RfD for each exposure pathway) the largest value was calculated for the drinking water exposure scenario, amine formulation, irrigation ditch scenario. This value was 3.09 x 10-01 and is still considered to be low (i.e., "safe") in light of the fact that the hazard quotient is "1" when the chronic daily intake and RfD are equal.

5.16 UNCERTAINTY ANALYSIS

Uncertainty is inherently introduced at a number of steps in the risk assessment process. Generally, sources of uncertainty include variability in exposure input parameters, contaminant transport modeling, toxicological evaluation of contaminants, and analytical data. To compensate for such uncertainties, risk assessments are commonly conducted by incorporating conservative assumptions and input parameters favoring the protection of public health. This same approach has been incorporated here. Although a rigorous quantitative evaluation of these uncertainties is beyond the scope of this assessment, it is important to consider, at least qualitatively, the effect various assumptions used throughout this analysis are likely to have on the final risk estimates.

Assumptions are a necessary and innate part of risk assessment, and each assumption usually has a technically correct alternative. This risk assessment required that a greater than usual number of assumptions be made regarding 2,4-D exposure scenarios, as no one particular site was designated to be evaluated. A discussion of this and other sources of uncertainty are discussed below.

5.17 ENVIRONMENTAL CONCENTRATIONS

Uncertainty in risk predictions was introduced during the calculation of expected environmental 2,4-D concentrations. 2,4-D concentrations in environmental media were calculated assuming particular application rates, depth of water, and type of sediment. Any variation of these parameters affect 2,4-D application, environmental concentrations and resulting risk assessments.

Sediment and water concentrations were calculated using published application and degradation rates. Application rates are expected to vary with site, method, and 2,4-D formulation. Thus, the calculated environmental concentrations and resulting risks represent values around which variation is expected to occur.

5.18 EXPOSURE SCENARIOS AND ASSUMPTIONS

The inhabitants or visitors to a specific geographical area are generally designated as the exposed population in a risk assessment. For example, residents living near a contaminated site, recreational users of a specific body of water, or people living within the zone of deposition of an incinerator all represent specific groups. As 2,4-D is proposed for use across the entire state of Washington, it was impossible to evaluate human health risks from exposures that take place at one specific site.

Thus, uncertainty is introduced through the use of the necessary "generic" exposure scenarios. These generic scenarios represent the most likely routes of public exposure and are not intended to be site-specific. Thus, they may underestimate risks if, for example, people live near a lake particularly well suited for swimming and are thus exposed for longer periods of time than are built into the swimming exposure scenario. Likewise, risks may be overestimated for a lake in which conditions (i.e., cold temperature) cause people to spend very little time swimming. However, margins of safety are so large for this pathway that any variation in exposure parameters is unlikely to influence them to any great extent.

For the ingestion of fish pathway, it is likely that the MOSs are slightly underestimated (i.e., risk is overestimated) due to the use of a BCF to calculate fish tissue concentration of 2,4-D. The bioconcentration of 2,4-D by fish is a dynamic process of uptake and elimination and may take hours to days before a fish reaches an equilibrium or "steady state" concentration. Thus, the 2,4-D tissue concentrations calculated for the "immediately after spraying" scenario may be overestimates of actual concentrations, as fish may eliminate 2,4-D very rapidly (Rand and Petrocelli, 1985). The calculation of fish tissue concentration also assumes that a fish is exposed to a constant concentration of 2,4-D in water, which is rarely the case in a natural setting. The ingestion of surface water pathway represents an extremely conservative assessment as it was assumed people drink 2,4-D containing water 365 days/year. Given that 2,4-D is not labeled for application on a daily basis and that it degrades rapidly in water it is most likely not found in potable surface water 365 days/year.

For chronic exposures, the hazard index is calculated by summing hazard quotients across exposure pathways. It is highly unlikely that a person will be chronically exposed to 2,4-D via every pathway. Thus, the hazard index represents a very conservative value.

5.19 RISK EVALUATION

It is important to recognize that acute and chronic non-carcinogenic risk was evaluated using two separate approaches: the MOS and RfD approach, respectively. Given this, the results of the separate assessments should be interpreted independently, without comparison of the results of an acute exposure to that of a chronic exposure.

5.20 MITIGATION MEASURES

The Margin of Safety (MOS) is a calculated value as to the degree of safety a person may expected from exposure to a chemical. The MOS is determined by dividing the calculated exposure or dose by the lowest animal toxicology study NOEL. A MOS above 100 is an indicator that the particular exposure may not expected to cause adverse health effects. Similarly, calculated MOSs below 100 serve as signs that an overexposure situation may exist that could potentially result in adverse health effects. MOSs calculated and presented in the following

Tables indicate that there is very little risk to public health associated with 2,4-D use. All the MOSs and hazard indices indicate that 2,4-D should not pose acute or chronic risk to the public.

5.21 SUMMARY

Results indicate that 2,4-D should present little or no risk to the public from acute exposures via dermal contact with sediment, dermal contact with water, or ingestion of fish. Dermal contact with vegetation may present limited risk if it is contacted one hour after application. By 24 hours post-application non-carcinogenic risk is essentially nonexistent, as 2,4-D is unavailable for dermal uptake. Margins of safety for all acute exposure scenarios are greater than "100", implying that risk of systemic, teratogenic, or reproductive effects to humans is negligible.

Results of chronic exposure assessments indicate that human health should not be adversely impacted from chronic 2,4-D exposure via ingestion of fish, ingestion of surface water, incidental ingestion of sediments, dermal contact with sediments, or dermal contact with water (swimming). Hazard quotients for every exposure pathway and scenario are small (8E-10 to 3E-01). Hazard quotients are consistently higher (i.e., higher risk) for the irrigation ditch scenario.

The overview presented in this document concerning the toxicology and risk assessment of 2,4-D in its use as an aquatic herbicide indicates that use of the chemical in accordance with label directions is not expected to result in adverse health effects. A review of the acute, subchronic and chronic toxicology investigations demonstrate that 2,4-D acid, amine salts and esters have similar degrees of low systemic toxicity. The amine salts and esters are metabolized to the acid and undergo rapid excretion by the kidneys. 2,4-D does not accumulate in the organism or environment, however when the administered dose exceeds the threshold for normal renal function, a decrease in excretion occurs resulting in possible systemic poisoning. Findings from subchronic and chronic toxicology studies and genotoxicity testing do not implicate that 2,4-D is a carcinogen or developmental or reproductive toxin in laboratory animals. A review of the epidemiology studies and opinions from scientific review panels indicate that some of the investigations present inconsistent results, design flaws, and contain confounding variables, associations between NHL and 2,4-D exposure are weak and conclusions by the investigators are conflicting. Therefore, based on the weight-of-the-evidence, label directed use of 2,4-D for aquatic herbicide control poses little concern for causing adverse health effects to people.

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APPENDICES

APPENDIX I: EPIDEMIOLOGY STUDY PARAMETERS

Types of Epidemiology Studies

There are two basic types of epidemiology studies: case-control and cohort.

- **Case-control epidemiology studies**

  In case-control studies, individuals with a specific diagnosed disease(s) are identified and compared to nondiseased control subjects. Case-control studies have the advantage of being able to identify cases diagnosed with the disease of interest, which is especially important when the disease is rare. Chemical exposure information is developed from records and questionnaires completed by the subjects. The information on frequency and duration of chemical(s) exposure is compared between the two groups and statistically adjusted by the investigators for other factors that may influence the disease. The disadvantage of case-control studies is that exposures to the chemical(s) usually occurred years before and the subjects are prone to errors of recall. It is often impossible to separate exposure to the chemical of interest from exposure to other chemicals. In cases where the subject is incapacitated or deceased, the exposure information may be obtained from the next-of-kin or neighbors who may have little knowledge of the chemicals used or degree of exposure. This type of data collection in epidemiology investigations is controversial because inaccurate or biased information may affect the results.

- **Cohort epidemiology studies**

  Cohort studies begin with a group of people that have a common occupation, e.g. wheat farmers of golf course superintendents. The subjects are evaluated over an extended period of time and findings of various disease states are compared to the general unexposed population. Subjects in both groups must be free of disease at the beginning of the study. During the course of the investigation their chemical exposures, lifestyles, and health are evaluated.

  Cohort studies can begin with the exposure point starting in the past or in the present. The former type is termed a retrospective study while the latter termed a prospective cohort epidemiology study.

  Cohort studies offer the advantage of following large groups of subjects with a specific exposure (i.e. pesticide plant workers). The disadvantages are the length of time and expense. For example, some rare diseases may require a large study population or cohort, e.g. 100,000 subjects, to yield an adequate number of diagnosed cases to determine a reliable calculated association between the incidence of disease and chemical exposure. Also, follow-up of individuals can be difficult with large populations over time because of subjects moving to other geographical areas or dropping out of the study.

Sources of Error in Epidemiologic Studies

Although numerous epidemiological studies on other chlorophenoxy herbicides have been conducted, it is important to understand that care must be taken by the study investigator(s) to ensure that accurate and complete data are gathered to provide reliable and correct answers as to cause and effect between exposure to a chemical(s) and the development of a disorder(s). Crucial information regarding exposure, e.g. to what specific chemical(s), duration and extent; accurate diagnosis of the disease and /or cause of death are essential in conducting a meaningful and scientifically sound epidemiology study (O'Brien, 1984). Before a casual relationship can be defined between a disease and a chemical the actual incidence of disease and a realistic estimation of exposure must first be determined for both case and control groups. Precautions must be taken to avoid bias introduced when individuals answer questions inaccurately or when diseases are misdiagnosed. Assessments of exposure to a specific chemical are difficult because almost all exposures involve multiple chemicals.

Often, living relatives or neighbors of a deceased person may be asked to recall complex lifetime herbicide exposures. Thus, every epidemiological study has some sort of criticism associated with it. In addition, there exists a publishing bias, in which authors of studies are more likely to publish studies with positive results than those with negative results. Results that are significant are more likely to be published than those that are non-significant (McNamee, 1989).

Expressing Epidemiology Study Results

The results of epidemiology studies are defined in terms of a disease rate within a defined population over a specified time period. In the case of cohort and case-control studies, the disease rate comparisons used are the relative risk ratio (RR) and the odds ratio (OR), respectively.

The RR is a numerical comparison of disease rates among exposed vs. nonexposed groups over a specified period of time. A RR of 1.0 means that the rate for a specific disease is the same for both the exposed and unexposed subjects. A RR greater than 1.0 indicates that the disease rate is higher in the exposed group and that there may be an association with the subjects exposure to the chemical(s) being studied. Similarly, a RR of less than 1.0 indicates a reduced disease rate in the exposed population and that the chemical exposure may not be associated with the disease in question.

The measure of the disease rate in a case-control study is the OR. The OR is the ratio of the odds of exposure in the diseased or case group to the odds of exposure in the nondiseased or control group. The OR and RR are essentially the same with regard to numerical definitions of degree of disease rate association with chemical exposure. An OR of 1.0 indicates the same rate of disease for both groups, while less than or greater than 1.0 indicates a possible negative or positive association, respectively.

Since the RR and OR are single point estimates of the disease rate ratios, they are vulnerable to statistical variability and the true number may be higher or lower than the calculated ratio. To integrate more precision into the ratio calculations, epidemiologists use confidence intervals (CI) to provide theoretical 95% upper and lower probability limits to the RR and OR. For example, an epidemiology study reports an OR of 1.8 and a 95% CI of 0.2 (lower) and 2.9 (higher) in comparing the incidence of prostate cancer between the general population and soybean farmers. Although the OR number indicates an increased incidence of prostate cancer in soybean farmers, the 95% CI reveals that the OR could be more precisely located between the lower interval of 0.2 and the upper limit of 2.9. Thus, the OR in this case with the lower CI being less than 1.0, could

be interpreted that the results are inconclusive. Conversely, a 95% CI greater than 1.0 may be reason to conclude that the exposure to the chemical caused the disease. In summary, the 95% CI is important in concluding the degree of association between the incidence of the disease and exposure to the chemical(s).

Some epidemiology studies utilize the standardized mortality ratio (SMR) to evaluate the number of deaths in the chemical(s) exposed group to the death rate in the unexposed general population. The SMR is calculated as the ratio of observed to expected deaths multiplied by 100. A SMR value of 100 indicates that there is a similar death rate for both the exposed and unexposed populations. Similarly, SMR findings below and above 100 indicate a lower and greater incidence of deaths in the chemicals(s) exposed group, respectively. Also, SMR calculations include the 95% CI so that the principles of interpreting the significance the study calculations, as previously discussed for OR and RR, apply to SMRs.

APPENDIX II: 2,4-D TOXICOLOGY PROFILE

STUDY	RESULTS	REFERENCE
Acute Oral – Rat	LD50 = ~700 mg/kg EPA Tox. Cat. III	Myer, 1981a
Acute Dermal – Rabbit	LD50 = >2000 mg/kg EPA Tox. Cat. III	Myer, 1981b
Acute Inhalation – Rat	LC50 = 1.79 mg/L EPA Tox. Cat. III	Auletta, 1986
Primary Eye Irritation – Rabbit	Severe Irritant EPA Tox. Cat. I	Schults, 1990a
Primary Skin Irritation – Rabbit	Slight Irritant EPA Tox. Cat. IV	Schults, 1990b
Dermal Sensitization – Guinea Pig	Non-Sensitizer	Schults, 1990c
Acute Neurotoxicity – Rat	Systemic Tox NOEL = 227 mg/kg LOEL = >227 mg/kg Neurobehavior NOEL = 67 mg/kg LOEL = 227 mg/kg	Mattson, 1994
90-Day Feeding – Mouse	NOEL = 15 mg/kg/dy LOEL = 100 mg/kg/dy	Schulze, 1991a
90-Day Feeding- Rat	NOEL = 15 mg/kg/dy LOEL = 100 mg/kg/dy	Schulze, 1991b
90-Day Feeding (capsule) – Dog	NOEL = 1.0 mg/kg/dy LOEL = 3.0 mg/kg/dy	Schulze, 1990a
90-Day Feeding (diet) – Dog	NOEL = 1.0 mg/kg/dy LOEL = 3.75 mg/kg/dy	Dalgard, 1993a Charles, 1996a
21-Day Dermal – Rabbit	Dermal and Systemic Toxicity NOEL = 1000 mg/kg/dy LOEL = >1000 mg/kg/dy	Schulze, 1990b
1-Year Neurotoxicity – Rat	NOEL = 75 mg/kg/dy LOEL = 150 mg/kg/dy	Jeffries, 1994
Chronic Toxicity – Dog	NOEL = 1.0 mg/kg/dy LOEL = 5.0 mg/kg/dy	Dalgard, 1993d Charles, 1996a
Carcinogenicity – Mice	Non-Carcinogenic Systemic NOEL = 5.0 mg/kg/dy Systemic LOEL = 62.5 mg/kg/dy (males) = 150 mg/kg/dy (females)	Sott, 1995
Carcinogenicity – Rat	Non-Carcinogenic Systemic NOEL = 75 mg/kg/dy (males) = 5.0 mg/kg/dy (females) Systemic LOEL = 150 mg/kg/dy (males) = 75 mg/kg/dy (females)	Jeffries, 1995
Developmental Toxicity – Rat	Maternal and Developmental NOEL = 25 mg/kg/dy LOEL = 75 mg/kg/dy	Nemec, 1984
Devel. Toxicity – Rabbit	Maternal NOEL = 30 mg/kg/dy Maternal LOEL = 90 mg/kg/dy Devel. NOEL = 90 mg/kg/dy Devel. LOEL = >90 mg/kg/dy	Hoberman, 1990
2-Generation Reproduction – Rat	Parental/Reproductive/Systemic NOEL = 5.0 mg/kg/dy LOEL = 20 mg/kg/dy	Rodwell, 1985
Mutagenicity	Considered Non-Mutagenic both i*n vitro and in vivo*	USEPA, 1994a, 1996a

APPENDIX III: 2,4-D HEALTH ADVISORIES

Drinking Water	
MCLG[a]	0.07 mg/L
MCL[b]	0.7 mg/L
DWEL[c]	0.4 mg/L (70 kg)
1-Day Ha[d]	1.0 mg/L (10 kg child)
10-Day Ha[e]	0.3 mg/L (10 kg child)
Longer term Ha[f]	0.1 mg/L (10 kg child)
	0.4 mg/L (70 kg)
Lifetime Ha[g]	0.07 mg/L (70 kg)
Dietary ADI[h]	0.01 mg/kg/dy
Tolerances	
Asparagus	5 ppm
Avocados	1
Citrus Fruits	1
Fish	1
Forage Grasses	1
Grain Crops	1
Leafy vegetables	1
Shellfish	1
Strawberries	0.05

[a] Maximum Contamination Level Goal – A non-enforceable concentration of a drinking water contamination that is protective of adverse human health effects and allows an adequate MOS.

[b] Maximum Contamination Level – Maximum permissible level of contamination in water, which is delivered to any user of public water system.

[c] Drinking Water Equivalent Level – A lifetime exposure concentration protective of adverse, noncancer health effects, that assumes all of the exposure to a contaminant that is from a drinking water source.

[d] One day Health Advisory – concentration of a chemical in drinking water that is not expected to cause any adverse noncarcinogenic effects for up to 5 consecutive days of exposure, with a MOS.

[e] Ten day Health Advisory – Same as one day HA for up to 14 consecutive days of exposure, with MOS.

[f] Longer Term Health Advisory – same as one day HA for up to 7 years (10% of lifetime of exposure) consecutive exposure, with MOS.

[g] Lifetime Health Advisory – same as one day HA for a lifetime of exposure, with MOS.

[h] Acceptable Daily Intake – dietary intake of a chemical that is not expected to cause any adverse health effects over a lifetime.

Reference: USEPA, 1987

APPENDIX IV: 2,4-D TOXICOLOGY QUANTITATIVE PARAMETERS

Toxicology

Acute NOEL[a,b]	67 mg/kg (Mattson, 1997)
Subchronic NOEL	1.0 mg/kg/dy/90 dy (Schulze, 1991a; Dalgard, 1993a)
Chronic NOEL	1.0 mg/kg/dy (Dalgard, 1993d)
Chronic RfD[d]	0.01 mg/kg/dy (1-Yr Dog Oral NOEAL[c]=1.0 mg/kg/dy)
Reproduction NOEL	5.0 mg/kg/dy (Rodwell, 1985)

Cancer Classification

EPA[e]	Group D (inadequate evidence in humans and animals)
IARC[f]	2B (possible carcinogenic to humans)

Dermal

Absorption = ~6%/24-hours (Feldman and Maibach, 1974)

Inhalation

ACGIH TLV[g] = 10 mg/M3

[a] NOEL – no observed effect level. A dose in an animal toxicology study where no changes or effects in the test animals was measured or observed. The effects do not always have to be considered adverse health effects.

[b] Acute dietary endpoint (l day) based on neurobehavioral effects observed in FOB and motor activity testing (US EPA, 1996c).

[c] NOAEL – no observed adverse effect level. A dose in an animal toxicology study where adverse health effects were measured or observed.

[d] RfD – Reference dose – represents a calculated lifetime daily exposure to a chemical that is not expected to result in any adverse health effects.

[e] EPA – US Environmental Protection Agency

[f] IARC – International Agency for Registration of Carcinogens

[g] ACGIH – American Conference of Governmental Industrial Hygienists – an organization devoted to the administrative and technical aspects of occupational and environmental health. The organization is a professional society, not a government agency. The organization establishes TLVs. TLV – Threshold Limit Value is an airborne concentration of a specific chemical expressed as a time-weighted average concentration for a conventional 8 hr/dy/40 hr/wk, where it is believed that nearly all workers may be repeatedly exposed, day after day, without adverse effects (ACGIH, 1997).

LIST OF TABLES

Table 1: Potential Exposure Routes

Application Method	Dermal	Oral	Inhalation
Helicopter	Contact with sediment/soil Contact with vegetation Contact with water	Ingestion of fish Incidental ingestion of sediment	Not Applicable
Helicopter with ball	Same as above	Same as above	Not Applicable
Backpack sprayer	Same as above	Same as above	Not Applicable
Wicking	Contact with vegetation	Not Applicable	Not Applicable

Table 2: Degradation Half Lives of 2,4-D in Water

Half life, days	Reference
2,4-D acid: 1 - 11	Reinert and Rodgers 1987
2,4-D DMA: 3.9	Robson 1968
2,4-D DMA: 10- 11	Schultz and Harman 1973
2,4-D DMA: 6.6	Westerdahl and Getsinger 1988
2,4-D DMA: 2.2-4.2	Averitt and Gangstad 1976
2,4-D DMA: 0.5-0.8	Gangstad 1983
2,4-D DMA: 2.5-6.2	Otto et al. 1983
2,4-D BEE: 3	Dynamac 1988
2,4-D BEE: 0.11-2.3	Reinert and Rodgers 1987
2,4-D BEE: 2.2-2.3	Oklahoma Water Research 1975; Frank and Comes 1967

Table 3: Three scenarios for 2,4-D application

Parameter	Scenario 1: Small Lake or Pond	Scenario 2: Wasteway (large irrigation canal)	Scenario 3: Portion of a Large Lake (e.g. Lake Washington)
Water dimensions	1 acre x 4 feet	200 feet x 3 miles x 3 feet	20 acre x 8 feet
Water volume	4,934,000 L	2.701×10^8 L	1.974×10^8 L
Treatment area	740 ft x 3 feet (0.051 acre)	100 feet x 3 miles (36 acres)	1, 100 feet (1/3 perimeter) x 12 feet (0.03 acre)

Table 4: Initial concentrations of 2,4-D DMA and 2,4-D BEE in Scenarios 1 - 3

Scenario	2,4-D DMA concentration (mg/L)	2,4-D BEE concentration (mg/L)
1 (small lake)	0.018	0.18
2 (wasteway)	0.24	2.3
3 (large lake)	0.00028	0.0026

Table 5: Sediment and Water Concentrations of DMA and BEE Immediately Following Application

	2,4-D DMA Concentration		2,4-D BEE Concentration	
Scenario	**Water (mg/L)**	**Sediment (mg/kg)**	**Water (mg/L)**	**Sediment (mg/kg)**
1	0.015	0.0028	0.0032	0.17
2	0.20	0.044	0.041	2.26
3	0.00024	0.00004	0.00004	0.0025

Table 6: Average Concentrations (A_{ave}) of 2,4-D Within a 22 Day Period Following Application

Scenario	2,4-D DMA Concentration		2,4-D BEE Concentration	
	Water (mg/L)	Sediment (mg/kg)	Water (mg/L)	Sediment (mg/kg)
1	8.10×10^{-3}	2.47×10^{-3}	1.73×10^{-3}	0.15
2	0.108	3.89×10^{-2}	2.22×10^{-2}	2.00
3	1.30×1^{-4}	3.53×10^{-5}	2.16×10^{-5}	$2.21 \times 10_{-3}$

Table 7: Single Doses from Dermal Contact with Water (Swimming)

Scenario 1: Small Pond

Days After Application	Water Concentration (mg/L)		Dose Swimming (mg/kg)	
	Amine	Ester	Amine	Ester
Immediately	1.50E-02	3.20E-03	4.93E-08	1.13E-05
1	1.41E-02	3.00E-03	4.63E-08	1.06E-05
2	1.32E-02	2.82E-03	4.34E-08	9.92E-06
3	1.24E-02	2.65E-03	4.08E-08	9.31E-06
4	1.17E-02	2.49E-03	3.83E-08	8.74E-06
5	1.09E-02	2.34E-03	3.60E-08	8.21E-06
6	1.03E-02	2.19E-03	3.38E-08	7.71E-06
7	9.64E-03	2.06E-03	3.17E-08	7.24E-06
8	9.06E-03	1.93E-03	2.98E-08	6.80E-06
9	8.51E-03	1.81E-03	2.80E-08	6.38E-06
10	7.99E-03	1.70E-03	2.62E-08	5.99E-06
11	7.50E-03	1.60E-03	2.46E-08	5.63E-06
12	7.04E-03	1.50E-03	2.31E-08	5.28E-06
13	6.61E-03	1.41E-03	2.17E-08	4.96E-06
14	6.20E-03	1.32E-03	2.04E-08	4.66E-06
15	5.83E-03	1.24E-03	1.92E-08	4.37E-06
16	5.47E-03	1.17E-03	1.80E-08	4.10E-06
17	5.14E-03	1.10E-03	1.69E-08	3.85E-06
18	4.82E-03	1.03E-03	1.59E-08	3.62E-06
19	4.53E-03	9.66E-04	1.49E-08	3.40E-06
20	4.25E-03	9.07E-04	1.40E-08	3.19E-06
21	3.99E-03	8.52E-04	1.31E-08	3.00E-06
22	3.75E-03	8.00E-04	1.23E-08	2.81E-06

Table 7: Single Doses from Dermal Contact with Water (Swimming) (Continued)

Scenario 2: Irrigation Ditch

Days After Application	Water Concentration (mg/L)		Dose Swimming (mg/kg)	
	Amine	Ester	Amine	Ester
Immediately	2.00E-01	4. 10E-02	6.57E-07	1.44E-04
1	1.88E-01	3.85E-02	6.17E-07	1.35E-04
2	1.76E-01	3.61E-02	5.79E-07	1.27E-04
3	1.66E-01	3.39E-02	5.44E-07	1.19E-04
4	1.55E-01	3.19E-02	5.11E-07	1.12E-04
5	1.46E-01	2.99E-02	4.80E-07	1.05E-04
6	1.37E-01	2.81E-02	4.50E-07	9.88E-05
7	1.29E-01	2.64E-02	4.23E-07	9.27E-05
8	1.21E-01	2.48E-02	3.97E-07	8.71E-05
9	1.13E-01	2.33E-02	3.73E-07	8.18E-05
10	1.07E-01	2.18E-02	3.50E-07	7.68E-05
11	1.00E-01	2.05E-02	3.29E-07	7.21E-05
12	9.39E-02	1.92E-02	3.09E-07	6.77E-05
13	8.82E-02	1.81E-02	2.90E-07	6.35E-05
14	8.28E-02	1.70E-02	2.72E-07	5.97E-05
15	7.77E-02	1.59E-02	2.55E-07	5.60E-05
16	7.30E-02	1.50E-02	2.40E-07	5.26E-05
17	6.85E-02	1.40E-02	2.25E-07	4.94E-05
18	6.43E-02	1.32E-02	2.11E-07	4.64E-05
19	6.04E-02	1.24E-02	1.98E-07	4.35E-05
20	5.67E-02	1.16E-02	1.86E-07	4.09E-05
21	5.33E-02	1.09E-02	1.75E-07	3.84E-05
22	5.00E-02	1.03E-02	1.64E-07	3.60E-05

Table 7: Single Doses from Dermal Contact with Water (Swimming) (Continued)

Scenario 3: Large Lake

Days After Application	Water Concentration (mg/L)		Dose Swimming (mg/kg)	
	Amine	Ester	Amine	Ester
Immediately	2.40E-04	4.00E-05	7.89E-10	1.41E-07
1	2.25E-04	3.76E-05	7.40E-10	1.32E-07
2	2.12E-04	3.53E-05	6.95E-10	1.24E-07
3	1.99E-04	3.31E-05	6.53E-10	1.16E-07
4	1.87E-04	3.11E-05	6.13E-10	1.09E-07
5	1.75E-04	2.92E-05	5.75E-10	1.03E-07
6	1.64E-04	2.74E-05	5.40E-10	9.64E-08
7	1.54E-04	2.57E-05	5.07E-10	9.05E-08
8	1.45E-04	2.42E-05	4.76E-10	8.49E-08
9	1.36E-04	2.27E-05	4.48E-10	7.98E-08
10	1.28E-04	2.13E-05	4.20E-10	7.49E-08
11	1.20E-04	2.00E-05	3.94E-10	7.03E-08
12	1.13E-04	1.88E-05	3.70E-10	6.60E-08
13	1.06E-04	1.76E-05	3.48E-10	6.20E-08
14	9.93E-05	1.36E-05	3.26E-10	4.77E-08
15	9.33E-05	1.55E-05	3.06E-10	5.46E-08
16	8.76E-05	1.46E-05	2.88E-10	5.13E-08
17	8.22E-05	1.37E-05	2.70E-10	4.82E-08
18	7.72E-05	1.29E-05	2.54E-10	4.52E-08
19	7.25E-05	1.21E-05	2.38E-10	4.25E-08
20	6.81E-05	1.13E-05	2.24E-10	3.99E-08
21	6.39E-05	1.07E-05	2.10E-10	3.74E-08
22	6.00E-05	1.00E-05	1.97E-10	3.52E-08

Table 8: Chronic Intake Values From Dermal Contact With Water (Swimming)

Scenario	Amine Average Concentration in Water (mg/L)	Ester Average Concentration in Water (mg/L)	Amine Intake (mg/kg-day)	Ester Intake (mg/kg-day)
1 (small pond)	8. 10 E-03	1.73 E-03	1.60 E-09	3.67 E-07
2 (irrigation ditch)	0.108	2.22 E-02	2.14 E-08	4.70 E-06
3 (large lake)	1.30 E-04	2.16 E-05	2.57 E-11	4.58 E-09

Table 9: Single Doses From Dermal Contact With Sediment

Scenario 1: Small Pond

Days After Application	Concentration in Sediment (mg/kg)		Dose Dermal Contact with Sediment (mg/kg)	
	Amine	Ester	Amine	Ester
Immediately	2.80E-03	1.70E-01	7.34E-09	4.46E-07
1	2.77E-03	1.68E-01	7.26E-09	4.41E-07
2	2.74E-03	1.66E-01	7.17E-09	4.36E-07
3	2.70E-03	1.64E-01	7.09E-09	4.31E-07
4	2.67E-03	1.62E-01	7.01E-09	4.26E-07
5	2.64E-03	1.60E-01	6.93E-09	4.21E-07
6	2.61E-03	1.59E-01	6.85E-09	4.16E-07
7	2.58E-03	1.57E-01	6.77E-09	4.11E-07
8	2.55E-03	1.55E-01	6.69E-09	4.06E-07
9	2.52E-03	1.53E-01	6.62E-09	4.02E-07
10	2.49E-03	1.51E-01	6.54E-09	3.97E-07
11	2.47E-03	1.50E-01	6.47E-09	3.93E-07
12	2.44E-03	1.48E-01	6.39E-09	3.88E-07
13	2.41E-03	1.46E-01	6.32E-09	3.84E-07
14	2.38E-03	1.45E-01	6.25E-09	3.79E-07
15	2.35E-03	1.43E-01	6.17E-09	3.75E-07
16	2.33E-03	1.41E-01	6.10E-09	3.71E-07
17	2.30E-03	1.40E-01	6.03E-09	3.66E-07
18	2.27E-03	1.38E-01	5.96E-09	3.62E-07
19	2.25E-03	1.36E-01	5.89E-09	3.58E-07
20	2.22E-03	1.35E-01	5.83E-09	3.54E-07
21	2.20E-03	1.33E-01	5.76E-09	3.50E-07
22	2.17E-03	1.32E-01	5.69E-09	3.46E-07

Table 9: Single Doses from Dermal Contact with Sediment (Continued)

Scenario 2: Irrigation Ditch

Days After Application	Concentration in Sediment (mg/kg)		Dose Dermal Contact with Sediment (mg/kg)	
	Amine	Ester	Amine	Ester
Immediately	2.80E-03	1.70E-01	1. 15E-07	5.93E-06
1	2.77E-03	1.68E-01	1. 14E-07	5.86E-06
2	2.74E-03	1.66E-01	1. 13E-07	5.79E-06
3	2.70E-03	1.64E-01	1. 11E-07	5.72E-06
4	2.67E-03	1.62E-01	1. 10E-07	5.66E-06
5	2.64E-03	1.60E-01	1.09E-07	5.59E-06
6	2.61E-03	1.59E-01	1.08E-07	5.53E-06
7	2.58E-03	1.57E-01	1.06E-07	5.47E-06
8	2.55E-03	1.55E-01	1.05E-07	5.40E-06
9	2.52E-03	1.53E-01	1.04E-07	5.34E-06
10	2.49E-03	1.51E-01	1.03E-07	5.28E-06
11	2.47E-03	1.50E-01	1.02E-07	5.22E-06
12	2.44E-03	1.48E-01	1.00E-07	5.16E-06
13	2.41E-03	1.46E-01	9.93E-08	5.10E-06
14	2.38E-03	1.45E-01	9.81E-08	5.04E-06
15	2.35E-03	1.43E-01	9.70E-08	4.98E-06
16	2.33E-03	1.41E-01	9.59E-08	4.93E-06
17	2.30E-03	1.40E-01	9.48E-08	4.87E-06
18	2.27E-03	1.38E-01	9.37E-08	4.81E-06
19	2.25E-03	1.36E-01	9.26E-08	4.76E-06
20	2.22E-03	1.35E-01	9.16E-08	4.70E-06
21	2.20E-03	1.33E-01	9.05E-08	4.65E-06
22	2.17E-03	1.32E-01	8.95E-08	4.60E-06

Table 9: Single Doses from Dermal Contact with Sediment (Continued)

Scenario 3: Large Lake

Days After Application	Concentration in Sediment (mg/kg)		Dose Dermal Contact with Sediment (mg/kg)	
	Amine	Ester	Amine	Ester
Immediately	2.80E-03	1.70E-01	1.05E-10	6.56E-09
1	2.77E-03	1.68E-01	1.04E-10	6.48E-09
2	2.74E-03	1.66E-01	1.02E-10	6.41E-09
3	2.70E-03	1.64E-01	1.01E-10	6.33E-09
4	2.67E-03	1.62E-01	1.00E-10	6.26E-09
5	2.64E-03	1.60E-01	9.90E-11	6.19E-09
6	2.61E-03	1.59E-01	9.79E-11	6.12E-09
7	2.58E-03	1.57E-01	9.67E-11	6.05E-09
8	2.55E-03	1.55E-01	9.56E-11	5.98E-09
9	2.52E-03	1.53E-01	9.45E-11	5.91E-09
10	2.49E-03	1.51E-01	9.34E-11	5.84E-09
11	2.47E-03	1.50E-01	9.24E-11	5.77E-09
12	2.44E-03	1.48E-01	9.13E- 11	5.71E-09
13	2.41E-03	1.46E-01	9.03E-11	5.64E-09
14	2.38E-03	1.45E-01	8.92E- 11	5.58E-09
15	2.35E-03	1.43E-01	8.82E-11	5.51E-09
16	2.33E-03	1.41E-01	8.72E- 11	5.45E-09
17	2.30E-03	1.40E-01	8.62E-11	5.39E-09
18	2.27E-03	1.38E-01	8.52E-11	5.32E-09
19	2.25E-03	1.36E-01	8.42E-11	5.26E-09
20	2.22E-03	1.35E-01	8.32E-11	5.20E-09
21	2.20E-03	1.33E-01	8.23E-11	5.14E-09
22	2.17E-03	1.32E-01	8.13E-11	5.08E-09

Table 10: Chronic Intake Values For Dermal Contact With Sediments

Scenario	Amine Average Concentration in Sediment (mg/kg)	Ester Average Concentration in Sediment (mg/kg)	Amine Intake (mg/kg-day)	Ester Intake (mg/kg-day)
1 (small pond)	2.47 E-03	0.15	3.90 E- 10	2.37 E-08
2 (irrigation ditch)	3.89 E-02	2	6.51 E-09	3.16 E-07
3 (large lake)	3.53 E-03	2.21 E-05	5.58 E-12	3.49 E-10

Table 11: Single Doses, from Ingestion of Fish

Scenario 1: Small Pond

Days After Application	Concentration In Water (mg/L)		BCF	Concentration in Fish (mg/kg)		Dose Ingestion of Fish (mg/kg)	
	Amine	Ester		Amine	Ester	Amine	Ester
Immediately	1.50E-02	3.20E-03	5	7.50E-02	1.60E-02	4.29E-04	9.14E-05
1	1.41E-02	3.00E-03	5	7.04E-02	1.50E-02	4.02E-04	9.58E-05
2	1.32E-02	2.82E-03	5	6.61E-02	1.41E-02	3.78E-04	8.06E-05
3	1.24E-02	2.65E-03	5	6.21E-02	1.32E-02	3.55E-04	7.57E-05
4	1.17E-02	2.49E-03	5	5.83E-02	1.24E-02	3.33E-04	7.11E-05
5	1.09E-02	2.34E-03	5	5.47E-02	1.17E-02	3.13E-04	6.67E-05
6	1.03E-02	2.19E-03	5	5.14E-02	1.10E-02	2.94E-04	6.26E-05
7	9.64E-03	2.06E-03	5	4.82E-02	1.03E-02	2,75E-04	5.88E-05
9	9.06E-03	1.93E-03	5	4.53E-02	9.66E-03	2.59E-04	5.52E-05
9	8.51E-03	1.81E-03	5	4.25E-02	9.07E-03	2.43E-04	5.19E-05
10	7.99E-03	1.70E-03	5	3.99E-02	8.52E-03	2.28E-04	4.87E-05
11	7.50E-03	1.60E-03	5	3.75E-02	8.00E-03	2.14E-04	4.57E-05
12	7.04E-03	1.50E-03	5	3.52E-02	7.51E-03	2.01E-04	4.29E-05
13	6.61E-03	1.41E-03	5	3.31E-02	7.05E-03	1.89E-04	4.03E-05
14	6.20E-03	1.32E-03	5	3.10E-02	6.62E-03	1.77E-04	3.78E-05
15	5.83E-03	1.24E-03	5	2.91E-02	6.22E-03	1.67E-04	3.55E-05
16	5.47E-03	1.17E-03	5	2.74E-02	5.84E-03	1.56E-04	3.34E-05
17	5.14E-03	1.10E-03	5	2.57E-02	5.48E-03	1.47E-04	3.13E-05
18	4.82E-03	1.03E-03	5	2.41E-02	5.15E-03	1.38E-04	2.94E-05
19	4.53E-03	9.66E-04	5	2.27E-02	4.83E-03	1.29E-04	2.76E-05
20	4.25E-03	9.07E-04	5	2.13E-02	4.54E-03	1.22E-04	2.59E-05
21	3.99E-03	9.52E-04	5	2.00E-02	4.26E-03	1.14E-04	2.43E-05
22	3.75E-03	8.00E-04	5	1.88E-02	4.00E-03	1.07E-04	2.29E-05

Table 11: Single Doses from Ingestion of Fish (Continued)

Scenario 2: Irrigation Ditch

Days After Application	Concentration In Water (mg/L)		BCF	Concentration in Fish (mg/kg)		Dose Ingestion of Fish (mg/kg)	
	Amine	Ester		Amine	Ester	Amine	Ester
Immediately	2.00E-01	4.10E-02	5	1.00E-00	2.05E-01	5.71E-03	1.17E-03
1	1.88E-01	3.85E-02	5	9.39E-01	1.92E-01	5.37E-03	1.10E-03
2	1.76E-01	3.61E-02	5	8.82E-01	1.81E-01	5.04E-03	1.03E-03
3	1.66E-01	3.39E-02	5	8.28E-01	1.70E-01	4.73E-03	9.70E-04
4	1.55E-01	3.19E-02	5	7.77E-01	1.59E-01	4.44E-03	9.10E-04
5	1.46E-01	2.99E-02	5	7.30E-01	1.50E-01	4.17E-03	8.55E-04
6	1.37E-01	2.81E-02	5	6.85E-01	1.40E-01	3.92E-03	8.03E-04
7	1.29E-01	2.64E-02	5	6.43E-01	1.32E-01	3.68E-03	7.54E-04
8	1.21E-01	2.48E-02	5	6.04E-01	1.24E-01	3.45E-03	7.05E-04
9	1.13E-01	2.33E-02	5	5.67E-01	1.16E-01	3.24E-03	6.64E-04
10	1.07E-01	2.18E-02	5	5.33E-01	1.09E-01	3.04E-03	6.24E-04
11	1.00E-01	2.05E-02	5	5.00E-01	1.03E-01	2.86E-03	5.86E-04
12	9.39E-02	1.92E-02	5	4.69E-01	9.62E-02	2.68E-03	5.50E-04
13	9.82E-02	1.81E-02	5	4.41E-01	9.04E-02	2.52E-03	5.16E-04
14	8.28E-02	1.70E-02	5	4.14E-01	8.48E-02	2.37E-03	4.85E-04
15	7.77E-02	1.59E-02	5	3.99E-01	7.97E-02	2.22E-03	4.55E-04
16	7.30E-02	1.50E-02	5	3.65E-01	7.48E-02	2.08E-03	4.27E-04
17	6.85E-02	1.40E-02	5	3.43E-01	7.02E-02	1.96E-03	4.01E-04
19	6.43E-02	1.32E-02	5	3.22E-01	6.59E-02	1.84E-03	3.77E-04
19	6.04E-02	1.24E-02	5	3.02E-01	6.19E-02	1.73E-03	3.54E-04
20	5.67E-02	1.16E-02	5	2.94E-01	5.81E-02	1.62E-03	3.32E-04
21	5.33E-02	1.09E-02	5	2.66E-01	5.46E-02	1.52E-03	3.12E-04
22	5.00E-02	1.03E-02	5	2.50E-01	5.13E-02	1.43E-03	2.93E-04

Table 11: Single Doses from Ingestion of Fish (Continued)

Scenario: Large Lake

Days After Application	Concentration In Water (mg/L)		BCF	Concentration in Fish (mg/kg)		Dose Ingestion of Fish (mg/kg)	
	Amine	Ester		Amine	Ester	Amine	Ester
Immediately	2.40E-04	4.00E-05	5	1.20E-03	2.00E-04	6.86E-06	1.14E-06
1	2.25E-04	3.76E-05	5	1.13E-03	1. 88E-04	6.44E-06	1.07E-06
2	2.12E-04	3.53E-05	5	1.06E-03	1.76E-04	6.05E-06	1.01E-06
3	1.99E-04	3.31E-05	5	9.93E-04	1.66E-04	5.68E-06	9.46E-07
4	1.87E-04	3.11E-05	5	9.33E-04	1.55E-04	5.33E-06	8.88E-07
5	1.75E-04	2.92E-05	5	8.76E-04	1.46E-04	5.00E-06	8.34E-07
6	1.64E-04	2.74E-05	5	8.22E-04	1.37E-04	4.70E-06	7.93E-07
7	1.54E-04	2.57E-05	5	7.72E-04	1.29E-04	4.41E-06	7.35E-07
8	1.45E-04	2.42E-05	5	7.25E-04	1.21E-04	4.14E-06	6.90E-07
9	1.36E-04	2.27E-05	5	6.82E-04	1.13E-04	3.90E-06	6.48E-07
10	1.28E-04	2.13E-05	5	6.39E-04	1.07E-04	3.65E-06	6.09E-07
11	1.20E-04	2.00E-05	5	6.00E-04	1.00E-04	3.43E-06	5.71E-07
12	1.13E-04	1.88E-05	5	5.63E-04	9.39E-05	3.22E-06	5.37E-07
13	1.06E-04	1.76E-05	5	5.29E-04	8.82E-05	3.02E-06	5.04E-07
14	9.93E-05	1.36E-05	5	4.97E-04	6.78E-05	2.84E-06	3.87E-07
15	9.33E-05	1.55E-05	5	4.66E-04	7.77E-05	2 66E-06	4.44E-07
16	8.76E-05	1.46E-05	5	4.38E-04	7.30E-05	2.50E-06	4.17E-07
17	8.22E-05	1.37E-05	5	4.11E-04	6.85E-05	2.35E-06	3.92E-07
18	7.72E-05	1.29E-05	5	3.86E-04	6.43E-05	2.21E-06	3.68E-07
19	7.25E-05	1.21E-05	5	3.62E-04	6.04E-05	2.07E-06	3.45E-07
20	6.81E-05	1.13E-05	5	3.40E-04	5.67E-05	1.94E-06	3.24E-07
21	6.39E-05	1.07E-05	5	3.20E-04	5.33E-05	1. 83E-06	3.04E-07
22	3.00E-04	5.00E-05	5	3.00E-04	5.00E-05	1.71E-06	2.86E-07

Table 12: Chronic Intake Values for Ingestion of Fish

Scenario	Amine Average Conc. in Water (mg/l)	Ester Average Conc. in Water (mg/l)	Amine Average Conc. in Fish (mg/kg)	Ester Average Conc. in Fish (mg/kg)	Amine Intake (mg/kg day)	Ester Intake (mg/kg day)
1 (small pond)	8. 10 E-03	1.73 E-03	4.05 E-02	8.65 E-03	3.30 E-05	7.04 E-06
2 (irrigation ditch)	0.108	2.22 E-02	0.54	1.11 E-01	4.40 E-04	9.04 E-05
3 (large lake)	1.30 E-04	2.16 E-05	6.5 E-04	1.10 E-04	5.29 E-07	8.79 E-08

Table 13: Chronic Intake Values for Ingestion of Surface Water

Scenario	Amine Average Concentration in Water (mg/kg)	Ester Average Concentration in Water (mg/kg)	Amine Intake (mg/kg-day)	Ester Intake (mg/kg-day)
1 (small pond)	8. 10 E-03	1.73 E-03	2.31 E-04	4.94 E-05
2 (irrigation ditch)	0.108	2.22 E-02	3.09 E-03	6.34 E-04
3 (large lake)	1.30 E-04	2.16 E-05	3.71 E-06	6.17 E-07

Table 14: Chronic Intake Values for Incidental Ingestion of Sediment

Scenario	Amine Average Concentration in Sediment (mg/kg)	Ester Average Concentration in Sediment (mg/kg)	Amine Intake (mg/kg-day)	Ester Intake (mg/kg-day)
1 (small pond)	2.47 E-03	0.15	5.67 E-10	3.44 E-08
2 (irrigation ditch)	3.89 E-02	2	8.93 E-09	4.59 E-07
3 (large lake)	3.53 E-05	2.21 E-03	8.11 E-12	5.07 E-10

Table 15: Acute Toxicity of 2,4-D, Amine Salts, and Esters in Male and Female Animals

Compound	Species	Route	LD_{50}(mg/kg bw) or LC_{50}(mg/litre)	Reference
2,4-D	Rat	Oral	699	Myer (1981a)
2,4-D	Rat	Oral	443	Squibb et al.(1983)
DEA salt	Rat	Oral	910	Schults et al. (1990a)
DMA salt	Rat	Oral	949	Myer (1989b)
IPA salt	Rat	Oral	2322 (m) 1646 (f)	Carreon et al. (1983)
TIPA salt	Rat	Oral	1220 (m) 1074 (f)	Berdasco et al. (1989a)
BEH ester	Rat	Oral	866	Jeffrey et al. (1987a)
EH ester	Rat	Oral	896	Myer (1981c)
2,4-D	Rabbit	Dermal	>2000	Myer (1981d)
DEA salt	Rabbit	Dermal	>2000	Shults et al. (1990b)
DMA salt	Rabbit	Dermal	>20000	Myer (1981e)
IPA salt	Rabbit	Dermal	>2000	Carreon et al. (1983)
TIPA salt	Rabbit	Dermal	>2000	Berdasco et al. (1989b)
BEH ester	Rabbit	Dermal	>2000	Jeffrey et al. (1987b)
EH ester	Rabbit	Dermal	>2000	Myer (1981f)
2,4-D	Rat	Inhalation	>1.8	Auletta & Daly (1986)
DEA salt	Rat	Inhalation	>3.5	Jackson & Hardy (1990)
DMA salt	Rat	Inhalation	>3.5	Streeter et al. (1990)
IPA salt	Rat	Inhalation	>3.2	Streeter et al. (1983)
TIPA salt	Rat	Inhalation	>0.84	Nitschke & Lomax (1990)
BEH ester	Rat	Inhalation	4.6	Streeter et al. (1987)
EH ester	Rat	Inhalation	>5.4	Cieszlak (1992)

DEA, diethanolamine; DMA, dimethylamine; IPA, isopropanolamine; TIPA, triisopropylamine; BEH, butoxyethylhexyl; EH, 2-ethylhexyl \

(WHO, 1996)

Table 16: Summary of Mammalian Systemic Toxicity Studies for 2,4-Dichlorophenoxyacetic Acid (2,4-D)*

Type of Test		*Effects*				
Formulation Species	Route of Exposure	NOEL	LEL	LD50	Comments	Reference
ACUTE , ORAL 2,4-D (Acid)		mg/kg				
Rat Mouse Guinea Pig	Single dose, administered by intubation in olive oil; observed two weeks; male rats, mice, male and female guinea pigs	-	-	375 (rat 368 (mouse) 469 (g.pig)	In general, adverse effects in animals, including rats, mice, guinea pigs, and dogs, exposed to acutely toxic doses of 2,4-D include anorexia, weight loss, dipsesis (excessive thirst), depression, roughness of coat, tremors, myasthenia (muscle weakness), rapid breathing and salivation. Post-mortem findings include stomach irritation, liver and kidney damage, and occasional lung congestion.	Rowe and Hymas, 1954
Rat	Single dose, administered by intubation in a 5% gelatin solution at dosage levels of 0, 333, 666 or 1,000 mg/kg; 4 rats/dose level.	-	-	666	Adverse effects for all species tested included myatonia, stiffness of extremities, ataxia, paralysis and coma with death caused by ventricular fibrillation.	Hill and Carlisle, 1947
Dog	Administered by capsule, at dosage levels of 1, 25, 100, 250 or 400 mg/kg; 2-4 dogs per dose.	25	100	100	Anorexia, weight loss, amyotonia, and pathological changes in the gastrointestinal tract, lungs, and liver with death resulting from hepatic congestion or pneumonia.	Drill and Hiratzka, 1953

Table 16: Summary of Mammalian Systemic Toxicity Studies for 2,4-Dichlorophenoxyacetic Acid (2,4-D)* (Continued)

Type of Test		Effects				
Formulation Species	**Route of Exposure**	**NOEL**	**LEL**	**LD50**	**Comments**	**Reference**
2,4-D (isopropyl ester)						
Monkey	Single injections either oral (up to 214 mg/kg) or intraperitoneal (up to 428 mg/kg); combined oral or intraperitoneal for 500 mg/kg total dose.	-	-	-	Tolerated 214 mg/kg by the oral route or 428 mg/kg by the intraperitoneal route; 500 mg/kg resulted in nausea, vomiting, lethargy and muscle incoordination.	Hill and Carlisle, 1947
Mouse Rat Guinea Pig	Single doses, administered by intubation in olive oil; observed two weeks. Male mice, guinea pigs, male and female rats.	-	-	541 (mouse) 700 (rat) 550 (g.pig)	-	Rowe and Hymas, 1954
ACUTE/ORAL 2,4-D (mixed butyl esters)		**mg/kg**				
Mouse Rat Guinea Pig Rabbit	Single doses, administered by intubation to females in corn oil; observed two weeks.	-	-	713 (mouse) 620 (rat) 848 (g.pig) 424 (rabbit)	-	Rowe and Hymas, 1954

Table 16: Summary of Mammalian Systemic Toxicity Studies for 2,4-Dichlorophenoxyacetic Acid (2,4-D)* (Continued)

Type of Test		Effects				
Formulation Species	**Route of Exposure**	**NOEL**	**LEL**	**LD50**	**Comments**	**Reference**
2,4-D (sodium salt)						
Mouse	Single dose, administered by intubation at dosage levels of 1,2.5,5.0, 7.5 or 10 mg (equivalent to 1, 125, 250, 375 or 500 mg/kg); 10 mice per dose level.	-	-	375	Adverse effects included myotonia, stiffness of extremities, ataxia, paralysis and coma with death caused by ventricular fibrillation.	Hill and Carlisle, 1947
Rat	Single doses by intubation in water; observed two weeks.	-	-	666	Adverse effects for all species tested included myotonia, stiffness of extremities, ataxia, paralysis and coma with death caused by ventricular fibrillation.	Hill and Carlisle, 1947
Rat	Single doses by intubation in water to females; observed two weeks.	-	-	805	-	Rowe and Hymas, 1954
Rabbit	Single dose, administered by intubation at 4 or 5 dosage levels; 4 rabbits per dose level.	-	-	800	Adverse effects included myotonia, stiffness of extremities, ataxia, paralysis and coma with death caused by ventricular fibrillation.	Hill and Carlisle, 1947

Table 16: Summary of Mammalian Systemic Toxicity Studies for 2,4-Dichlorophenoxyacetic Acid (2,4-D)* (Continued)

Type of Test		Effects				
Formulation Species	**Route of Exposure**	**NOEL**	**LEL**	**LD50**	**Comments**	**Reference**
2,4-D (sodium salt)						
Guinea Pig	Single doses by intubation in water to moles; observed two weeks.	-	-	551	-	Rowe and Hymas, 1954
Guinea Pig	Single dose, administered by intubation at dosage levels of 0, 100, 150, 200, 250 or 300 mg (equivalent to 0, 330, 500, 666, 833 or 1,000 mg/kg); 6 guinea pigs per dose level.	-	-	1,000	Adverse effects included myotonia, stiffness of extremities, ataxia, paralysis and coma with death caused by ventricular fibrillation.	Hill and Carlisle, 1947
ACUTE, DERMAL				**mg/kg**		
2,4-D (acid)						
Rat	Details not specified.	-	-	1,500	-	USDA, 1984a
Mouse	Details not specified.	-	-	1,400	-	USDA, 1984a

Table 16: Summary of Mammalian Systemic Toxicity Studies for 2,4-Dichlorophenoxyacetic Acid (2,4-D)* (Continued)

Type of Test		Effects				
Formulation Species	**Route of Exposure**	**NOEL**	**LEL**	**LD50**	**Comments**	**Reference**
2,4-D (butyl ester or diethanolomine salt						
Rabbit	Applied to shaved skin of rabbits.	-	-	>3,980	-	Kay et al., 1965
Inhalation **2,4-D (sodium salt)**						
Guinea Pig	Exposed to 2,4-D dust; no details specified.	-	-	-	No signs of adverse systemic effects.	Hill and Carlisle, 1947
Eye Irritation **2,4-D (acid salt, and esters)**						
Species not specified	Applications of dry powder or highly concentrated solutions	-	-	-	Produced irritation of conjunctival membranes and possible corneal damage.	Gehring and Betso, 1978 in USDA, 1984a
Subchronic, Dermal **2,4-D (dimethyl-amine salt, and isoctyl or butyl esters)**				**% of solution**		
Rabbit	Formulations of 2,4-D were applied to intact and abraded rabbit skin at 0.636% and 3.13% 7 hours/day, 5 days/week for 3 weeks.	No NOEL	0.636	-	Adverse reactions were limited to localized skin inflammations in both treated and control groups that were apparently produced by the oil-based solvents used as the vehicle. Treated animals had an increased incidence and severity of subepithelia fibrosis and accompanying mononuclear infiltration of the skin.	Kay et al., 1965

Table 16: Summary of Mammalian Systemic Toxicity Studies for 2,4-Dichlorophenoxyacetic Acid (2,4-D)* (Continued)

Type of Test		Effects				
Formulation Species	**Route of Exposure**	**NOEL**	**LEL**	**LD50**	**Comments**	**Reference**
Subchronic and Chronic, Oral 2,4-D (sodium salt)				**mg/kg/day a.e.**		
Mouse	Fed at dosage levels up to 93 mg/kg/day for 3 weeks to 3 months.	>93	-	-	No adverse effects measured.	Bucher, 1946
Guinea Pig	Groups of 6 guinea pigs were fed 0, 50 or 100 mg daily for 10 days over a 12-day period (doses equivalent to 0, 167 or 333 mg/kg).	333	-	-	No adverse treatment-related effects reported.	Hill and Carlisle, 1947
2,4-D (acid)						
Mouse	Once or twice daily subcutaneous injections at dosage levels of 50 to 90 mg/kg or 3 weeks to 90 days.	<70	70	-	No adverse effects below 70 mg/kg; levels of 70 mg/kg and above resulted in growth retardation.	Bucher, 1946
Mouse	Fed diet containing 0, 5 45 or 90 mg/kg/day for 90 days.	No NOEL	-	-	Histopathological changes in renal tubules occurred in both sexes at the lowest dose tested.	EPA, 1984

Table 16: Summary of Mammalian Systemic Toxicity Studies for 2,4-Dichlorophenoxyacetic Acid (2,4-D)* (Continued)

Type of Test		Effects				
Formulation Species	**Route of Exposure**	**NOEL**	**LEL**	**LD50**	**Comments**	**Reference**
2,4-D (acid)						
Rat	Fed diets containing 0, 100, 200 or 400 ppm daily for 30 days (equivalent to up to 20 mg/kg/day); 7 rats/dose level.	20	-	-	Doses up to 400 ppm (20 mg/kg/day) produced no adverse effects. One death occurred at 400 ppm.	Hill and Carlisle, 1947
Rat	Groups of 5 or 6 female rats fed dosage levels of 0, 3, 10, 30, 100 or 300 mg/kg 5 days/week, for 4 weeks	30	100	-	At dosage levels of 30 mg/kg/day and below, no adverse effects were observed. At doses of 100 mg/kg/day, varying degrees of gastrointestinal irritation, depressed growth rates, and cloudy swelling of the liver were reported. At 300 mg/kg/day animals failed rapidly and died.	Rose and Hymas, 1954
Rat	Groups of 5 female rats fed diets containing 0, 100, 300, 1,000, 3,000 or 10,000 ppm daily for 113 days; doses equivalent to 0, 5, 15, 50, 150 or 500 mg/kg/day.	15	50	-	No adverse effects at doses below 300 ppm. At 1,000 ppm toxic effects were characterized by depressed growth rate, increased mortality, increased liver weights, and cloudy swelling of the liver.	Rowe and Hymas, 1954

Table 16: Summary of Mammalian Systemic Toxicity Studies for 2,4-Dichlorophenoxyacetic Acid (2,4-D)* (Continued)

Type of Test		Effects				
Formulation Species	**Route of Exposure**	**NOEL**	**LEL**	**LD50**	**Comments**	**Reference**
2,4-D (acid)						
Rat	Fed at dietary levels of 0, 5, 25, 125, or 1,250 ppm (equivalent to 0, 0.25, 1.25, 6.25 or 62.5 mg/kg/day) daily for two years, 25/sex/group.	62.5	-	-	No significant differences in growth rate, survival rate, organ weights, or hematological values as compared to controls were observed.	Hansen et al., 1971
Rat	Fed diets containing 0, 1, 5, 15 or 45 mg/kg/day for 90 days.	No NOEL	-	-	Histopathological changes in renal cortical tubules and increased thyroid weight occurred in the 1 mg/kg/day dose group.	EPA, 1984
Dog	Fed capsules containing 0, 2, 5, 10 or 20 mg/kg daily for 5 days/week for 13 weeks, four/group.	10	20	-	All dogs survived dosages up to 10 mg/kg/day and no significant changes in body weight, organ weights, or blood counts weights, or blood counts were observed. At 20 mg/kg/day, three or four dogs died between 18 and 49 days. All four dogs displayed ataxia, weakness, dysphagia, (difficulty in swallowing), bleeding gums, buccal mucosa necrosis, stiff hind legs, liver and kidney alterations, and decreased lymphocyte counts.	Drill and Hiratzka, 1953

Table 16: Summary of Mammalian Systemic Toxicity Studies for 2,4-Dichlorophenoxyacetic Acid (2,4-D)* (Continued)

Type of Test		Effects				
Formulation Species	**Route of Exposure**	**NOEL**	**LEL**	**LD50**	**Comments**	**Reference**
2,4-D (acid)						
Dog	Groups of male and female dogs were fed 0, 10, 50, 100 or 500 ppm daily in the diet for two years (equivalent to 0, 0.4, 2, 4 or 20 mg/kg/day); three/sex/group.	20	-	-	No treatment-related effects were noted at any dose tested.	Hansen et al., 1971

Table 17: Summary[2] of Subchronic Studies on 2,4-D Tested in Various Animal Species

Chemical Species	Route	Dose (mg/kg bw/d) [in acid equivalents]	NOEL/NOAEL[b] (mg/kg/bw/d)	Reference
13-week:Rat				
2,4-D (100% pure)	diet	0, 15, 60, 100, 150	15 (females only)	Gorzinski et al., 1981a
2,4-D (97.5% pure)	diet	0, 15, 60, 100, 150	15 (females only)	Gorzinski et al., 1981b
2,4-D (97.5% pure)	diet	0, 1, 5, 15, 45	1 (males only)	Serota, 1983a
2,4-D (96.1% pure)	diet	0, 1, 15, 100, 300	15	Charles et al., 1996a
2,4-D ethylhexyl ester	diet	0, 1, 15, 100, 300	15	Charles et al., 1996a
2,4-D dimethylamine salt	diet	0, 1, 15, 100, 300	15	Charles et al., 1996a
2,4-D butoxyethyl ester	diet	0, 1, 15, 100, 300	15	Dow, 1991a
2,4-D triisopropanolamine salt	diet	0, 1, 15, 100, 300	15	Dow, 1991b
2,4-D isopropylamine salt	diet	0, 1, 15, 100, 300	15	Dow, 1991c
12-week: Rat				
2,4-D sodium salt	intraperitoneal	0, 100, 150	-	Lukowicz-Ratajczak and Krechniak, 1988
13-week: Mouse				
2,4-D acid	diet	0, 5, 15, 45, 90	-	Serota, 1983b
2,4-D acid	diet	0, 1, 15, 100, 300	15	Schulze, 1991
14-day: Mouse				
2,4-D acid	oral intubation	50, 100, 200	-	Kuntz et al., 1990
4-day: Mouse				
2,4-D acid	diet	100	-	Lundgren et al., 1987
13-week: Dog				
2,4-D acid	gelatin capsule	0, 0.3, 1, 3, 10	1	ITF, 1990
2,4-D acid	diet	0, 0.5, 1.0, 3.75, 7.5	1	Charles et al. 1996c
2,4-D dimethylamine salt	diet	0, 1.0, 3.75, 7.5	1	Charles et al. 1996c
2,4-D 2-ethylhexyl ester	diet	0, 1.0, 3.75, 7.5	1	Charles et al. 1996c

Table 17: Summary[2] of Subchronic Studies on 2,4-D Tested in Various Animal Species (Continued)

Chemical Species	Route	Dose (mg/kg bw/d) [in acid equivalents]	NOEL/NOAEL[b] (mg/kg/bw/d)	Reference
21-day: Rabbit				
2,4-D acid	dermal	0, 10, 100, 1000	1000	Schulze, 1990a
2,4-D dimethylamine salt	dermal	0, 10, 100, 300	10	Schulze, 1990b
2,4-D 2-ethylhexyl ester	dermal	0, 10, 100, 1000	10	Schulze, 1990c
2,4-D triisopropanolamine salt	dermal	0, 55, 193, 553	553	Mizell et al., 1989a
2,4-D isopropylamine salt	dermal	0, 39, 98, 275	275	Mizell et al., 1989b
2,4-D butoxyethyl ester	dermal	0, 32, 96, 321	321	Mizell et al., 1989c

[a]adapted from Munro et al. (1992)
[b]NOEL = no-observed-effect level; NOAEL = no-observed-adverse-effect level

Table 18: Summary of Developmental and Reproductive Toxicity Studies on 2,4-D Tested in Various Animal Species

Chemical Species	**Route**	**Exposure Duration**	**Dose (mg/kg bw/d)**	**NOEL/NOAEL[a] (mg/kg bw/d)**	**Reference**
Developmental: Rabbit					
2,4-D triisopropanolamine salt	gavage	GD[b] 7-19	0, 10, 30, or 75[c]	10[d]; >75[e]	Liberacki et al., 1994
2,4-D isopropylamine salt	gavage	GD 7-19	0, 10, 30, or 75[c]	10[d]; >75[e]	Liberacki et al., 1994
2,4-D butoxyethyl ester	gavage	GD[b]7-19	0, 10, 30, or 75[c]	10[d]; >75[e]	Liberacki et al., 1994
2,4-D acid	gavage	GD 6-18	0, 10, 30, or 90	30[d];>90[e]	Hoberman, 1990
2,4-D dimethylamine salt	gavage	GD 6-18	0, 10, 30, or 90[c]	30[d];>90[e]	Martin, 1991
2,4-D ethylhexyl ester	gavage	GD 6-18	0, 10, 30, or 75[c]	30[d];>75[e]	Martin, 1992a
Developmental: Rat					
2,4-D isooctyl ester	oral	GD 6-15	0, 12.5, 25, 50, 75, or 87.5[c]	>87.5[d]; 25[e]	Schwetz et al. 1971
2,4-D propylene glycol butyl ether	oral	GD 6-15	0, 12.5, 25, 50, 75, or 87.5[c]	>87.5[d]; 25[e]	Schwetz et al. 1971
2,4-D acid	oral	GD 6-15	0, 12.5, 25, 50, 75, or 87.5	>87.5[d]; 25[e]	Schwetz et al. 1971
2,4-D isoctyl ester	oral	GD 5-8	0, or 87.5[c]	>87.5[d,e]	Schwetz et al., 1971
2,4-D propylene glycol butyl ether	oral	GD 5-8	0, or 87.5[c]	>87.5[d,e]	Schwetz et al., 1971
2,4-D isoctyl ester	oral	GD 8-11	0, 50, or 87.5[c]	>87.5[d];<50[e]	Schwetz et al., 1971
2,4-D isoctyl ester	oral	GD 12-15	0, 50, or 87.5[c]	>87.5[d]	Schwetz et al., 1971
2,4-D isoctyl ester	oral	GD 6-15	0, 50, or 150	>150[d];50[e]	Khera and McKinley, 1972
2,4-D butyl ester	oral	GD 6-15	0, 50, or 150	>150[d];50[e]	Khera and McKinley, 1972
2,4-D butoxyethynol	oral	GD 6-15	0, 50, or 150	>150[d];50[e]	Khera and McKinley, 1972
2,4-D dimethylamine salt (49.5%)	oral	GD 6-15	0, 100, or 300	>300[d];50[e]	Khera and McKinley, 1972
2,4-D acid	oral	GD 6-15	0, 50, or 100	>100[d];50[e]	Khera and McKinley, 1972
2,4-D acid	oral	GD 6-15	0, 25, 50, or 100	>100[d];50[e]	Khera and McKinley, 1972
2,4-D acid	oral	GD 6-15	0, 25, 50, 100, or 150	>150[d];50[e]	Khera and McKinley, 1972
2,4-D propylene glycol butyl ether	oral	GD 6-15	0, 6.25, 12.5, 25, or 87.5[c]	>87.5[d];25[e]	Unger et al., 1981
2,4-D isoctyl ester	oral	GD 6-15	0, 6.25, 12.5, 25, or 87.5[c]	>87.5[d];25[e]	Unger et al., 1981
2,4-D acid	gavage	GD 6-15	0, or 115	<115[d,e]	Chernoff et al., 1990
2,4-D ethylhexyl ester	gavage	GD 6-15	0, 10, 30, or 90[c]	10[d,e]	Martin, 1992b
2,4-D dimethylamine salt	gavage	GD 6-15	0, 12.5, 50, or 100[c]	12.5[d]; 50[c]	Lochry, 1990

Table 18: Summary of Developmental and Reproductive Toxicity Studies on 2,4-D Tested in Various Animal Species (Continued)

Chemical Species	**Route**	**Exposure Duration**	**Dose (mg/kg bw/d)**	**NOEL/NOAEL[a] (mg/kg bw/d)**	**Reference**
Developmental: Mouse					
2,4-D acid	gavage	GD 6-15	0, 8, 25, or 75	25[d,e]	Nemec et al., 1984
2,4-D acid	oral	GD 7-15	0, or 0.56 mM/kg bw	<0.56[d];>0.56[e]	Courtney, 1977
2,4-D acid	oral	GD 11-14	0, or 0.80 mM/kg bw	<0.80[d,e]	Courtney, 1977
2,4-D acid	oral	GD 12-15	0, or 1 mM/kg bw	<1[d,e]	Courtney, 1977
2,4-D acid	subcutaneous	GD 12-15	0, or 1 mM/kg bw	<1[d,e]	Courtney, 1977
2,4-D isopropyl ester	oral	GD 7-15	0, or 0.56 mM/kg bw	<0.56[d,e]	Courtney, 1977
2,4-d n-butyl ester	oral	GD 7-15	0, 0.56 mM/kg bw	>0.56[d,e]	Courtney, 1977
2,4-d n-butyl ester	oral	GD 12-15	0, or 1 mM/kg bw	<1[d,e]	Courtney, 1977
2,4-D isoctyl ester	oral	GD 7-15	0, or 0.56 mM/kg bw	<0.56[d,e]	Courtney, 1977
2,4-D propylene glycol butyl ether	oral	GD 7-15	0, or 0.56 mM/kg bw	<0.56[d,e]	Courtney, 1977
2,4-D propylene glycol butyl ether	oral	GD 12-15	0, or 1 mM/kg bw	>1[d];<1[e]	Courtney, 1977
2,4-D acid	oral	GD 8-12	0, or 87.5	>87.5[d,e]	Kavlock et al., 1987
2,4-D propylene glycol butyl ether	oral	GD 8-12	0, or 87.5	>87.5[d,e]	Kavlock et al., 1987
2,4-D isoctyl ester	oral	GD 8-12	0, or 87.5	>87.5[d,e]	Kavlock et al., 1987
2,4-D acid	subcutaneous	GD 6-14	0, or 100	<100[d,e]	Bionetics, 1968
2,4-D acid	subcutaneous	GD 6-14	0, or 98	<98[d,e]	Bionetics, 1968
2,4-D acid	subcutaneous	GD 6-14	0, or 215	>215[d,e]	Bionetics, 1968
2,4-D acid	subcutaneous	GD 6-14	0, or 50	>50[d,e]	Bionetics, 1968
2,4-D acid	oral	GD 6-14	0, or 100	<100[d,e]	Bionetics, 1968
Developmental: Hamster					
2,4-D	oral	GD 6-10	0, 20, 40, 60, or 100	>100[e]	Collins and Williams, 1971
2,4-D	oral	GD 6-10	0, 40, 60, or 100	>100[e]	Collins and Williams, 1971
2,4-D	oral	GD 6-10	0, 40, 60, or 100	40	Collins and Williams, 1971

Table 18: Summary of Developmental and Reproductive Toxicity Studies on 2,4-D Tested in Various Animal Species (Continued)

Chemical Species	**Route**	**Exposure Duration**	**Dose (mg/kg bw/d)**	**NOEL/NOAEL[a] (mg/kg bw/d)**	**Reference**
Reproductive Toxicity: Rat					
2,4-D acid	diet	2-generation	0, 5, 20, or 80	20[d];5[e]	Rodwell, 1984

[a]NOEL=no-observed-effect level; NOAEL=no-observed-adverse-effect level
[b]GD=gestational days
[c]2,4-D acid equivalents
[d]maternal
[e]fetal

Adapted from Kennepohl and Muro (2000)

Table 19: Summary of Mammalian Carcinogenicity Studies for 2,4-Dichlorophenoxyacetic Acid (2,4-D)

Type of Test		Effects				
Formulation Species	**Nature of Exposure**	**NOEL**	**LEL**	**LD50**	**Comments**	**Reference**
CARCINOGENIC		**mg/kg/day**				
2,4-D (acid or isopropyl, n-butyl, and isoctyl esters)						
Mouse (C57BL/6xC3H/Aanl)F_1 or (C57BL/6xAFR)F_1	Mice were administered by gavage doses of 46.4 or 100 mg/kg/day from 7 to 28 days, then maintained on diets containing 149 or 323 ppm 2,4-D until termination of the experiment at 78 weeks. Mice treated with isopropyl, n-butyl, or isoctyl esters were initially treated with 46.4 mg/kg/day for days 7 to 28, then fed diets containing 111, 149 or 130 ppm respectively, until termination at 78 weeks.	Highest Dose Treated	-	-	The tumor incidence in any group was not significantly different from that in control animals.	Innes et al., 1969
CARCINOGENIC 2,4-D (acid)						
Dog	Groups of male and female beagle dogs, three per sex dose group, were fed 0, 10, 50, 100 or 500 ppm 2,4-D in the diet for two years (equivalent to 0, 0.4, 2, 4 or 20 mg/kg/day).	20	-	-	Although one dog at the 500 ppm dosage level developed an adrenal hemangioma, the authors stated that there were no treatment-related lesions or tumors.	Hansen et al., 1971

Table 19: Summary of Mammalian Carcinogenicity Studies for 2,4-Dichlorophenoxyacetic Acid (2,4-D) (continued)

Type of Test		Effects				
Formulation Species	**Nature of Exposure**	**NOEL**	**LEL**	**LD50**	**Comments**	**Reference**
CARCINOGENIC 2,4-D (acid)						
Rat	Groups of 25 rats/sex/dose group were fed diets containing 0, 5, 25, 125, 625 or 1,250 ppm for two years (equivalent to 0, 0.25, 1.25, 6.25, 31.25 or 62.5 mg/kg/day)	31.25	62.5	-	No target organ tumors were observed and the individual tumor types, normally age-related in this strain, were randomly and widely distributed. A dose-related increase in total tumors and malignant tumors occurred in both make and females. Only the 1,250 ppm level in males showed a significant increase in the incidence of malignant tumors. IARC considered the study inadequate for assessing carcinogenicity (IARC, 1977 and 1982)	Hansen et al., 1971
CARCINOGENIC 2,4-D (acid)						
Rat	Groups of 10 male and 12 female offspring of dams treated during pregnancy received 0 or 1,000 ppm in drinking water for two years (doses equivalent to 0 or 35 mg/kg/day).	35	-	-	No significant differences in hematology or clinical chemistry values or in pathology. Elevated mortality, decreased food and water intake, poor general condition observed in treated group.	Bjorklund and Erne, 1966

Table 19: Summary of Mammalian Carcinogenicity Studies for 2,4-Dichlorophenoxyacetic Acid (2,4-D) (continued)

Type of Test		Effects				
Formulation Species	**Nature of Exposure**	**NOEL**	**LEL**	**LD50**	**Comments**	**Reference**
CARCINOGENIC 2,4-D (acid)						
Rat	Groups of 50 males and 50 females fed diets containing 0, 1, 5, 15 or 45 mg/kg/dy/2 yrs	1.0	5	-	No treatment related neoplastic lesions were seen at any dose. There was an increase in the incidence of brain astrocytomas in the high dose group males. The brain astrocytomas were not attributable to treatment because they did not occur earlier in treated rats than in controls. The NOAEL was 1 mg/kg/dy/2yrs.	Serota, 1986
Rat	Groups of 50 males and 50 females fed diets containing 0, 5, 75 or 150 mg/kg/dy/2 yrs	M: 75 F: 5	150 75		No brain tumors or any evidence of carcinogenicity was seen in the same strain of rats used in the Serota study. The high dose level in Jefries study was more than three times that to the high dose Serota study animals. Malignant tumors at different sites were seen in both the control and treated rats.	Charles et al, 1996
Mice	Groups of 50 males and 50 females fed diets containing 0, 1, 15 or 45 mg/kg/dy/2 yrs	1.0	15		No evidence of carcinogenicity was seen; the tumor types and incidence were similar in the treated and control groups. The NOAEL was based on lack of increase in kidney weights and renal lesions.	Serota, 1987

Table 19: Summary of Mammalian Carcinogenicity Studies for 2,4-Dichlorophenoxyacetic Acid (2,4-D) (continued)

Type of Test		Effects				
Formulation Species	**Nature of Exposure**	**NOEL**	**LEL**	**LD50**	**Comments**	**Reference**
2,4-D (acid)						
Mice .	Groups of 50 males and 50 females. Males were fed diets containing 0, 5, 62.5 or 125 mg/kg/dy/2 yrs; females were fed diets containing 0, 5, 150 or 300 mg/kg/dy/2 yrs	M: 5 F: 5	62.5 150		No oncogenic effect was noted in the study. No statistically significant treatment related changes in hematology parameters, histological treatment related changes included minimal degeneration with regeneration of the descending portion of the renal proximal tubules	Charles et al, 1996; Stott et al, 1995
Dogs	Groups of 5 males and 5 females fed diets containing 0, 1, 5 or 7.5 mg/kg/dy/1 year	1.0	5.0		The clinical pathology findings included reduction in body weight gain, decreased food consumption and minor increases in blood urea nitrogen, creatinine and alanine aminotransferase. No evidence of carcinogenic findings.	Charles et al, 1996
2,4-D (dimethylamine salt)						
Rat	Groups of 120 male and 45 female rats were fed diets containing no 2,4-D or levels equivalent to one-tenth the LD50 for 27 months.	-	-	-	No treatment-related adverse effects reported.	Archipov and Kozlova, 1974
Mice	Groups of 100 female mice were fed diets containing no 2,4-D or levels equivalent to one-tenth the LD50 for 27 months	-	-	-	No treatment-related adverse effects reported.	Archipov and Kozlova, 1974

[a]The NOEL, LEL and LD50s for 2,4-D amines and esters expressed as the acid equivalent.

Table 20: NOEL and LEL Used to Calculate Margins of Safety for Noncarcinogenic Effects Resulting From Exposure to 2,4-D

Effect	Study	NOEL	LEL	Reference
		mg/kg-day		
Systemic Toxicity	Subchronic-oral dogs. Fed diets containing 0, 0.5, 1.0, 3.75, 7.5 mg/kg/day for 90 days.	1.0	3.75[a]	Charles, et al., 1996
Reproductive	2-generation rat reproduction. Groups of 30 males and 30 females maintained on diets containing 0, 5, 20, 80 mg/kg/day throughout life.	5	20[b]	Rodwell, 1985
Teratogenic	Oral (gavage) rat dosage levels of 0, 8, 25, 75 mg/kg/day on days 6 through 15 of gestation.	25	75[c]	Rodwell, 1983

[a]Significant increase in blood urea nitrogen, creatinine and alanine transaminase
[b]Decreased pup weights. Increased nuclear density in renal medullary tubules of Fo Generation
[c]At 75 mg/kg/day level, increase in fetal skeletal variations. The increases were not statistically significant, but considered treatment related.

Table 21: Margins of Safety for Various Effects From a Single Exposure to 2,4-D Through Dermal Contact With Vegetation

Days After Application	Single Human Dose (mg/kg)		MOS Systemic Effects NOEL = 1 mg/kg/day		MOS Reproductive and Teratogenic Effects NOEL = 25 mg/kg/day	
	Amine (low dose)	Amine (high dose)	Amine (low dose)	Amine (high dose)	Amine (low dose)	Amine (high dose)
Immediately	1.50E-03	6.00E-03	667	167	16667	4167
1	NA	NA	NA	NA	NA	NA

Table 22: Margins of Safety for Various Effects from a Single Exposure to 2,4-D Through Dermal Contact with Water (Swimming)

Scenario 1: Small Pond

Days After Application	Single Human Dose (mg/kg)		MOS Systemic Effects NOEL = 1 mg/kg/day		MOS Reproductive/ Teratogenic Effects NOEL = 25 mg/kg/day	
	Amine	Ester	Amine	Ester	Amine	Ester
Immediately	4.93E-08	1.13E-05	2.03E+07	8.85E+04	5.07E+08	2.21E+06
1	4.63E-08	1.06E-05	2.16E+07	9.47E+04	5.40E+08	2.37E+06
2	4.34E-08	9.92E-06	2.30E+07	1.01E+05	5.76E+08	2.52E+06
3	4.08E-08	9.31E-06	2.45E+07	1.07E+05	6.13E+08	2.68E+06
4	3.83E-08	8.74E-06	2.61E+07	1.14E+05	6.53E+08	2.86E+06
5	3.60E-08	8.21E-06	2.78E+07	1.22E+05	6.95E+08	3.05E+06
6	3.38E-08	7.71E-06	2.96E+07	1.30E+05	7.40E+08	3.24E+06
7	3.17E-08	7.24E-06	3.16E+07	1.38E+05	7.89E+08	3.45E+06
8	2.98E-08	6.80E-06	3.36E+07	1.47E+05	8.40E+08	3.68E+06
9	2.80E-08	6.38E-06	3.58E+07	1.57E+05	8.94E+08	3.92E+06
10	2.62E-08	5.99E-06	3.81E+07	1.67E+05	9.53E+08	4.17E+06
11	2.46E-08	5.63E-06	4.06E+07	1.78E+05	1.01E+09	4.44E+06
12	2.31E-08	5.28E-06	4.32E+07	1.89E+05	1.08E+09	4.73E+06
13	2.17E-08	4.96E-06	4.60E+07	2.02E+05	1.15E+09	5.04E+06
14	2.04E-08	4.66E-06	4.91E+07	2.15E+05	1.23E+09	5.37E+06
15	1.92E-08	4.37E-06	5.22E+07	2.29E+05	1.31E+09	5.72E+06
16	1.80E-08	4.10E-06	5.56E+07	2.44E+05	1.39E+09	6.09E+06
17	1.69E-08	3.85E-06	5.92E+07	2.59E+05	1.48E+09	6.49E+06
18	1.59E-08	3.62E-06	6.31E+07	2.76E+05	1.58E+09	6.91E+06
19	1.49E-08	3.40E-06	6.72E+07	2.94E+05	1.68E+09	7.36E+06
20	1.40E-08	3.19E-06	7.16E+07	3.13E+05	1.79E+09	7.84E+06
21	1.31E-08	3.00E-06	7.62E+07	3.34E+05	1.91E+09	8.35E+06
22	1.23E-08	2.81E-06	8.12E+07	3.56E+05	2.03E+09	8.89E+06

Table 22: Margins of Safety for Various Effects from a Single Exposure to 2,4-D Through Dermal Contact with Water (Swimming) (Continued)

Scenario 2: Irrigation Ditch

Days After Application	Single Human Dose (mg/kg)		MOS Systemic Effects NOEL = 1 mg/kg/day		MOS Reproductive/ Teratogenic Effects NOEL = 25 mg/kg/day	
	Amine	Ester	Amine	Ester	Amine	Ester
Immediately	6.57E-07	1.44E-04	1.52E+06	6.94E+03	3.81E+07	1.74E+05
1	6.17E-07	1.35E-04	1.62E+06	7.39E+03	4.05E+07	1.85B+05
2	5.79E-07	1.27E-04	1.73E+06	7.87E+03	4.32E+07	1.97E+05
3	5.44E-07	1.19E-04	1.84E+06	8.38E+03	4.60E+07	2.10E+05
4	5.11E-07	1.12E-04	1.96E+06	8.93E+03	4.89E+07	2.23E+05
5	4.80E-07	1.05E-04	2.09E+06	9.51E+03	5.21E+07	2.38E+05
6	4.50E-07	9.88E-05	2.22E+06	1.01E+04	5.55E+07	2.53E+05
7	4.23E-07	9.27E-05	2.37E+06	1.08E+04	5.91E+07	2.70E+05
8	3.97E-07	8.71E-05	2.52E+06	1.15E+04	6.30E+07	2.87E+05
9	3.73E-07	8.18E-05	2.68E+06	1.22E+04	6.71E+07	3.06E+05
10	3.50E-07	7.68E-05	2.86E+06	1.30E+04	7.14E+07	3.26E+05
11	3.29E-07	7.21E-05	3.04E+06	1.39E+04	7.61E+07	3.47E+05
12	3.09E-07	6.77E-05	3.24E+06	1.48E+04	8.10E+07	3.69E+05
13	2.90E-07	6.35E-05	3.45E+06	1.57E+04	8.63E+07	3.93E+05
14	2.72E-07	5.97E-05	3.68E+06	1.68E+04	9.19E+07	4.19E+05
15	2.55E-07	5.60E-05	3.92E+06	1.79E+04	9.79E+07	4.46E+05
16	2.40E-07	5.26E-05	4.17E+06	1.90E+04	1.04E+08	4.75E+05
17	2.25E-07	4.94E-05	4.44E+06	2.03E+04	1.11E+08	5.06E+05
18	2.11E-07	4.64E-05	4.73E+06	2.16E+04	1.18E+08	5.39E+05
19	1.98E-07	4.35E-05	5.04E+06	2.30E+04	1.26E+08	5.74E+05
20	1.86E-07	4.09E-05	5.37E+06	2.45E+04	1.34E+08	6.12E+05
21	1.75E-07	3.84E-05	5.72E+06	2.61E+04	1.43E+08	6.51E+05
22	1.64E-07	3.60E-05	6.09E+06	2.77E+04	1.52E+08	6.94E+05

Table 22: Margins Of Safety For Various Effects From A Single Exposure To 2,4-D Through Dermal Contact With Water (Swimming) (Continued)

Scenario 3: Large Lake

Days After Application	Single Human Dose (mg/kg)		MOS Systemic Effects NOEL = 1 mg/kg/day		MOS Reproductive/ Teratogenic Effects NOEL = 25 mg/kg/day	
	Amine	Ester	Amine	Ester	Amine	Ester
Immediately	7.89E-10	1.41E-07	1.27E+09	7.09E+06	3.17E+10	1.77E+08
1	7.40E-10	1.32E-07	1.35E+09	7.57E+06	3.38E+10	1.89E+08
2	6.95E-10	1.24E-07	1.44E+09	9.07E+06	3.60E+10	2.02E+08
3	6.53E-10	1.16E-07	1.53E+09	8.59E+06	3.83E+10	2.15E+08
4	6.13E-10	1.09E-07	1.63E+09	9.15E+06	4.09E+10	2.29E+08
5	5.75E-10	1.03E-07	1.74E+09	9.74E+06	4.34E+10	2.44E+08
6	5.40E-10	9.64E-08	1.85E+09	1.04E+07	4.63E+10	2.59E+08
7	5.07E-10	9.05E-08	1.97E+09	1.11E+07	4.93E+10	2.76E+08
8	4.76E-10	8.49E-08	2.10E+09	1.18E+07	5.25E+10	2.94E+08
9	4.48E-10	7.98E-08	2.23E+09	1.25E+07	5.58E+10	3.13E+08
10	4.20E-10	7.49E-08	2.38E+09	1.34E+07	5.95E+10	3.34E+08
11	3.94E-10	7.03E-08	2.54E+09	1.42E+07	6.34E+10	3.56E+08
12	3.70E-10	6.60E-08	2.70E+09	1.51E+07	6.75E+10	3.79E+08
13	3.48E-10	6.20E-08	2.88E+09	1.61E+07	7.19E+10	4.03E+08
14	3-26E-10	4.77E-08	3.06E+09	2.10E+07	7.66E+10	5.25E+08
15	3.06E-10	5.46E-08	3.26E+09	1.83E+07	8.16E+10	4.57E+08
16	2.88E-10	5.13E-08	3.48E+09	1.95E+07	8.69E+10	4.87E+08
17	2.70E-10	4.82E-08	3.70E+09	2.08E+07	9.25E+10	5.19E+08
18	2.54E-10	4.52E-08	3.94E+09	2.21E+07	9.86E+10	5.53E+08
19	2.38E-10	4.25E-08	4.20E+09	2.35E+07	1.05E+11	5.89E+08
20	2.24E-10	3.99E-08	4.47E+09	2.51E+07	1.12E+11	6.27E+08
21	2. 10E-10	3.74E-08	4.76E+09	2.67E+07	1.19E+11	6.68E+08
22	1.97E-10	3.52E-08	5.07E+09	2.84E+07	1.27E+11	7.11E+08

Table 23: Margins of Safety for Various Effects from a Single Exposure to 2,4-D Through Dermal Contact with Sediments

Scenario 1: Small Pond

Days After Application	Single Human Dose (mg/kg)		MOS Systemic Effect NOEL = 1 mg/kg/day		MOS Reproductive/ Teratogenic Effect NOEL = 25 mg/kg/day	
	Amine	Ester	Amine	Ester	Amine	Ester
Immediately	7.34E-09	4.46E-07	1.36E+08	2.24E+06	3.41E+09	5.61E+07
1	7.26E-09	4.41E-07	1.38E+08	2.27E+06	3.44E+09	5.67E+07
2	7.17E-09	4.36E-07	1.39E+08	2.30E+06	3.48E+09	5.74E+07
3	7.09E-09	4.31E-07	1.41E+08	2.32E+06	3.53E+09	5.81E+07
4	7.01E-09	4.26E-07	1.43E+08	2.35E+06	3.57E+09	5.87E+07
5	6.93E-09	4.21E-07	1.44E+08	2.38E+06	3.61E+09	5.94E+07
6	6.85E-09	4.16E-07	1.46E+08	2.40E+06	3.65E+09	6.01E+07
7	6.77E-09	4.11E-07	1.48E+08	2.43E+06	3.69E+09	6.08E+07
8	6.69E-09	4.06E-07	1.49E+08	2.46E+06	3.73E+09	6.15E+07
9	6.62E-09	4.02E-07	1.51E+08	2.49E+06	3.78E+09	6.22E+07
10	6.54E-09	3.97E-07	1.53E+08	2.52E+06	3.82E+09	6.30E+07
11	6.47E-09	3.93E-07	1.55E+08	2.55E+06	3.87E+09	6.37E+07
12	6.39E-09	3.88E-07	1.56E+08	2.58E+06	3.91E+09	6.44E+07
13	6.32E-09	3.84E-07	1.58E+08	2.61E+06	3.96E+09	6.52E+07
14	6.25E-09	3.79E-07	1.60E+08	2.64E+06	4.00E+09	6.59E+07
15	6.17E-09	3.75E-07	1.62E+08	2.67E+06	4.05E+09	6.67E+07
16	6.10E-09	3.71E-07	1.64E+08	2.70E+06	4.10E+09	6.75E+07
17	6.03E-09	3.66E-07	1.66E+08	2.73E+06	4.14E+09	6.83E+07
18	5.96E-09	3.62E-07	1.68E+08	2.76E+06	4.19E+09	6.91E+07
19	5.89E-09	3.58E-07	1.70E+08	2.79E+06	4.24E+09	6.99E+07
20	5.83E-09	3.54E-07	1.72E+08	2.83E+06	4.29E+09	7.07E+07
21	5.76E-09	3.50E-07	1.74E+08	2.86E+06	4.34E+09	7.15E+07
22	5.69E-09	3.46E-07	1.76E+08	2.89E+06	4.39E+09	7.23E+07

Table 23: Margins of Safety for Various Effects from a Single Exposure to 2,4-D Through Dermal Contact with Sediments (Continued)

Scenario 2: Irrigation Ditch

Days After Application	Single Human Dose (mg/kg)		MOS Systemic Effect NOEL = 1 mg/kg/day		MOS Reproductive/ Teratogenic Effect NOEL = 25 mg/kg/day	
	Amine	Ester	Amine	Ester	Amine	Ester
Immediately	1.15E-07	5.93E-06	8.67E+06	1.69E+05	2.17E+08	4.22E+06
1	1.14E-07	5.86E-06	8.77E+06	1.71E+05	2.19E+08	4.27E+06
2	1.13E-07	5.79E-06	8.87E+06	1.73E+05	2.22E+08	4.32E+ 06
3	1.11E-07	5.72E-06	8.97E+06	1.75E+05	2.24E+08	4.37E+06
4	1.10E-07	5.66E-06	9.08E+06	1.77E+05	2.27E+08	4.42E+06
5	1.09E-07	5.59E-06	9.18E+06	1.79E+05	2.30E+08	4.47E+06
6	1.08E-07	5.53E-06	9.29E+06	1.81E+05	2.32E+08	4.52E+06
7	1.06E-07	5.47E-06	9.40E+06	1.83E+05	2.35E+08	4.57E+06
8	1.05E-07	5.40E-06	9.51E+06	1.85E+05	2.38E+09	4.63E+06
9	1.04E-07	5.34E-06	9.62E+06	1.87E+05	2.40E+08	4.68E+06
10	1.03E-07	5.28E-06	9.73E+06	1.89E+05	2.43E+08	4.74E+06
11	1.02E-07	5.22E-06	9.84E+06	1.92E+05	2.46E+08	4.79E+06
12	1.00E-07	5.16E-06	9.96E+06	1.94E+05	2.49E+08	4.85E+06
13	9.93E-08	5.10E-06	1.01E+07	1.96E+05	2.52E+08	4.90E+06
14	9.81E-08	5.04E-06	1.02E+07	1.98E+05	2.55E+08	4.96E+06
15	9.70E-08	4.98E-06	1.03E+07	2.01E+05	2.58E+08	5.02E+06
16	9.59E-08	4.93E-06	1.04E+07	2.03E+05	2.61E+08	5.08E+06
17	9.48E-08	4.87E-06	1.05E+07	2.05E+05	2.64E+08	5.13E+06
18	9.37E-08	4.81E-06	1.07E+07	2.08E+05	2.67E+08	5.19E+06
19	9.26E-08	4.76E-06	1.08E+07	2.10E+05	2.70E+08	5.25E+06
20	9.16E-08	4.70E-06	1.09E+07	2.13E+05	2.73E+08	5.32E+06
21	9.05E-08	4.65E-06	1.10E+07	2.15E+05	2.76E+08	5.38E+06
22	8.95E-08	4.60E-06	1.12E+07	2.18E+05	2.79E+08	5.44E+06

Table 23: Margins of Safety for Various Effects from a Single Exposure to 2,4-D Through Dermal Contact with Sediments (Continued)

Scenario 3: Large Lake

Days After Application	Single Human Dose (mg/kg)		MOS Systemic Effect NOEL = 1 mg/kg/day		MOS Reproductive/ Teratogenic Effect NOEL = 25 mg/kg/day	
	Amine	Ester	Amine	Ester	Amine	Ester
Immediately	1.05E-10	6.56E-09	9.53E+09	1.53E+08	2.38E+11	3.81E+09
1	1.04E-10	6.48E-09	9.65E+09	1.54E+08	2.41E+11	3.86E+09
2	1.02E-10	6.41E-09	9.76E+09	1.56E+08	2.44E+11	3.90E+09
3	1.01E-10	6.33E-09	9.87E+09	1.58E+08	2.47E+11	3.95E+09
4	1.00E-10	6.26E-09	9.99E+09	1.60E+08	2.50E+11	3,99E+09
5	9.90E-11	6.19E-09	1.01E+10	1.62E+08	2.53E+11	4.04E+09
6	9.79E-11	6.12E-09	1.02E+10	1.64E+08	2.55E+11	4.09E+09
7	9.67E-11	6.05E-09	1.03E+10	1.65E+08	2.58E+11	4.14E+09
8	9.56E-11	5.98E-09	1.05E+10	1.67E+08	2.61E+11	4.18E+09
9	9.45E-11	5.91E-09	1.06E+10	1.69E+08	2.64E+11	4.23E+09
10	9.34E-11	5.84E-09	1.07E+10	1.71E+08	2.68E+11	4-28E+09
11	9.24E-11	5.77E-09	1.08E+10	1.73E+08	2.71E+11	4.33E+09
12	9.13E-11	5.71E-09	1.10E+10	1.75E+08	2.74E+11	4.38E+09
13	9.03E-11	5.64E-09	1.11E+10	1.77E+08	2.77E+11	4.43E+09
14	8.92E-11	5.58E-09	1.12E+10	1.79E+08	2.80E+11	4.48E+09
15	8.82E-11	5.51E-09	1.13E+10	1.81E+08	2.83E+11	4.54E+09
16	8.72E-11	5.45E-09	1.15E+10	1.84E+08	2.87E+11	4.59E+09
17	8.62E-11	5.39E-09	1.16E+10	1.96E+08	2.90E+11	4.64E+09
18	8.52E-11	5.32E-09	1.17E+10	1.88E+08	2.93E+11	4.70E+09
19	8.42E-11	5.26E-09	1.19E+10	1.90E+08	2.97E+11	4.75E+09
20	8.32E-11	5.20E-09	1.20E+10	1.92E+08	3.00E+11	4.81E+09
21	8.23E-11	5.14E-09	1.22E+10	1.94E+08	3.04E+11	4.86E+09
22	8.13E-11	5.08E-09	1.23E+10	1.97E+08	3.07E+11	4.92E+09

Table 24: Margins of Safety for Various Effects From a Single Exposure to 2,4-D Through Ingestion of Fish

Scenario 1: Small Pond

Days After Application	Single Human Dose (mg/kg)		MOS Systemic Effects NOEL = 1 mg/kg/day		MOS Reproductive/ Teratogenic Effects NOEL = 25 mg/kg/day	
	Amine	Ester	Amine	Ester	Amine	Ester
Immediately	4.29E-04	9.14E-05	2.33E+03	1.09E+04	5.83E+04	2.74E+05
1	4.02E-04	8.58E-05	2.49E+03	1.17E+04	6.22E+04	2.91E+05
2	3.78E-04	8.06E-05	2.65E+03	1.24E+04	6.61E+04	3.10E+05
3	3.55E-04	7.57E-05	2.82E+03	1.32E+04	7.04E+04	3.30E+05
4	3.33E-04	7.11 E-05	3. 00E+03	1.41E+04	7.51E+04	3.52E+05
5	3.13E-04	6.67E-05	3.19E+03	1.50E+04	7.99E+04	3.75E+05
6	2.94E-04	6.26E-05	3.40E+03	1.60E+04	8.50E+04	3.99E+05
7	2.75E-04	5.88E-05	3.40E+03	1.70E+04	9.09E+04	4.25E+05
8	2.59E-04	5.52E-05	3.64E+03	1.81E+04	9.65E+04	4.53E+05
9	2.43E-04	5.19E-05	3.86E+03	1.93E+04	1.03E+05	4.82E+05
10	2.28E-04	4.87E-05	4.12E+03	2.05E+04	1.10E+05	5.13E+05
11	2.14E-04	4.57E-05	4.39E+03	2.19E+04	1.17E+05	5.47E+05
12	2.01E-04	4.29E-05	4.67E+03	2.33E+04	1.24E+05	5.83E+05
13	1.89E-04	4.03E-05	5.29E+03	2.48E+04	1.32E+05	6.20E+05
14	1.77E-04	3.78E-05	5.65E+03	2.65E+04	1.41E+05	6.61E+05
15	1.67E-04	3.55E-05	5.99E+03	2.82E+04	1.50E+05	7.04E+05
16	1.56E-04	3.34E-05	6.41E+03	2.99E+04	1.60E+05	7.49E+05
17	1.47E-04	3.13E-05	6.80E+03	3.19E+04	1.70E+05	7.99E+05
18	1.38E-04	2.94E-05	7.25E+03	3.40E+04	1.81E+05	8.50E+05
19	1.29E-04	2.76E-05	7.75E+03	3.62E+04	1.94E+05	9.06E+05
20	1.22E-04	2.59E-05	8.20E+03	3.86E+04	2.05E+05	9.65E+05
21	1.14E-04	2.43E-05	8.77E+03	4.12E+04	2.19E+05	1.03E+06
12	1.07E-04	2.29E-05	9.35E+03	4.37E+04	2.34E+05	1.09E+06

Table 24: Margins of Safety for Various Effects from a Single Exposure to 2,4-D Through Ingestion of Fish (Continued)

Scenario 2: Irrigation Ditch

Days After Application	Single Human Dose (mg/kg)		MOS Systemic Effects NOEL = 1 mg/kg/day		MOS Reproductive/ Teratogenic Effects NOEL = 25 mg/kg/day	
	Amine	Ester	Amine	Ester	Amine	Ester
Immediately	5.71E-03	1.17E-03	1.75E+02	8.55E+02	4.38E+03	2.14E+04
I	5.37E-03	1.10E-03	1.86E+02	9.09E+02	4.66E+03	2.27E+04
2	5.04E-03	1.03E-03	1.98E+02	9.71E+02	4.96E+03	2.43E+04
3	4.73E-03	9.70E-04	2.11E+02	1.03E+03	5.29E+03	2.58E+04
4	4.44E-03	9. 10E-04	2.25E+02	1.10E+03	5.63E+03	2.75E+04
5	4.17E-03	8.55E-04	2.40E+02	1.17E+03	6.00E+03	2.92E+04
6	3.92E-03	8.03E-04	2.55E+02	1.25E+03	6.38E+03	3.11E+04
7	3.68E-03	7.54E-04	2.72E+02	1.33E+03	6.79E+03	3.32E+04
9	3.45E-03	7.08E-04	2.90E+02	1.41E+03	7.25E+03	3.53E+04
9	3.24E-03	6.64E-04	3.09E+02	1.51E+03	7.72E+03	3.77E+04
10	3.04E-03	6.24E-04	3.29E+02	1.60E+03	8.22E+03	4.01E+04
I I	2.86E-03	5.86E-04	3.50E+02	1.71E+03	8.74E+03	4.27E+04
12	2.68E-03	5.50E-04	3.73E+02	1.82E+03	9.33E+03	4.55E+04
13	2.52E-03	5.16E-04	3.97E+02	1.94E+05	9.92E+03	4.84E+04
14	2.37E-03	4.85E-04	4.22E+02	2.06E+03	1.05E+04	5.15E+04
15	2.22E-03	4.55E-04	4.50E+02	2.20E+03	1.13E+04	5.49E+04
16	2.08E-03	4.27E-04	4.81E+02	2.34E+03	1.20E+04	5.85E+04
17	1.96E-03	4.01E-04	5.10E+02	2.49E+03	1.28E+04	6.23E+04
18	1.94E-03	3.77E-04	5.43E+02	2.65E+03	1.36E+04	6.63E+04
19	1.73E-03	3.54E-04	5.78E+02	2.82E+03	1.45E+04	7.06E+04
20	1.62E-03	3.32E-04	6.17E+02	3.01E+03	1.54E+04	7.53E+04
21	1.52E-03	3.12E-04	6.58E+02	3.21E+03	1.64E+04	8.01E+04
22	1.43E-03	2.93E.04	6.99E+02	3.41E+03	1.75E+04	8.53E+04

Table 24: Margins of Safety for Various Effects from a Single Exposure to 2,4-D Through Ingestion of Fish (Continued)

Scenario 3: Large Lake

Days After Application	Single Human Dose (mg/kg)		MOS Systemic Effects NOEL = 1 mg/kg/day		MOS Reproductive/ Teratogenic Effects NOEL = 25 mg/kg/day	
	Amine	Ester	Amine	Ester	Amine	Ester
Immediately	6.86E-06	1.14E-06	1.46E+05	8.77E+05	3.64E+06	2.19E+07
I	6.44E-06	1.07E-06	1.55E+05	9.35E+05	3.88E+06	2.34E+07
2	6.05E-06	1.01E-06	1.65E+05	9.90E+05	4.13E+06	2.48E+07
3	5.68E-06	9.46E-07	1.76E+05	1.06E+06	4.40E+06	2.64E+07
4	5.33E-06	8.88E-07	1.88E+05	1.13E+06	4.69E+06	2.82E+07
5	5.00E-06	8.34E-07	2.00E+05	1.20E+06	5.00E+06	3.00E+07
6	4.70E-06	7.83E-07	2.13E+05	1.28E+06	5.32E+06	3.19E+07
7	4.41E-06	7.35E-07	2.27E+05	1.36E+06	5.67E+06	3.40E+07
8	4.14E-06	6.90E-07	2.42E+05	1.45E+06	6.04E+06	3.62E+07
9	3.90E-06	6.48E-07	2.56E+05	1.54E+06	6.41E+06	3.86E+07
10	3.65E-06	6.09E-07	2.74E+05	1.64E+06	6.85E+06	4.11E+07
11	3.43E-06	5.71E-07	2.92E+05	1.75E+06	7.29E+06	4.38E+07
12	3.22E-06	5.37E-07	3.11E+05	1.86E+06	7.76E+06	4.66E+07
13	3.02E-06	5.04E-07	3.31E+05	1.98E+06	8.28E+06	4.96E+07
14	2.84E-06	3.87E-07	3.52E+05	2.58E+06	8.80E+06	6.46E+07
15	2.66E-06	4.44E-07	3.76E+05	2.25E+06	9.40E+06	5.63E+07
16	2.50E-06	4.17E-07	4.00E+05	2.40E+06	1.00E+07	6.00E+07
17	2.35E-06	3.92E-07	4.26E+05	2.55E+06	1.06E+07	6.38E+07
18	2.21E-06	3.68E-07	4.52E+05	2.72E+06	1.13E+07	6.79E+07
19	2.07E-06	3.45E-07	4.83E+05	2.90E+06	1.21E+07	7.25E+07
20	1.94E-06	3.24E-07	5.15E+05	3.09E+06	1.29E+07	7.72E+07
21	1.83E-06	3.04E-07	5.46E+05	3.29E+06	1.37E+07	8.22E+07
22	1.71E-06	2.86E-07	5.85E+05	3.50E+06	1.46E+07	8.74E+07

Table 25: Hazard Quotients* for all Pathways

Scenario	Ingestion of Fish		Dermal Contact With Water (Swimming)		Dermal Contact With Sediments		Incidental Ingestion of Sediments		Drinking Water		Cumulative Hazard Index	
	Amine	Ester	Amino	Ester	Amine	Ester	Amine	Ester	Amine	Ester	Amine	Ester
1: Small Pond	3.30E-03	7.04E-04	1.69E-07	3.86E-05	4.11E-08	2.50E-06	5.67E-08	3.44E-06	2.31E-02	4.94E-03	2.64E-02	5.69E-03
2: Irrigation Ditch	4.40E-04	9.04E-03	2.25E-06	4.95E-04	6.47E-07	3.33E-05	8.939-07	4.59E-05	3.09E-01	6.34E-02	3.53E-01	7.30E-02
3: Large Lake	5.29E-05	8.79E-06	2.71E-09	4.82E-07	5.87E-10	3.68E-08	8.11E-10	5.07E-08	3.71E-04	6.17E-05	4-26E-04	7.11 E-05

*Hazard Quotients calculated using USEPA-derived RfD's (IRIS, 1992)
Oral RfD = 1.00E-02 mg/kg/day
Dermal RfD = 9.50E-03 mg/kg/day