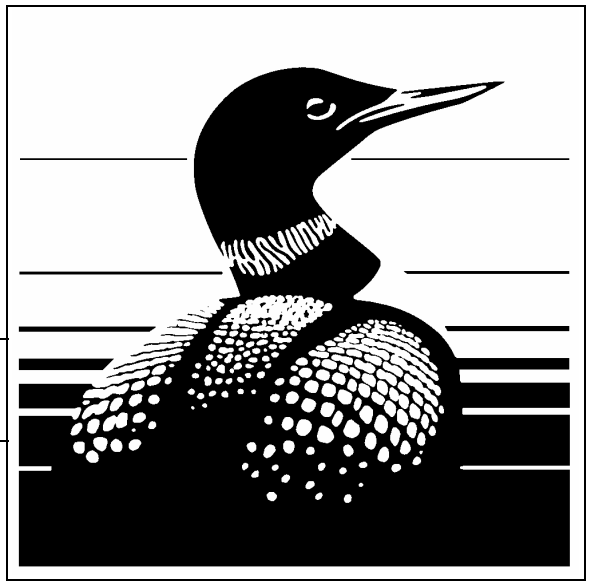
PESTICIDES IN ONTARIO: A CRITICAL ASSESSMENT OF POTENTIAL TOXICITY OF URBAN USE PRODUCTS TO WILDLIFE, WITH CONSIDERATION FOR ENDOCRINE DISRUPTION

VOLUME 3: Phenoxy herbicides, chlorothalonil and chlorpyrifos

J. Grabusky, P.A. Martin, J. Struger

Canadian Wildlife Service 2004
Environmental Conservation Branch
Ontario Region



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EXECUTIVE SUMMARY

This series of reports provides an in-depth review of the environmental toxicity, environmental concentrations and potential for endocrine disruption of a selection of pesticides used in Ontario. Our goal was to identify the potential for adverse effects to wildlife at environmentally relevant concentrations of the compounds extensively used in agricultural and urban landscapes.

There is ample evidence that many environmental pollutants have mechanisms of action that directly or indirectly disrupt/modulate the endocrine system. Such pollutants have been termed **endocrine disrupting chemicals (EDCs)**; for the purposes of this assessment, the working definition of an EDC is:

> *An exogenous agent that directly interferes with the synthesis, secretion, transport, binding action, or elimination of endogenous hormones and neurohormones, resulting in physiological manifestations of the neuroendocrine, reproductive or immune systems in an intact organism*.

Pesticides or pesticide families were selected from 1993 and 1998 Ontario agricultural and 1993 urban use estimates. This report is based on published information from the period 1978 to 2001. Compounds were chosen for review based on comparative use and toxicity class information. Only those compounds whose estimated use within Ontario exceeded 10 000 kilograms in 1993 were evaluated. Review results of the selected compounds are reported in several volumes. Volume 1 included the assessments of eight compounds within five pesticide families: an organochlorine insecticide (endosulfan), three ethylenebisdithiocarbamate fungicides (maneb, mancozeb, metiram), two dinitroaniline herbicides (trifluralin, pendimethalin), a halogenated hydrocarbon nematocide (1,3-dichloropropene), and an organophosphorus insecticide (azinphos-methyl). Pesticide mixtures are also discussed. Volume 2 examined the triazine herbicides, glyphosate, and metolachlor. In Volume 3, pesticides important for urban usage were examined. The four pesticides include two phenoxy herbicides, chlorothalonil fungicide, and the organophosphorus insecticide, chlorpyrifos.

Profiles for each pesticide or pesticide family include a brief description of active ingredients; use patterns in Ontario; occurrence in surrounding natural environments; associated acute and chronic toxicity and potential for endocrine disruption; evaluation of risk to wildlife; and recommendations for further research and monitoring.

Phenoxy herbicides:

- 2,4-D and mecoprop were measured in Ontario urban streams (1998-2000): 2,4-D was detected in nine of 133 samples, with a maximum concentration of 2.1 ug/L; mecoprop was detected in 41 of 133 samples with a maximum concentration of 1.77 ug/L.
- The Canadian Water Quality Guideline for the Protection of Aquatic Life for combined phenoxy herbicides in freshwater is 4.0 ug/L.
- Phenoxy herbicides degrade quickly in the aquatic environment and are rapidly excreted from organisms.
- Effects were seen at dose levels well above those found in the environment.
- The action of 2,4-D on the central nervous system and the thyroid indicates the potential for endocrine disruption at levels of 10 mg/kg or more indicating very low risk to aquatic organisms.
- The information needed to determine if low level exposure to mecoprop will affect wildlife is not available since there is a lack of studies examining the acute and chronic toxicity and effects of mecoprop, particularly its salt formulations.

- As phenoxy herbicides are moderately persistent on land, animals such as squirrels and birds that traverse and feed on treated urban landscapes may be exposed to low levels throughout their active season.

Chlorothalonil:

- Chlorothalonil was not detected in 96 samples collected in urban streams of Ontario and exposure to aquatic organisms appears minimal.
- The Canadian Water Quality Guideline for the Protection of Aquatic Life for chlorothalonil in freshwater is 0.18 ug/L.
- The low acute toxicity of chlorothalonil to birds and laboratory mammals, coupled with its low rate of detection and short persistence in the environment suggests minimal risk to most terrestrial wildlife.
- Studies on the potential of chlorothalonil to interfere with endogenous hormones and neurohormones, and its potential for immunomodulation suggest that chlorothalonil is an endocrine disrupting compound.

Chlorpyrifos:

- Chlorpyrifos is not commonly detected in urban watersheds of Ontario: in a recent survey of urban streams, chlorpyrifos was not detected in any of 136 surface water samples collected. However, chlorpyrifos was detected in two out of eight water samples taken from two urban stormwater detention ponds with an average concentration of 0.148 ug/L.
- The Canadian Water Quality Guideline for the Protection of Aquatic Life for chlorpyrifos in freshwater is 0.0035 ug/L.
- As a developmental neurotoxin, chlorpyrifos meets our criteria as an endocrine disruptor.
- As neurotoxic effects can be detected at low levels, some aquatic species may be at risk.
- Mixtures of pesticides also pose a risk to wildlife. Other organophosphates are known to co-occur with chlorpyrifos and additive toxicity of chlorpyrifos and diazinon has been observed in *Ceriodaphnia dubia.* Atrazine paired with chlorpyrifos has shown greater than additive toxicity in a study of 4th instar larvae of the aquatic midge (*C. tentans*) and a study by Jett *et al.* found that four common PAHs (pyrene, benzo(a)pyrene, anthracene, fluoranthene), together with chlorpyrifos, inhibited AChE in an additive manner.

Priorities for further research and recommendations:

1. Better information is needed on usage of urban pesticides, including that by homeowners: no accurate information currently exists.
2. Continued environmental monitoring of water bodies draining urban environments is essential to determine exposure of wildlife.
3. Research is needed to determine acute and chronic toxicity on the salt and ester formulations of mecoprop as well as its degradates, using environmentally relevant concentrations and including endpoints assessing endocrine disruption potential.
4. Assessments of the effects of phenoxy acid herbicides on terrestrial organisms of the urban landscape.
5. Further study is needed on the ecological effects of mixtures of organophosphate pesticides and other pesticides (such as atrazine) on the various components of natural ecosystems.
6. Assessment of acute and chronic exposure to chlorpyrifos to evaluate the cellular, synaptic, and behavioural consequences and potential for endocrine disruption in wildlife.
7. Analytical improvements in the extraction efficiency and possible preservation techniques of chlorothalonil should be investigated to obtain reliable field results.

8. Studies on how other pesticides occurring in the environment affect the toxicity of chlorothalonil, since chemicals that utilize or deplete tissue glutathione may predispose organisms to acute toxic effects.
9. Further study is needed to determine the potential for endocrine disruption in wildlife to chronic chlorothalonil/DS-3701 exposure, and on the potential for immunomodulation.
10. Best management practices, such as the use of buffer strips and grassy swales, proper application and disposal practices, and integrated pest management techniques, should be implemented to reduce pesticide inputs into urban surface waters.

SOMMAIRE EXÉCUTIF

La présente série de rapports offre un examen en profondeur de la toxicité et des concentrations dans l'environnement de certains pesticides utilisés en Ontario, ainsi que de leurs effets perturbateurs possibles sur le système endocrinien. Notre objectif consistait à déterminer les effets nocifs possibles sur la faune, à des concentrations observées dans l'environnement, de composés largement utilisés dans les paysages agricoles et urbains.

Il y a amplement de preuves selon lesquelles de nombreux polluants dans l'environnement utilisent des mécanismes qui, directement ou indirectement, peuvent perturber ou moduler le système endocrinien. Ces polluants ont été nommés « **substances chimiques perturbatrices du système endocrinien » (SPSE)**; aux fins de la présente évaluation, la définition ad hoc d'une SPSE est la suivante :

> *Un agent exogène interférant directement avec la synthèse, la sécrétion, le transport, l'activité de liaison ou l'élimination des hormones endogènes et des neurohormones, qui ont des effets physiologiques sur les systèmes neuro-endocrinien, reproducteur ou immunitaire dans un organisme intact*.

On a sélectionné des pesticides ou familles de pesticides selon des estimations des utilisations agricoles en Ontario pour 1993 et 1998 et des utilisations urbaines en Ontario pour 1993. Ce rapport repose sur des informations publiées entre 1978 et 2001. On a choisi les composés à examiner d'après des informations sur leurs catégories relatives d'utilisation et de toxicité. On n'a évalué que les composés sélectionnés dont le volume d'utilisation estimé en Ontario dépassait les 10 000 kilogrammes en 1993. Les résultats des examens des composés sélectionnés sont publiés dans plusieurs volumes. Le volume 1 rassemble les évaluations de huit composés de cinq familles de pesticides : un insecticide organochloré (endosulfan), trois fongicides de type éthylène-bis-dithiocarbamate (manèbe, mancozèbe, métirame), deux herbicides de type dinitroaniline (trifluraline, pendiméthaline), un nématocide hydrocarboné halogéné (1,3-dichloropropène) et un insecticide organophosphoré (azinphos-méthyl). On y traite également de mélanges de pesticides. Le volume 2 porte sur les herbicides de type triazine, le glyphosate et le métalochlore. Les quatre pesticides comprennent deux herbicides du type phénoxy, un fongicide chlorothalonil et l'insecticide organophosphoré, le chlorpyrifos.

Les profils de chaque pesticide ou famille de pesticides comportent notamment une brève description des ingrédients actifs; les tendances de leur utilisation en Ontario; leur occurrence dans les milieux naturels avoisinants; leur toxicité aiguë et chronique ainsi que leur potentiel de perturbation du système endocrinien; une évaluation du risque pour la faune; et des recommandations aux fins de recherche et de surveillance supplémentaires.

Herbicides du type phénoxy :

- On a mesuré le 2,4-D et le mécoprop dans les cours d'eau urbains de l'Ontario (1998 à 2000) : le 2,4-D a été décelé dans 9 des 133 échantillons, la concentration maximale étant de 2,1 ug/L; le mécoprop a été décelé dans 41 des 133 échantillons, la concentration maximale étant de 1,77 ug/L.
- La recommandation canadienne pour la qualité des eaux en vue de protéger la vie aquatique pour ce qui est des herbicides du type phénoxy combinés dans l'eau douce est de 4,0 ug/L.
- Les herbicides du type phénoxy se dégradent rapidement dans le milieu aquatique et sont rapidement excrétés par les organismes.
- On a observé des effets à des doses supérieures à celles que l'on retrouve dans l'environnement.
- L'action du 2,4-D sur le système nerveux central et la thyroïde indique une possibilité de perturbation du système endocrinien à des niveaux de 10 mg/kg ou plus, indiquant un très faible risque pour les organismes aquatiques.

- Les renseignements requis pour déterminer si une faible exposition au mécoprop nuira à la faune ne sont pas disponibles puisqu'il n'y a pas suffisamment d'études examinant la toxicité aiguë et chronique et les effets du mécoprop, particulièrement ses formulations de sel.
- Comme les herbicides du type phénoxy sont modérément persistants sur le sol, les animaux comme les écureuils et les oiseaux qui traversent les paysages urbains traités et s'y nourrissent peuvent être exposés à de faibles niveaux tout au long de leur saison active.

Chlorothalonil :

- Aucun chlorothalonil n'a été décelé dans 96 échantillons recueillis dans des cours d'eau urbains de l'Ontario, et l'exposition aux organismes aquatiques semble minime.
- La recommandation canadienne pour la qualité des eaux en vue de protéger la vie aquatique pour ce qui est du chlorothalonil dans l'eau douce est de 0,18 ug/L.
- La faible toxicité aiguë du chlorothalonil pour les oiseaux et les mammifères de laboratoire, associée à son faible taux de détection et à sa faible persistance dans l'environnement, dénote un risque minimal pour la plupart de la faune terrestre.
- Des études sur le potentiel d'immunomodulation du chlorothalonil et la possibilité qu'il perturbe les hormones endogènes et les neurohormones dénotent que le chlorothalonil est un dérégulateur endocrinien.

Chlorpyrifos :

- Le chlorpyrifos n'est pas couramment décelé dans les bassins versants urbains de l'Ontario : dans un récent sondage sur les cours d'eau urbains, aucun chlorpyrifos n'a été décelé dans aucun des 136 échantillons d'eau de surface recueillis. Toutefois, le chlorpyrifos a été décelé dans 2 des 8 échantillons d'eau provenant de deux bassins de retenue des eaux pluviales urbaines dont la concentration moyenne était de 0,148 ug/L.
- La recommandation canadienne pour la qualité des eaux en vue de protéger la vie aquatique pour ce qui est du chlorpyrifos dans l'eau douce est de 0.0035 ug/L.
- En tant que toxine agissant sur le développement neurologique, le chlorpyrifos répond à nos critères de dérégulateur endocrinien.
- Puisque certains effets neurotoxiques peuvent être décelés à de faibles niveaux, certaines espèces aquatiques peuvent être en péril.
- Les mélanges de pesticides présentent également un risque pour la faune. D'autres composés organophosphorés sont connus pour se manifester simultanément avec le chlorpyrifos, et on a observé une toxicité additive du chlorpyrifos et du diazinon sur le *Ceriodaphnia dubia.* L'atrazine conjuguée au chlorpyrifos a révélé une toxicité supérieure à une toxicité additive dans une étude sur des larves du quatrième stade du moucheron aquatique (*C. tentans*), et une étude de Jett et coll. a révélé que quatre HAP communs (pyrène, (a)-benzopyrènepyrene, anthracène, fluoranthène), de concert avec le chlorpyrifos, ont inhibé l'AChE de façon additive.

Priorités pour d'autres recherches et recommandations :

1. Il faut de meilleurs renseignements sur l'usage des pesticides urbains, incluant l'usage qu'en font les propriétaires : aucun renseignement précis à ce sujet n'existe en ce moment.
2. Une surveillance environnementale continue des plans d'eau qui s'écoulent dans les milieux urbains est essentielle pour déterminer l'exposition de la faune.
3. Il faut effectuer une recherche afin de déterminer la toxicité aiguë et chronique des formulations de sel et d'ester du mécoprop ainsi que de ses produits de dégradation, utilisant des concentrations présentes dans l'environnement et comprenant des mesures terminales évaluant le potentiel de perturbation des fonctions endocriniennes.

4. Il faut des évaluations des effets des herbicides du type phénoxy sur les organismes terrestres du paysage urbain.
5. Il faut effectuer d'autres recherches sur les effets écologiques des mélanges de pesticides organophosphorés et d'autres pesticides (comme l'atrazine) sur les divers éléments des écosystèmes naturels.
6. Il faut une évaluation de l'exposition aiguë et chronique au chlorpyrifos visant à évaluer les conséquences sur les cellules, les synapses et les comportements ainsi que la possibilité de perturbation du système endocrinien dans la faune.
7. Il faudrait examiner les améliorations des méthodes d'analyse relativement à l'efficacité de l'extraction et aux techniques de préservation possibles du chlorothalonil afin d'obtenir des résultats fiables sur le terrain.
8. Il faut des études sur les effets d'autres pesticides dans l'environnement sur la toxicité du chlorothalonil, puisque les produits chimiques qui utilisent ou réduisent le glutathion tissulaire peuvent prédisposer les organismes à des effets toxiques aigus.
9. Il faut effectuer d'autres études afin de déterminer la possibilité de perturbation du système endocrinien dans la faune par suite d'une exposition chronique au chlorothalonil/DS-3701, ainsi que le potentiel d'immunomodulation.
10. Dans le but de réduire l'utilisation des pesticides dans les eaux de surface urbaines, il faudrait mettre en œuvre de meilleures pratiques de gestion, dont l'utilisation de bandes tampons et de fossés herbeux, de pratiques d'application et d'élimination appropriées et de techniques de lutte intégrée contre les parasites.

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1.0 INTRODUCTION

1.1 PURPOSE OF REPORT

The purpose of this report is to provide an in-depth review of the environmental toxicity, environmental concentrations, and potential for endocrine disruption of a selection of pesticides used in Ontario. Our particular goals were to identify adverse effects at environmentally relevant concentrations of these compounds and to prioritize compounds that may pose a risk to populations of non-target vertebrate and invertebrate wildlife. Further, based on our findings, we sought to point out data gaps in specific areas and to recommend directions for future research and monitoring.

1.2 OVERVIEW OF THE ENDOCRINE DISRUPTION ISSUE

The last decade has been one of intense focus on pollutants that may affect the function of the endocrine system in humans, fish and other wildlife. This report places some emphasis on potential endocrine disrupting properties of pesticides in use in Ontario. There has been a long-term concern regarding contaminant-induced reproductive effects in wildlife and humans resident in the Great Lakes region (Foster 1995, Fry 1995, Hose and Guillette 1995, Gilbertson 1997).

Although many of the adverse physiological effects of chemicals affecting the neuroendocrine system have been known for over three decades, widespread attention to this issue only materialized in the early 1990s. Before this time, some of the effects that are now considered endocrine disruption were classified as sublethal or chronic toxicity and for the most part were not used for regulatory purposes, with the exception of some reproductive endpoints.

The events that brought endocrine disruption to the forefront of environmental toxicology were reports of reproductive abnormalities in American alligators (Vonier *et al.*1996, Crain *et al.* 1997, Guillette *et al.* 1994); declining sperm counts in humans (Cooper and Kavlock 1997; Carlson *et al.* 1992); induction of female-specific protein production in males and the lack of gonad development in both sexes of fish (Tyler *et al.* 1998; Van Der Kraak *et al.* 1998; Ankley *et al.* 1998); masculinization of marine snails (Bryan *et al.* 1988), and; endocrine toxicity and vaginal cancer in the daughters of diethylstilbestrol (DES) treated women (Poskanzer and Herbst 1997).

The heightened interest in endocrine-related effects of pollution in recent years is evidenced by the vast number of articles and reviews published about various aspects of endocrine disruption (see Tyler *et al.* 1998, Ankley *et al.* 1998, Barton and Andersen 1998, Chapin *et al.* 1996, Golden *et al.* 1998, Gillesby and Zacharewski 1998, Arcand-Hoy and Benson 1998, Safe and Gaido 1998, Stahlschmidt-Allner *et al.* 1997, Campbell and Hutchinson 1998, Palmer *et al.* 1998, Kime 1999, Kendall *et al.* 1998, U.S. EPA 1997, Cooper *et al.* 1999, Porter *et al.* 1999, Short and Colborn 1999, Ashby *et al.* 1997.) and the numerous meetings and workshops that have been held (Kavlock *et al.* 1996, Ankley *et al.* 1996).

In the United States (U.S.), congress passed the *Food Quality Protection Act* (1996) and the *Safe Drinking Water Act* (1996), which mandated the U.S. EPA to establish an advisory committee to assist in developing a screening and testing strategy for evaluating chemicals for their potential to cause effects via endocrine disruption. This strategy had to be developed and implemented within three years (i.e., by 1999) (U.S. EPA 1997, Cooper and Kavlock 1997). A more extensive exploration of environmental endocrine disruption will be completed by the National Academy of Science.

The U.S. EPA Science Policy Council interim position (U.S. EPA 1997) on endocrine disruptors states that:

> *The EPA is aware of and concerned about information indicating the possibility of adverse effects on human health and the environment associated with exposure to endocrine disrupters* and that *The agency does not consider endocrine disruption to be an adverse endpoint per se but rather to be a mode or mechanism of action potentially leading to other outcomes, for example carcinogenic, reproductive, or developmental effects routinely considered in reaching regulatory decisions*.

The above-noted U.S. legislation focus primarily on human health consequences of endocrine-active chemicals; however, the U.S. EPA has included wildlife health as an endpoint of concern. In this context, wildlife includes vertebrate and invertebrate species (Ankley *et al.* 1998).

1.2.1 Definitions and Terms of Endocrine Disruption

The U.S. EPA (U.S. EPA 1997) uses the terms "endocrine disrupting chemical (EDC)", "hormone disrupter", and "environmental endocrine disrupter" synonymously, and defines them as:

> *An exogenous agent that interferes with the synthesis, transport, secretion, binding-action or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development and/or behavior.*

This definition, however, does not directly address the interactions of the nervous and endocrine systems and therefore allows for some ambiguity. The neuroendocrine system is any of the systems of dual control of certain activities in the body of some higher animals by nervous and hormonal stimulation. For example, the posterior pituitary gland and the medulla of the adrenal gland receive direct nervous stimulation to secrete their hormones, whereas the anterior pituitary gland is stimulated by releasing hormones from the hypothalamus.

In addition, neurohormones are not specified in the above definition. Neurohormones are hormones that are produced not by an endocrine gland but by a specialized nerve cell and are secreted from nerve endings into the bloodstream or directly to the tissue or organ whose growth or function it controls. Examples of neurohormones are norepinephrine, vasopressin, insect juvenile hormone, and ecdysone.

As stated by the U.S. EPA (U.S. EPA 1997), of importance here is the concept that endocrine disrupting chemicals encompass more than just environmental estrogens and include any agents that adversely affect any aspect of the entire endocrine system. The endocrine system includes a number of central nervous system (CNS)-pituitary target organ feedback loops involved in regulating a multitude of bodily functions and maintaining homeostasis, and there are potentially several target sites through which environmental endocrine disruptors could act. Thus, impaired hormonal action could result as a consequence of altered hormone synthesis, release, clearance, or binding, regardless of the initial site of action. Effects may be acute, although are more likely to be delayed or not expressed for a period of time. Emphasis is placed on disruption of CNS-pituitary integration of hormonal and sexual behaviour, female and male reproductive system development and function, and thyroid function.

The European community (Ankley *et al.* 1996) defines an EDC more broadly as:

> *An exogenous substance that causes adverse health effects in an intact organism or its progeny, secondary to changes in endocrine function.*

For the purposes of the current assessment, the following definition of an EDC is used:

> *An exogenous agent that directly interferes with the synthesis, secretion, transport, binding action, or elimination of endogenous hormones and neurohormones, resulting in physiological manifestations of the neuroendocrine, reproductive or immune systems in an intact organism.*

1.2.2 Mechanisms of Action of Endocrine Disruption

In order to elucidate the mechanisms of action of endocrine active compounds, background information on endocrine physiology in the current context is provided by several recent reviews (Carlsen *et al.* 1992, Poskanzer and Herbst 1997, Safe and Gaido 1998, Kime 1999). The modes of action of EACs can be categorized into five groups (Poskanzer and Herbst 1997); they may
act as:

1. Alternate ligands that can bind in place of endogenous hormones.
2. Modulators of hormone metabolism.
3. Modifiers of signalling occurring subsequent to receptor-ligand binding.
4. Modulators of CNS components responsible for neuroendocrine regulation.
5. Target organ toxicants.

Other modes of action are also possible, including those not associated with the endocrine system. Some chemicals are active through several modes of action that may vary depending on the dose or other conditions. For example, several phytoestrogens bind to the estrogen receptor, inhibit the enzymatic conversion of estradiol to estrone and/or androgens to estrogens (Barton and Andersen 1998).

The estrogen and androgen receptors are nuclear receptors. As a result, the steroid hormones that bind them, as well as any estrogen or androgen mimic, must diffuse through the plasma membrane. Following steroid receptor binding, the activated receptor complex seeks out specific DNA motifs, termed hormone response elements, upstream of hormone responsive genes (Gillesby and Zacharewski 1998). This results in mRNA production followed by protein expression.

Estrogen mimics act within the cell via three main mechanisms (Gillesby and Zacharewski 1998):

1. Direct binding and activation of the estrogen receptor.
2. Binding to other nuclear receptors that then interact with an estrogen responsive element.
3. Through other receptor and/or signal transduction pathways that alter estrogen signalling.

Some endocrine disrupters, such as the fungicide vinclozolin, cause estrogenic effects although they are antiandrogens (U.S. EPA 1997, Barton and Andersen 1998) while others may be estrogenic or antiestogenic depending on the cellular environment (Gillesby and Zacharewski 1998). Other EDCs act on the aryl hydrocarbon receptor (AhR), including dioxins and polychlorinated biphenyls (PCBs) (Tyler *et al.* 1998).

Non-receptor mediated mechanisms of action of EDCs are exemplified by the phytoestrogen β-sitosterol and the organotin tributyltin (TBT). The former reduces the biosynthetic capacity of gonadal steroids while the latter inhibits the enzymatic conversion of androgens to estrogens.

The U.S. EPA (U.S. EPA 1997) states that:

> *For virtually all toxic chemicals, the toxic action or stress imparted on an organism will likely be moderated by endocrine and immune processes that exist to maintain homeostasis. Because of this, it is difficult to elucidate whether a toxic action is directed specifically at an endocrine function or whether an endocrine process disruption is an indirect consequence of some other stress to the immune, nervous and /or reproductive system.*

The evaluation of endocrine disruption in wildlife is further complicated by critical windows during development and species-specific endocrine function. The developing fetus is extremely sensitive to the hormonal environment in the uterus for example, and natural differences in hormone levels surrounding rat or mouse fetuses of only 10^{-12} M influence the timing of sexual development and the behaviour of the animal in adult life (Tyler *et al.* 1998).

Similarly for fish, exposure to estrogen mimics during a narrow window spanning just 10 days (d) either side of hatching can cause feminization of the subsequent fry, whereas at high doses the exposure period need only be two hours in some fish (Tyler *et al.* 1998). In birds, sexual differentiation is estrogen dependent but this is not the case in mammals. Consequently, birds may be more sensitive to EDCs than mammals during embryonic development.

1.3 PESTICIDE USE IN ONTARIO

Pesticides are applied to terrestrial and aquatic ecosystems within Ontario. The agriculture and forest industries rely heavily on pesticides, even though integrated pest management programs have been implemented to reduce their use. Many green areas in urban environments are maintained with herbicides, and certain water bodies are periodically directly sprayed with lampricides to control sea lamprey populations in the Great Lakes Basin.

Pesticides were first applied in Ontario in 1885, when acetoarsenite and copper sulphate were sprayed in apple orchards to control insect pests (Frank *et al.* 1989). Since then, pesticides have gone through several transitions: from primarily inorganic and organometallic formulations to organochlorine insecticides, triazine herbicides, organophosphorous, carbamate, and later, pyrethroid insecticides; and, more recently, to sulfonylurea and imidazolinone herbicides. Many of the earlier products, such as the organochlorine DDT, have been withdrawn from use in Canada and other developed countries because of unacceptable levels of toxicity or environmental persistence. In general, pesticides have become less persistent, lower in toxicity to non-target organisms with greater specificity, but with high efficiency at very low volumes.

Unlike most developed countries, Canada no longer requires pesticide manufacturers to provide public access to records of product sales, so there is no direct way to obtain accurate information on pesticide use. However, every five years since 1973, the Ontario Ministry of Agriculture and Rural Affairs (OMAFRA) has conducted a voluntary survey of Ontario farmers regarding pesticide use, in an effort to estimate this information. Products and quantities used for different crop types reported through the survey are used in conjunction with known areas of each crop type per county, as attained through use of Ontario Ministry of Agriculture and Rural Affairs (OMAFRA) annual statistical crop surveys (OMAFRA 1994) to extrapolate countywide estimates of pesticide use. The two most recent surveys conducted were in 1993 (Hunter and McGee 1994)

and 1998 (Hunter and McGee 1999). In 1993, pesticide use estimates were based on 1,800 farm surveys out of a provincial total of 61 432 farms (2.9 percent) whereas in 1998, only 1,200 farms responded. Rapidly growing segments of the agricultural industry, such as greenhouse and nursery operations, were not surveyed prior to 1998. The 1998 survey included nursery, sod and ginseng farms but still did not include greenhouses. Treatments with surfactants and oils, livestock sprays and rodenticides were also not included.

In 1998 [38], the estimated total pesticide active ingredients that had been applied to field crops, fruits and vegetables that year, was 5 214 402 kg. The previous survey of 1993 (Hunter and McGee 1994) estimated a total pesticide use of 6 246 442 kg, representing a reduction in the volume of pesticide use by 16.5 percent. The 1993 and 1998 surveys show a general decline in pesticide use from earlier surveys. Newer, high efficacy pesticides are being used more widely. These are applied at grams per hectare instead of kg per hectare, which is reflected in the reduced total volume of pesticides used. For example, the sulfonylurea and imidazolinone herbicides have acute phytotoxicity to non-target plants at concentrations below the limits of analytical chemical detection (Fletcher *et al.* 1996, Boutin *et al.* 2000). The inability to detect these compounds in environmental samples is just one of the many factors that makes environmental risk assessment very challenging.

Pesticide use in urban areas is more poorly understood than use in agriculture. The Ontario Ministry of the Environment (MOE) surveys individuals (voluntary participation) receiving commercial pesticide applicators licenses every five years and includes pesticides applied to roadsides, residential/industrial lawns, golf courses and parks by these applicators (Hunter and McGee 1994). In 1993, MOE reported that of 1 302 086 kg of active pesticide ingredient applied that year, 62 percent was on residential lawns, 17.4 percent to roadsides, 7.9 percent to industrial lawns, and 6.9 and 4.6 percent to parks and golf courses, respectively (Hunter and McGee 1994). The remaining was applied to school and cemetery grounds. However, totals obtained through these surveys represent an unknown proportion of the total urban use of pesticides, given that most domestic-use pesticides may be purchased and applied by unlicensed homeowners for which there are no sales records. MOE did not conduct this survey in 1998 or 2003.

Inputs of pesticides into urban surface waters are most commonly the result of run-off from impermeable surfaces such as sidewalks, driveways, patios and parking lots, which have had pesticides applied to them accidentally because of spray drift or misdirected application. Proper application to well-maintained turf results in very low levels of run-off even in subsequent storm events, indicating that simple attention to details during application can be very effective in reducing environmental inputs of urban pesticides (Hoffman *et al.* 2000). Due to a greater awareness of environmental and health issues, public concern has grown recently regarding the use of pesticides in urban environments. In Canada, some urban jurisdictions have recently enacted bylaws restricting the cosmetic use of pesticides on both public and private property. As well, some municipalities have incorporated the use of Integrated Pest Management techniques in the management of their public green spaces.

1.4 ASSESSMENT METHODS

A subset of the pesticides used in Ontario agriculture (Hunter and McGee 1994) has been selected for critical evaluation in this series of reports. Selection was based on comparative use patterns and toxicity class data (Table 1.1). As part of the toxicity characterization, the inclusion or exclusion of each pesticide from the lists of potential endocrine disrupters drafted by the World Wildlife Fund (http://www.wwfcanada.org/hormone-disruptors/) and the U.S. EPA (U.S. EPA 1997) was considered. With these use and toxicity guidelines, 12 compounds from eight pesticide families were selected for review (Table 1.1) in Volumes 1 and 2, concentrating on

pesticides of particular importance in agriculture in Ontario. In Volume 3, four pesticides within three families, which had both considerable agricultural use as well as being important in the urban usage, including golf course and turf usage, were reviewed.

Each chapter profiles a pesticide or family of pesticides that is of relevance in Ontario either because of high combined agricultural and/or urban usage or for showing a relatively high level of toxicity to non-target organisms.

Each chapter contains the following components:

- A basic description of the pesticide, including the nature of the active ingredient and any information on other surfactants or inert ingredients in applied formulations.
- Use patterns in Ontario agriculture and, where information is available, in the urban landscape.
- Occurrence in surrounding natural environments.
- Associated acute and chronic toxicity, and a critical assessment of the chemical's potential for endocrine disruption.
- A critical evaluation of risk posed to Ontario wildlife by continued use.
- Recommendations for research and monitoring relevant to Ontario environments.

For Volume 1, the agricultural use pattern maps were derived using 1993 data from Hunter and McGee (Hunter and McGee 1994) for Ontario, and from Gianessi and Anderson (Gianessi and Anderson 1995) for U.S. states in the Great Lakes Basin. In Volume 2, these maps were derived using data from the 1998 census (Hunter and McGee 1999) for Ontario only. These use patterns reflect agricultural use only and similar geographic data are not available for urban use and are therefore not included in Volume 3. Assessment of toxicity was conducted by examining peer-reviewed publications and government reviews of pesticides and endocrine disruption, and by systematically searching for all combinations of active ingredient and/or chemical family names in the Life Sciences Index, Environment Abstracts, Water Resources Abstracts, and Pollution Abstracts (1978 - 2001). Historical monitoring by Frank and associates (Braun and Frank 1980, Frank *et al.* 1979, Frank *et al.* 1981, Frank *et al.* 1982, Frank *et al.* 1987a, Frank *et al.* 1987b, Frank and Logan 1988, Frank *et al.* 1990, Frank *et al.* 1991) in the 1970s and 1980s documented pesticide concentrations in surface water, groundwater and Great Lakes tributaries in southern Ontario. More recent surface water quality monitoring has focused on pesticides in agricultural ecosystems of concern (i.e., fruit, tobacco, muck crops, row crops) and the urban environment (Bishop *et al.* 1999, Harris *et al.* 1998, Struger *et al.* 1994, Merriman *et al.* 1991, Struger *et al.* 2001, Struger 1999, Struger *et al.* 2002).

1.5 REALISTIC ASSESSMENT OF RISK: MIXTURES, UNKNOWN INGREDIENTS, SURROGATE DOSES, AND HISTORICAL PERSISTENCE

Before continuing with independent evaluations of the selected pesticides, the influence of mixtures, surfactants, and historical loads of banned compounds on the assessment of overall risk should be briefly addressed. A more detailed discussion of pesticide mixtures present in Ontario may be found in Chapter 7 of Volume 1.

A comprehensive review of pesticide use in England, Scotland and Wales found that 59 different combinations of two or more active ingredients were applied to 30 000 ha or more, and that an average of two mixtures per crop were sprayed simultaneously (Thompson 1996). Spray recommendations published by OMAFRA for local fruit, vegetable, and flower (greenhouse) production (OMAFRA 1997, OMAFRA 1998abc) also refer frequently to co-formulations and mixtures of formulations, suggesting there is also widespread use of pesticide combinations in

this country. Since most toxicity testing is completed with one treatment ingredient at a time, the toxic behaviour of mixtures is largely unknown (Thompson 1996).

Pesticides also contain what have traditionally been referred to as "inert ingredients", which may be surfactants, solvents, emulsifiers, adjuvants, stabilizers, etc... The use of the term "inert" has been criticized (Tobin 1986), since breakdown products of some of these components have been shown to disrupt the endocrine system; for example, the breakdown of ethoxylated nonylphenols to nonylphenol. Typically, only information on the active ingredient is divulged by the manufacturer, with information on other ingredients considered proprietary. Sometimes limited data are available (e.g., the diazinon formulation, Basudin 500EC, contains a petroleum derivative solvent), usually because legislation demands that all hazardous ingredients be listed on material safety data sheets. In other instances, impurities may be surmised by knowing something of the manufacturing process; for example, when organochlorines like endosulfan are synthesized, dioxins, furans and hexachlorobenzene are sometimes present in small quantities in the commercial formulation (Foster 1995, Tobin 1986). In addition, alkyl phenols (including nonylphenols) are commonly used as surfactants for insoluble pesticides. The small quantities of inert ingredients and impurities in formulations likely preclude acutely toxic effects, but their presence may contribute to additivity or synergy, particularly with responses of the endocrine system. Polycyclic aromatic hydrocarbons (PAHs) from petroleum solvents, dioxins, furans, hexachlorobenzene and nonylphenol have all been implicated as potential endocrine disrupters (U.S. EPA 1997).

In addition to these factors that complicate assessments of toxicity, others complicate assessments of exposure. Although water concentrations are important components in determining environmental exposure, they generally provide a poor estimate of internal dose of toxicant. They may be too low to be detected by standard analytical methods, yet be bioconcentrated in sensitive species over time. These and other factors make chemical concentrations in abiotic media a poor choice for pesticide risk assessment. Body residues generally provide a better surrogate of the dose at the site of toxic action. A body residue known to be associated with a particular biological response (e.g., acute lethality) is termed a critical body residue. By comparing critical body residues to known tissue concentrations, it may be possible to improve determinations of environmental risk for those compounds. Existing tissue and sediment analyses have verified that several banned chlorinated compounds persist in Ontario environments (Bishop *et al.* 1991, Bishop *et al.* 1992, Struger *et al.* 1993, Russell *et al.* 1995, Russell *et al.* 1997). These chemicals include DDT, mirex, dieldrin, and PCBs. Their presence in the environment, in combination with mixtures of in-use pesticides, might contribute to triggers of toxic response pathways in wildlife. By nature of their persistence, these chemicals are more likely to affect species higher in the food chain. The persistent organochlorines are also those that are most frequently discussed as potential or known endocrine disrupters (Foster 1995, U.S. EPA 1997).

Table 1.1 A selection of agricultural and urban pesticides used in Ontario. Compounds that are underlined were reviewed in Volumes 1 and 2; those in BOLD font are reviewed in Volume 3.

	1993 Agric. Use (kg ai.)[a]	1998 Agric. Use (kg ai.)[a]	1993 Urban Use (kg ai.)[a]	Potential Endocrine Disrupter[b]	Soil DT50 (days)[c]	Solubility (mg/L)[c]	Mammalian Toxicity[c,d]	Fish LC_{50} (mg/L)[c,e]
Metolachlor (H)	1 327 315	1 376 570	0		20	488	III	3.9
Atrazine (H)	589 852	598 206	0	X*	16-77	33	III	4.5-11
Glyphosate (H)	414 821	647 494	18 556		3-174	11 600	III	86
Dichloropropene (N)	410 512[f]	177 000	0		2-17	2 000	III	3.9
Dicamba (H)	255 528	205 522	190 296		<14	6 500	III	135
Metribuzin (H)	254 276	71 761	0	X	-	1 050	III	76
2,4-D[g] (H)	222 746	145 720	328 523		<7	311	II	>100
Cyanazine (H)	215 480	49 038	0	X*	14	171	II	16[h]
MCPA (H)	161 605[i]	119 700	48 404		<7	734	III	232
Mancozeb (F)	155 463	156 269	486	X	6-15	6.2	III	2.2
Captan (F)	151 468	101 276	0		1	3.3	III	0.072[j]
Chlorothalonil (F)	115 613	120 751	5 831	X*	5-36	0.81	III	0.049
EPTC[k] (H)	113 030	46 312	0		-	375	II	19
Trifluralin (H)	83 945	23 250	0	X	57-126	0.184	III	0.01-0.04
Sulphur (F)	72 338	55 670			-	insoluble	III	non-toxic
Azinphos-methyl (I)	71 983	14 120	0		weeks	28	Ib	0.02
Metiram (F)	57 230	131 113	333	X	-	insoluble	III	1.1
Pendimethalin (H)	51 414	115 687	0	X[t]	90-120	0.3	III	0.14
Maneb (F)	49 440	1 873	0	X	25	insoluble	III	1.8[l]
Bromoxynil (H)	45 317	58 854	0	X*	10	130	II	0.46[m]
Terbufos (I)	38 282	1 297	0		9-27	4.5	Ia	0.01
Endosulfan (I)	25 930	6 909	0	X	30-70	0.32	II	0.002[n]
Carbaryl (I)	16 882	15 334	2 099	X	7-28	20	II	1.3
Carbofuran (I)	15 213	2 652	0	X	30-60	320-350	Ib	22-29
Cypermethrin (I)	12 780	6 310	0	X	5	0.004	II	0.001
Mecoprop (H)	not available	2 899	251 879		13	734	III	124.8
Chlorpyrifos (I)	1 353	7 311	125 766	X*	30	0.40	II	0.082

[a] Agricultural use estimate based on 1,800 farm surveys (1993) (Hunter and McGee 1994) and 1,200 surveys (1998) (Hunter and McGee 1999); urban use estimate based on reported use by Ontario Ministry of the Environment licensed pesticide applicators (1993) (Hunter and McGee 1999). [b] X indicates potential for endocrine disruption as per the U.S. EPA (U.S. EPA 1997) and the World Wildlife Fund (http://www.wwfcanada.org/hormone-disruptors/); * signifies that it was only listed by WWF, whereas [t] signifies that it was only listed by the U.S. EPA. [c] Source: Tomlin (Tomlin 1994); [d] Toxicity class based on World Health Organization (WHO) classification; Ia = extremely hazardous, Ib = highly hazardous, II = moderately hazardous, III = slightly hazardous.
[e] Rainbow trout 96 h LC_{50} unless otherwise indicated. [f] the sum of 1,3-dichloropropene and dichloropropene. [g] the sum of amines and butyl esters of 2,4-D ([2,4-dichlorophenoxy]acetic acid). [h] Fathead minnow 96 h LC_{50}. [i] the sum of MCPA ([4-chloro-2-methylphenoxy]acetic acid) and MCPA/MCPB. [j] Bluegill sunfish 96 h LC_{50}. [k] EPTC = dipropylcarbamothioic acid S-ethyl ester = dipropylthiocarbamic acid S-ethyl ester. [l] Carp 96 h LC_{50}. [m] Goldfish 48 h LC_{50}. [n] Golden orfe 96 h LC_{50}.
(H)-herbicide, (I)-insecticide, (F)-fungicide, (N)-nematocide, (-)-not available.

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2.0 THE PHENOXY HERBICIDES: 2,4-D AND MECOPROP

Phenoxy herbicides were introduced in the 1940s as highly selective, broadleaf-weed killers, which were translocated throughout the plant. Several compounds belong to this class, of which 2,4-D and 2,4,5-T are the most familiar. Others include 2,4-DB, MCPA, mecoprop (MCPP) and silvex. Phenoxy herbicides have complex mechanisms of action resembling those of plant growth hormones (auxins) which affect cellular division, activate phosphate metabolism, and modify nucleic acid metabolism (Ware 2000).

2.1 DESCRIPTION AND USE

2.1.1 2, 4-D

The phenoxy herbicide 2,4-D (2,4-dichlorophenoxyacetic acid, CAS No. 94-75-7) is primarily used for post-emergence control of unwanted broad-leaved plants like dandelion, lamb's quarters, mustards and pigweeds (OMAFRA 2000a), but is also used to a lesser extent as a growth regulator (Seiler 1978, Munro *et al.* 1992). It is used on corn, berries, pasture and oats, as well as on golf courses, lakes and ponds, government right-of-ways and in forestry applications. Various 2,4-D formulations are available to homeowners to eliminate broadleaf weeds from their lawns and gardens.

There are many commercial formulations of 2,4-D which can be divided into four major categories: 2,4-D acid, 2,4-D mixed butyl or isooctyl esters (BE or IOE), 2,4-D as low-volatile esters, and 2,4-D as the dimethylamine (DMA), diethalonamine (DEA) or other amine salts (Buzik 1992). These forms are also currently available in mixtures with other herbicides including mecoprop and dicamba. Some trade names include ESTASOL (EC 564 g/L), ESTEMINE 2,4-D (Sn 470 g/L), ESTER 600 (EC 564 g/L) as well as KIL-MOR (2,4-D/dicamba/mecoprop Sn 485 g/L), KILLEX 500 (Sn 479 g/L) and PAR III (Sn 308 g/L) (OMAFRA 2000a).

Application rates for 2,4-D are listed in Table 2.1 and vary depending on the target species. In Ontario agriculture, 96 384 kg of 2,4-D were applied to crops such as corn, hay and mixed barley in 1998 compared to 134 869 kg in 1993, a reduction of almost 30 percent (Hunter and McGee 1999, Hunter and McGee 1994). However, 2,4-D is used more widely outside the agricultural sector. In 1993, 225 052 kg of 2,4-D were applied to roadsides, residential/industrial lawns, golf courses and parks according to a MOE survey of registered pesticide applicators (Hunter and McGee 1994). Estimates on the amount of 2,4-D used in urban areas do not include the amount applied by private homeowners treating their property with mixtures of 2,4-D/mecoprop or 2,4-D/mecoprop/dicamba and there is no way to obtain an estimate of this use.

2.1.2 Mecoprop

Mecoprop ([R,S]-2-[4-chloro-2-methylphenoxy] propionic acid) is a phenoxyacid herbicide used for post-emergent control of broad-leaved weeds, including clover, chickweed, ground ivy, and black medick (OMAFRA 2000a). Application rates and formulations used are listed in Table 2.1.

Mecoprop (MCPP) is available under many trade names and formulations, as well as in mixtures with 2,4-D and dicamba. Trade names include COMPITOX (150 g/L), MECOPROP (150 g/L), MECOTURF PLUS 2,4-D (1:1 400 g/L), and MECO-D (1:1.9 435 g/L) (OMAFRA 2000a,b). MCPP is available as the parent acid or more commonly as a salt or ester formulation (potassium salt, diethanolamine salt, dimethylamine salt, isooctyl ester). The presence of a chiral

carbon atom in the aliphatic side chain gives two stereo-isomeric forms of mecoprop with only the (R)-(+)-enantiomer possessing herbicidal properties (Fletcher *et al.* 1995, Tett *et al.* 1994). Enantiopure products (R-mecoprop) have been developed and registered for use but racemic mixtures are still available.

In Ontario agriculture, only 2,899 kg of mecoprop were applied to crops like corn, pasture and mixed barley in 1998 compared to 11 065 kg in 1993 (Hunter and McGee 1999, Hunter and McGee 1994). However, mecoprop is used more widely outside the agricultural sector. In 1993, 251 879 kg of mecoprop were applied to roadsides, residential/industrial lawns, golf courses and parks (Hunter and McGee 1994). Estimates on the amount of mecoprop used in urban areas do not include the amount applied by homeowners treating their properties with mixtures of 2,4-D/mecoprop or 2,4-D/mecoprop/dicamba.

Table 2.1 Recommended applications of 2,4-D, and mecoprop formulations for use in Ontario turf and agriculture (OMAFRA 2000a, OMAFRA 2000b).

Plant(s) Protected	Weed(s) Controlled	Formulation	Application Rate
2,4-D			
Turfgrass			
Turfgrass (lawns, parks, golf courses, sod farms)	- Many broadleaf weeds (bentgrass, dandelion, black medick)	2,4-D (470 g/L)	1.7-3.0 L/ha
Agricultural Crops			
strawberries	- Post-emergent broadleaf weeds	2,4-D	1.17 L/ha
raspberries (caneberries and blackberries)	- Post-emergent broadleaf weeds (dandelions)	2,4-D	1.2 L/ha
cranberries	- Post-emergent broadleaf weeds (hardhack, St. John's wort, alder, purple aster)	2,4-D AMINE 500 (470 g/L)	1 L/2 L water
pears, peaches, cherries, plums	- Post-emergent broadleaf weeds (dandelion, seedling Canada thistle, sow-thistle)	2,4-D AMINE 600 (560 g/L)	1.7 L/ha
asparagus	- Post-emergent broadleaf weeds	2,4-D	2-2.75 L/ha
wheat (winter)	- Post-emergent broadleaf weeds	2,4-D KIL-MOR (485 g/L)	0.75-1.17 L/ha 0.85-1.1 L/ha
oats	- Post-emergent broadleaf weeds	2,4-D	0.75-1.06 L/ha
pasture	- Chicory - Yellow rocket - Goldenrod - Blueweed, burdock, wild carrot, milkweed, water hemlock, dandelion - Ox-eye daisy, hawks-beard - Tansy ragwort	2,4-D	1.8 L/ha 1.8-2.34 L/ha 2.15 L/ha 2.15 L/ha 2.34-3.72 L/ha 4.5 L/ha
field corn	- Post-emergent broadleaf weeds	2,4-D KIL-MOR	0.6-1.2 L/ha 0.85-1.1 L/ha
Mecoprop			
Turfgrass			
Turfgrass (lawns, parks, golf courses, sod farms)	- Broadleaf weeds including dandelion, bentgrass, black medick	COMPITOX (150 g/L), MECOPROP (150 g/L)	5.5-8.5 L/ha
Agricultural Crops			
wheat, rye, oats, barley	- Post-emergent broadleaf weeds	COMPITOX (150 g/L), MECOPROP (150 g/L)	5.5-7 L/ha

2.2 ENVIRONMENTAL FATE AND CONCENTRATIONS

2.2.1 2,4-D

The herbicide 2,4-D is applied to turfgrass and crops by foliar sprays to control terrestrial broadleaf weeds. It is also applied in granular formulation directly to water bodies for control of aquatic weeds like Eurasian watermillfoil (*Myriophyllum spicatum*). It may enter the environment directly through application and indirectly via spray drift, foliar wash-off, run-off, soil erosion, and volatilization.

The acid form of 2,4-D is moderately polar, slightly water soluble (0.09 percent or 900 mg/L) and volatile (0.01 mm Hg) at 25^{o}C. It is rarely sorbed (5 percent) to soil (Obenshain *et al.* 1997), unless those soils are high in organic material or have a low pH (Benoit *et al.* 1996, Greer and Shelton 1992). Commercial forms consist of the more water-soluble alkali or amine salts (300 percent), and organic solvent soluble esters (Health and Welfare Canada 1996). 2,4-D esters are more volatile than the acid or salt formulations with vapour pressures ranging between $1.1x10^{-3}$ - $2x10^{-6}$ mm Hg (Health and Welfare Canada 1996).

Despite having the potential to migrate through the soil profile (leaching) or soil surface (run-off), 2,4-D has rarely been detected at significant levels in the environment (Grover *et al.* 1997, Miller *et al.* 1995, Ryals *et al.* 1998, Stearman and Wells 1997), with the exception of one agricultural study (Nicholaichuk and Grover 1983). The Canadian Water Quality Guideline for the protection of aquatic life, for all phenoxy herbicides combined is 4.0 ug/L (CCME 1999). Struger *et al.* (2002a) measured phenoxy herbicides in Ontario urban streams in 1998-2000 and detected 2,4-D in nine of 133 samples, with a maximum concentration of 2.1 ug/L. Even combined with the other phenoxy herbicides, no samples exceeded the Water Quality Guideline. In addition, Canadian tributaries draining watersheds having both agricultural and urban inputs of lakes Erie and Ontario were monitored for phenoxy herbicides (Struger *et al.* 2002b). 2,4-D was detected in five of 75 samples in Lake Ontario tributaries with a maximum of 1.6 ug/L; in Lake Erie tributaries, it was detected in four of 83 samples with a maximum concentration of 2.0 ug/L. Total phenoxys did not exceed the Water Quality Guideline.

While spring and fall-applied 2,4-D (ester) on nursery plots did leach to 50-65 ng/g at a soil profile of 60-90 cm after the first rainfall, by d 21 (post-application) 2,4-D was at detection limits except in the surface 0-10 cm (Stearman and Wells 1997). In a surface water study of three pesticide-treated North Carolina golf courses, 83 percent of water samples contained 2,4-D residues with the highest level being 1.08 ug/L (Ryals *et al.* 1998). In prairie farm ponds 2,4-D was detected in 93-100 percent of samples, with a maximum concentration of 2.67 ug/L in the spring following the snowmelt (Grover *et al.* 1997). Soil and groundwater samples collected almost two years after last application from agricultural plots with a 10 to 13 year history of regular 2,4-D weed control applications, did not contain 2,4-D residues (Miller *et al.* 1995). The six-year average loss of fall-applied 2,4-D (dimethylamine salt) in spring run-off from treated fallow (control) and wheat stubble plots was 0.3 percent and 4.1 percent of the applied (3.0 and 31.2 ug/L), respectively (Nicholaichuk and Grover 1983).

While photodecomposition and volatilization of 2,4-D and some of its degradates may occur, the primary mode of breakdown appears to be microbial. Many organisms are capable of degrading 2,4-D, including the bacteria *Ralstonia eutrophus* (formerly *Alcaligenes eutrophus*) and *Burkholderia* sp. (Saari and Hausinger 1998, Matheson *et al.* 1996), the nonmycorrhizal fungus *Phanerochaete chrysosporium* (Donnelly *et al.* 1993, Yadav and Reddy 1993) and the algae *Scenedesmus quadricauda* (Valentine and Bingham 1974). The catabolic genes of the degrading bacteria are encoded on plasmids and chromosomes that may be horizontally transferred between bacterial species thus conferring the ability to degrade 2,4-D (Matheson *et al.*

1996). The genes may also be transferred to crop species to confer pesticide resistance (Lauren *et al.* 2000).

Microbial proliferation (and therefore 2,4-D degradation/mineralization) is dependent on environmental factors like pH, temperature, aeration, nutrients, organic matter and moisture content (Birmingham and Colman 1985, Estrella *et al.* 1993, Entry 1999, Entry and Emmingham 1995, Greer and Shelton 1992, Han and New 1994). Bioaugmentation of a contaminated environment with a suitable degrading strain of bacteria enhanced 2,4-D degradation (Dejonghe *et al.* 2000, Ka *et al.* 1994, Kandel *et al.* 1992) by eliminating the lag period during which the indigenous degrading population must become sufficiently large to cause detectable loss of pesticide (Chen and Alexander 1989, Ka *et al.* 1994). Repeat applications of 2,4-D resulted in degrading bacteria maintaining the ability to rapidly degrade 2,4-D for at least 204 weeks after the final application of the herbicide (Smith and Aubin 1994).

The half-life of 2,4-D acid in soil ranged from 1-135 d depending on formulation, application rate, and previous use history (Smith and Aubin 1991, Wilson *et al.* 1997). In water, 2,4-D formulations (esters and amines) are rapidly converted to the free acid form of 2,4-D (Grover 1973, Zepp *et al.* 1975), particularly under alkaline conditions, and with the presence of fish/plants (Birmingham and Colman 1985, Hoeppel and Westerdahl 1983).

The half-life of 2,4-D residues in the water column ranged from 2-34 d depending on application rate, formulation, environmental conditions (pH, temperature and oxygen), microbial/algal population (acclimation/lag period) and amount bound to sediment (Birmingham and Colman 1985, Hoeppel and Westerdahl 1983, Kandel *et al.* 1992, McMartin *et al.* 2000, Zepp *et al.* 1975). Birmingham and Colman (1985) found that although 24.2 percent of 2,4-D residues were initially associated with sediment within 1 d of application to a water body, they had desorbed by 50 percent within 13-16 d. Myers *et al.* (1994) found that anaerobic degradation of 2,4-D in sediment slurries was slow with half-lives >80 weeks.

2.2.2 Mecoprop

MCPP is typically applied by ground or aerial equipment as a broadcast, band or foliar spray. It enters the natural environment directly through application and indirectly via spray drift, foliar washoff, runoff, soil erosion and leaching.

MCPP is non-volatile, as shown by an experiment with the potassium salt where only 0.8 percent of applied mecoprop volatilized off treated turfgrass (Murphy *et al.* 1996). It is not highly sorbed to soil with K_d=0.07-0.20 and K_{oc}=5.3-13.3 (Helweg 1993) and is water soluble, particularly as a salt (Fletcher *et al.* 1995). Leaching studies conducted with the potassium salt of MCPP found that mecoprop was most mobile in sandy soils with 43-640 ppb detected in leachate depending on the interval between application (2.26 kg/ha) and leachate collection (Petrovic *et al.* 1993). A later study by Petrovic and Larsson-Kovach (1996) confirmed these findings. Fletcher *et al.* (1995) found levels of MCPP ranging from 0.5-25.8 ug/L in a salt marsh receiving run-off from a recently treated agricultural field. Another source of MCPP in the environment is the run-off from roofs treated with the roof protection agent Preventol B2. When hydrolyzed, Preventol B2 releases (R,S)-mecoprop, which has been found in Swiss roof run-off samples at concentrations up to 500 ug/L (Bucheli *et al.* 1998).

MCPP is stable to hydrolysis and undergoes minimal photodegradation (U.S. EPA 1990a). Degradation is primarily microbial as previously discussed, and half-lives in soil range from 3-50 d (Helweg 1993, Romero *et al.* 2001, Reffstrup *et al.* 1998) while the half-life in groundwater ranges from 35-120 d with an adaptation period of up to 56 d (Agertved *et al.* 1992, Heron and Christensen 1992, Klint *et al.* 1993). The rate of degradation decreases with decreasing temperature, increased organic matter or clay, under flooded/anaerobic conditions, higher MCPP concentrations and increased soil depth (Helweg 1993, Reffstrup *et al.* 1998). There is enhanced degradation of MCPP in soil with repeat applications. Residues on plants are

relatively short-lived. MCPP residues on barley, wheat and oat (1.1 kg/ha application rate) decreased by two orders of magnitude (from on the order of 100 mg/kg) within three to six weeks of application (Cessna 1992a, Cessna 1992b). Mixed and single bacterial cultures degrade racemic MCPP. *Sphingomonas herbicidovorans* degrades racemic MCPP by two iron- and alpha-ketoglutarate-dependent dioxygenase activities: one specific to (R)-mecoprop and one specific to (S)-mecoprop (Nickel *et al.* 1997). Degradation of enantiopure (R)-mecoprop by mixed bacterial cultures results in the concurrent formation and subsequent degradation of (S)-MCPP and vice versa (Buser and Muller 1998, Muller and Buser 1997). Incubation experiments suggest a preference for the (S)-enantioners in aquatic environments with the rate of inversion of (R)- to (S)-mecoprop three to 10 times faster than the inversion of (S)- to (R)-mecoprop (Buser and Muller 1998). Intermediates in the aerobic transformation of MCPP in soil include 4-chloro-2-methylphenol and pyruvate (Nickel *et al.* 1997).

The Canadian Water Quality Guideline for the protection of aquatic life for all phenoxy herbicides combined is 4.0 ug/L (CCME 1999). In Ontario, MCPP seems to appear in surface water slightly more frequently than 2,4-D. Struger *et al.* (2002a) measured phenoxy herbicides in Ontario urban streams in 1998-2000 and detected MCPP in 41of 133 samples (compared to nine for 2,4-D), with a maximum concentration of 1.77 ug/L. Even combined with the other phenoxy herbicides, no samples exceeded the Water Quality Guideline. In addition, Canadian tributaries draining watersheds having both agricultural and urban inputs of lakes Erie and Ontario were monitored for phenoxy herbicides (Struger *et al.* 2002b). 2,4-D was detected in 21 (compared to five for 2,4-D) of 75 samples in Lake Ontario tributaries with a maximum of 0.95 ug/L; in Lake Erie tributaries it was detected in 10 (compared to five for 2,4-D) of 83 samples with a maximum concentration of 1.9 ug/L. Total phenoxys did not exceed the Water Quality Guidelines.

2.3 BIOCONCENTRATION AND METABOLISM

2.3.1 2,4-D

2.3.1.1 Plants

2,4-D is absorbed by plant roots and leaves and moves upward in the phloem where it accumulates at the meristematic regions of the roots and shoots (Donnelly *et al.* 1993). Experiments in soybean callus tissue (*Glycine max*) and cucumber explants (*Cucumis sativus*), showed that 2,4-D is metabolized to ring-hydroxylated derivatives and amino acid conjugates (Feung *et al.* 1973, Klems *et al.* 1998), some of which retain growth stimulatory activity (Feung *et al.* 1973). Uptake and metabolism is rapid with <30 percent free 2,4-D remaining in cucumber explant tissue after 20 h exposure (Klems *et al.* 1998).

2.3.1.2 Vertebrates

Absorption of 2,4-D is rapid in fish and mammals (Erne 1966, Schultz 1973, Sikka *et al.* 1977). Inside the organism, amines and esters of 2,4-D are hydrolyzed to the free acid form (Finlayson and Verrue 1985, Knopp and Schiller 1992, Schulze *et al.* 1985). The absorbed 2,4-D binds to plasma proteins (Arnold and Beasley 1989, Erne 1966, Guarino *et al.* 1977, Plakas *et al.* 1992), but this process is saturable (Plakas *et al.* 1992). As plasma protein binding becomes saturated, plasma levels rise (Kay *et al.* 1965, Orberg 1980). Organic anion transport systems play a role in the distribution of 2,4-D to the kidney, liver, brain and fetus (Berndt and Koschier 1973, Erne 1966, Gorzinski *et al.* 1987, Pritchard 1980, Pritchard and James 1979, Sandberg *et al.* 1996). These transport systems are also saturable and, when this occurs, 2,4-D accumulates in tissue (Gorzinski *et al.* 1987, Tyynela *et al.* 1990). Elimination of 2,4-D is primarily renal with

most excreted as unchanged parent compound (Carpenter and Eaton 1983, Gorzinski *et al.* 1987, Griffin *et al.* 1997b, Khanna and Fang 1966, Plakas *et al.* 1992, Schulze *et al.* 1985, Sikka *et al.* 1977). This is a dose-dependent and saturable process with the threshold for renal tubular excretion in rats found to be 50 mg/kg (Gorzinski *et al.* 1987).

Various tests on the uptake and elimination of 2,4-D in channel catfish, bluegill sunfish, steelhead-rainbow trout and winter flounder were conducted and the half-life of 2,4-D was found to be 0.76-6 h (Plakas *et al.* 1992, Carpenter and Eaton 1983, Pritchard and James 1979). However, unlike the fish above, the marine dogfish shark extensively metabolized and renally excreted 90-95 percent of 2,4-D as the taurine conjugate (Koschier and Pritchard 1980, Guarino *et al.* 1977, James and Bend 1976). The marine crustacean (*Panulirus argus*) also metabolized a significant amount of an injected dose of 2,4-D to the taurine conjugate but the conjugated 2,4-D was excreted much more slowly than the unchanged acid (James 1982).

Rats absorbed and eliminated 90 percent of an oral dose of 2,4-D within 6-48 h depending on the formulation and concentration (Khanna and Fang 1966, Knopp and Schiller 1992, Pelletier *et al.* 1989, Schulze *et al.* 1985). Dermal absorption is more variable and may be affected by formulation and species (Wester *et al.* 1996, Moody and Nadeau 1997), vehicle and contact time (Wester *et al.* 1996), and pre-treatment of application site (Moody and Nadeau 1997, Pelletier *et al.* 1990). Pelletier *et al.* (1989) found a four to 10 percent absorption/elimination factor for 2,4-D dimethylamine (DMA) through rat skin. Other studies in rats and rhesus monkeys using 2,4-D DMA and 2,4-D acid indicate that dermal absorption/elimination is eight to 15 percent within 24-116 hours (Knopp and Schiller 1992, Wester *et al.* 1996). There was a "washing-in" effect of soap observed in dermal studies where a second cleansing of dosed skin favoured absorption of additional amounts of 2,4-D (Moody and Nadeau 1997, Pelletier *et al.* 1990). While most 2,4-D is excreted unchanged in mammals, hydrolysis and conjugation does occur at low rates (Griffin *et al.* 1997a, Mehmood *et al.* 1996).

Based on the short retention time and lack of metabolism of 2,4-D in fish and mammals, the risk of bioconcentration is expected to be low although no measured or calculated bioconcentration factors (BCFs) were found in the reviewed literature.

2.3.1.3 Invertebrates

Slugs (*Deroceras reticulatum*) fed 2,4-D for 5-10 d rapidly excreted about 80 percent of the ingested dose, 55 percent of which contained metabolites (Haque and Ebing 1983). Further experimentation showed that slugs could also dermally absorb 2,4-D from soil reaching equilibrium within 15 d (Haque and Ebing 1983). Caterpillars (*Eupackardia calleta*) fed 2,4-D (U46-D Fluid) at field application levels metabolized and excreted 2,4-D primarily in the feces; however, an intra-individual transfer of 2,4-D was observed when the resulting moths were found to contain 2,4-D (Deml and Dettner 2001).

2.3.2 Mecoprop

Leaves of *Stellaria media* (chickweed) rapidly absorbed 14C-MCPP with a maximum absorption by 72 h (Coupland *et al.* 1990). Most of the absorbed radiolabel remained in the leaves with less than 17 percent of the absorbed radioactivity in the apical, basal and root samples (Coupland *et al.* 1990). MCPP is converted to polar metabolites and this is proposed as a resistance mechanism in resistant plants (Coupland *et al.* 1990).

MCPP is not likely to bioconcentrate or bioaccumulate in animal tissues. In a 28-d flowthrough test, the bioconcentration factor for MCPP acid in bluegill sunfish was found to be three, and residues were depurated quickly (U.S. EPA 1988). The major degradate found in tissues was "mecoprop methyl" (U.S. EPA 1988). Mammals are able to eliminate MCPP as the parent compound in urine (Extoxnet 1995).

2.4 TOXIC MECHANISM OF ACTION

2.4.1 2,4-D

2,4-Dichlorophenoxyacetic acid (2,4-D) is a synthetic auxin analog that at low doses promotes growth in a manner similar to the naturally occurring auxin indole acetic acid (Boyle 1980). At higher doses, this growth becomes abnormal and kills the plant (Donnelly *et al.* 1993). Its selectivity against broad-leaved plants is thought to be due to their larger leaf area and better 2,4-D absorption (Seiler 1978).

The mode of action of 2,4-D has not been completely explained but many theories exist. These include cellular accumulation (Kasai and Bayer 1995, Tittle *et al.* 1990), modification of nucleic acid and protein synthesis (Fabra *et al.* 1993, Mori *et al.* 1995, Rivarola and Balegno 1991a, Rivarola and Balegno 1991b, Rivarola *et al.* 1992a, Rivarola *et al.* 1992b), and the inhibition of glutathione-S-transferase, the alteration of mitochondrial bioenergetics and glutathione depletion (Droog *et al.* 1995, Dierickx 1983, Dierickx 1985, Hietanen *et al.* 1983, Mustonen *et al.* 1989, Palmeira *et al.* 1994a, Palmeira *et al.* 1994b, Palmeira *et al.* 1995, Singh and Awasthi 1985, Vessey and Boyer 1984, Zychlinski and Zolnierowicz 1990).

Exposure results in increased ethylene production which, though not directly linked to killing susceptible plants, may be responsible for some of the observed morphological effects (Tittle *et al.* 1990). This increased production of ethylene resulted in the increased production of cyanide by soybean plants (*Glycine max*), which was proposed to accumulate in the cytoplasm and adversely affect the activity of cyanide-sensitive cytoplasmic enzymes (Tittle *et al.* 1990). Kasai and Bayer (1995) theorize that the accumulation of 2,4-D in the cytoplasm of corn (*Zea mays*) and the resulting cytoplasmic acidification may also be a factor in toxicity.

Studies in the bacteria *Azospirillum brasilense* and Chinese hamster ovary cells (CHO) indicate that the inhibition of cell growth, DNA, RNA and protein synthesis caused by 2,4-D is a result of altered polyamine metabolism (Fabra *et al.* 1993, Mori *et al.* 1995, Rivarola and Balegno 1991a, Rivarola and Balegno 1991b, Rivarola *et al.* 1992a, Rivarola *et al.* 1992b). Polyamines are molecules necessary in the processes of DNA replication, RNA transcription, and protein synthesis and include putrescine, spermidine and spermine (Fabra *et al.* 1993, Rivarola and Balegno 1991a). This inhibition of polyamine metabolism results from the decreased activity of the limiting enzyme of polyamine biosynthesis, ornithine decarboxylase (Fabra *et al.* 1993, Mori *et al.* 1995, Rivarola and Balegno 1991a, Rivarola and Balegno 1991b).

While studies in pumpkin culture cells (*Cucurbita maxima*) and tobacco (*Nicotiana tabacum*) indicate that 2,4-D induces the genes encoding glutathione S-transferase (GST) thus elevating the levels of GST proteins, 2,4-D is also capable of inhibiting the *in vitro* activity of these enzymes (Droog *et al.* 1995, Fujita *et al.* 1995, Fujita and Adachi 1996). The inhibition of GST activity by 2,4-D has also been observed in hepatic rat, human and steelhead-rainbow trout cells (Dierickx 1983, Dierickx 1985, Hietanen *et al.* 1983, Singh and Awasthi 1985, Vessey and Boyer 1984). This inhibition could alter the ability of GST to detoxify xenobiotics and thus impair the defence mechanisms against lipid peroxidation (Heitanen *et al.* 1983, Singh and Awasthi 1985, Vessey and Boyer 1984). *In vitro* tests in rat liver mitochondria with 2,4-D indicate an uncoupling of oxidative phosphorylation (Zychlinski and Zolnierowicz 1990) and inhibition of the redox chain (Palmeira *et al.* 1994a). Palmeira *et al.* (1994b) found that 2,4-D reduced rat hepatocyte glutathione (GSH), ATP and NADH levels *in vitro*. Palmeira *et al.* (1995) confirmed 2,4-D depletion of hepatic GSH, and protein thiols and also showed that 2,4-D induced lipid peroxidation. Cellular damage is thought to be based upon, or preceded by, injury of the bioenergetic functions of mitochondria (Palmeira *et al.* 1994a, Zychlinski and Zolnierowicz 1990), and related to GSH depletion, and consequent ATP and NADH depletion (Palmeira *et al.* 1994b, Palmeira *et al.* 1995). However, Mustonen *et al.* (1989) found no effect on thiol enzyme activities or GSH in *in vivo* tests with 2,4-D fed rats.

2.4.2 Mecoprop

The herbicide MCPP is also a synthetic auxin (plant hormone). The most important auxin produced by plants is indole-3-acetic acid (IAA). IAA plays important roles in a number of plant activities, including phototropism, gravitropism, apical dominance, fruit development, abscission, and root initiation.

MCPP stimulates nucleic acid and protein synthesis, affecting the efficacy of enzymes (U.S. EPA 1990a), causing a hormonal imbalance that distorts normal growth patterns (Coupland and Jackson 1991). This results in leaf epinasty, necrosis, chlorosis, and increased ethylene production (Coupland and Jackson 1991, Coupland *et al.* 1990).

2.5 ACUTE/SUBACUTE TOXICITY

2.5.1 2,4-D

2.5.1.1 Aquatic Organisms

Clinical symptoms of 2,4-D intoxication in spotted snakeheads were observed at 600 mg/L and included erratic swimming, difficult respiration, and excessive mucous secretion (Singh and Bhati 1994). Symptoms of acute toxicity in Indian toad tadpoles include rapid and erratic swimming and barrel-rolling, leading to fatigue and sluggishness, and finally death (Vardia *et al.* 1984). Temporary nest abandonment by nesting bluegill and redear sunfish and avoidance by steelhead-rainbow trout fry was observed upon application of 2,4-D to water bodies at levels of four and one mg/L respectively (Bettoli and Clark 1992, Folmar 1976). No avoidance of 2,4-D DMA was observed by mayfly nymphs (*Ephemerella walkeri*) at 10 or 100 mg/L, although 70 percent mortality was observed at the latter concentration (Folmar 1978).

Clinical symptoms may be accompanied by morphological and histopathological changes in the gills, kidney and liver (McBride *et al.* 1981, Neskovic *et al.* 1994, Pacces Zaffaroni *et al.* 1986, Singh and Bhati 1994). McBride *et al.* (1981) found that interrenal hypertrophy resulted from 48-h exposure of sockeye salmon smolts to 1.0 mg/L Aquakleen (2,4-D BEE). Spotted snakeheads exposed to 600 mg/L 2,4-D for 24-96 h experienced a significant decrease in total liver protein content (Singh and Bhati 1994). The adult crested newt experienced some morphological alterations of the kidney and liver after 72-h exposure to Agroxone 5 (IOE of 2,4-D) at lethal levels of 100-150 parts per million (ppm) (Pacces Zaffaroni *et al.* 1986). However, exposure of frog tadpoles (*Bufo bufo*) to up to 50 ppm 2,4-D for 48 h resulted in no visible effect or tissue residues (Cooke 1972).

Concentration dependent developmental effects were observed upon the acute exposure of bleak embryos to 50-3 200 mg/L Dikonirt (2,4-D Na) for 12-48 h (Biro 1979).

Table 2.2 lists a number of acute toxicity tests conducted on aquatic organisms. 2,4-D seems to be quite toxic to aquatic life with a number of experiments finding LC50s less than one mg/L. In general, it appears that the ester forms of 2,4-D are much more toxic to aquatic organisms than the free acid or 2,4-D salts with 96 h LC50s frequently under one mg/L (Alexander *et al.* 1985, Meehan *et al.* 1974, Wan *et al.* 1990, Wan *et al.* 1991), and toxicity increases with decreasing pH (Wan *et al.* 1990, Wan *et al.* 1991). The 96-h LC50 for *Gammarus lacustris* was 2.4 mg/L or less for three ester forms of 2,4-D and over 100 mg/L for the dimethylamine (DMA) formulation (Sanders 1969). Experiments in rainbow trout, coho and pink salmon (Wan *et al.* 1991) show LC50s for the 2,4-D diethylamine (DEA) formulation of 291 mg/L or more while experiments in steelhead-rainbow trout, channel catfish, and fathead minnows with the acid form of 2,4-D show a similar toxic range of concentrations (Alexander *et al.* 1985, McCorkle *et al.* 1977, Schultz 1973). Among the experiments with ester formulations,

tests in cutthroat, rainbow and steelhead-rainbow trout, chinook, chum, coho, pink and sockeye salmon indicate that the BE, BEE and PGBEE esters are generally more toxic (with 96-h LC50s less than 4.3 mg/L) than the IOE (Finlayson and Verrue 1985, Meehan *et al.* 1974, Wan *et al.* 1990, Wan *et al.* 1991, Woodward 1982). In actuality, the free acid is the physiologically toxic entity but the organic soluble ester forms are more readily taken up by fish.

Difficulty in comparing the toxicity tests is due to a number of factors. One of these is the use of commercial formulations like Weedestroy and Agroxone 5, which contain unknown additives, impurities and stabilizers, instead of pure compounds. Another is the hydrolysis of 2,4-D esters to the free acid form in non-renewed static tests, which may give an incorrect assessment of toxicity. Finlayson and Verrue (1985) found that 21-38 percent of BEE and PGBEE esters hydrolyzed to the free acid form within the three-hour 50 percent water volume replacement time in continuous flow tests.

2.5.1.2 Terrestrial Organisms

Clinical signs of acute toxicity in animals included muscle weakness, decreased activity, and motor incoordination (Beasley *et al.* 1991, Mattson *et al.* 1997, Morgulis *et al.* 1998, Oliveira and Palermo-Neto 1993, Paulino *et al.* 1994, Paulino and Palermo-Neto 1995, Paulino *et al.* 1996). There were often accompanying time and dose-dependent changes in serum components and enzyme activities including serum creatine kinase (CK), alkaline phosphatase (AP), creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and urea (Morgulis *et al.* 1998, Paulino and Palermo-Neto 1995, Paulino *et al.* 1996). Acute toxic effects were observed in broiler chicks fed 300 mg/kg 2,4-D or more and histopathological and biochemical alterations were observed at dose levels above 500 mg/kg (Morgulis *et al.* 1998). Holstein steers suffered acute toxicity at doses of 300 mg/kg or more (Paulino *et al.* 1994). Toxic effects were seen in rats at dose levels above 100 mg/kg (Oliveira and Palermo-Neto 1993); in addition, a dose of 600 mg/kg altered blood biochemistry (Paulino *et al.* 1996). English pointer dogs exhibited acute toxicity symptoms after a dose of 175 mg/kg 2,4-D (Beasley *et al.* 1991), and even a seven-day exposure to a properly treated lawn (4.26 L/ha) resulted in detectable 2,4-D levels in the serum, urine and kidney (Arnold *et al.* 1991). Acute dermal exposure of rabbits to 2,4-D DMA, IOE and BE (0.626-3.13 percent acid equivalents) resulted in no significant adverse effects (clinical, histopathological or blood chemistry) apart from skin irritation (Kay *et al.* 1965).

Acute intoxication effects (lethargy, motor dysfunction) are evidence of central nervous system depression resulting from a break in the blood-brain barrier and subsequent 2,4-D accumulation in the brain. Oliveira and Palermo-Neto (1995) found 1.6-5.6 percent of the dose of 2,4-D DMA (U46D Fluid) reached the CNS (rat brains) in relation to that detected in the serum. Tyynela *et al.* (1990) also found that 2,4-D (100-250 mg/kg) accumulated in rat brains. Pre-treatment of animals with 2,4-D caused an increase in cerebral 2,4-D concentration in rats (Elo and Ylitalo 1979), mice and rabbits (Kim *et al.* 1988). It was suggested that the organic anion transport system of 2,4-D at the blood-brain barrier became saturated resulting in accumulation in the rat brain (Kim *et al.* 1988, Tyynela *et al.* 1990). Exposure to high doses of 2,4-D Na salt (>30 mg/kg) and dimethylamine (>10 mg/kg) was found to alter the concentrations of neurotransmitters (serotonin and 5-hydroxyindoleacetic acid) in the rat brain and cerebrospinal fluid (Elo and MacDonald 1989, Oliveira and Palermo-Neto 1993). Experiments in rats treated with 50 or 100 mg/kg 2,4-D and then challenged with amphetamine, demonstrated that 2,4-D acts through serotonergic and dopaminergic mechanisms in a sex-dependent manner (Bortolozzi *et al.* 1998, Evangelista de Duffard *et al.* 1995). High dose acute exposure to 2,4-D or its formulations can also result in myotonia. Mice exposed to 50 mg/kg of topically applied 2,4-D BE showed evidence of CNS depression and myotonia (Blakley and Schiefer 1986). Experiments in rats, mice and dogs showed evidence of 2,4-D induced transient myotonia at levels of 100 mg/kg per d., 200 mg/kg and 8.8 mg/kg respectively (Beasley *et al.* 1991, Blakley and Schiefer 1986,

Mattson *et al.* 1997, Steiss *et al.* 1987, Toyoshima *et al.* 1985), but no evidence of polyneuropathy (Mattson *et al.* 1997, Steiss *et al.* 1987, Toyoshima *et al.* 1985). However, four cases of peripheral neuropathy were reported in humans following acute exposure to 2,4-D (Berkley and Magee 1963, Goldstein *et al.* 1959).

Myopathy is a muscle/muscle tissue disorder that develops under conditions of subacute intoxication (Muhlrad and Friedman 1978). Subacute exposure of guinea pigs and rats to 2,4-D (150-250 mg/kg) induced skeletal myopathy (Danon *et al.* 1978, Muhlrad and Friedman 1978). Based on a number of studies, including their own conducted in rats, Bernard *et al.* (1985) suggest that the morphologic development of myopathy is not associated with the progression of myotonia, but that the observed 20 to 30 percent decrease in muscle acetylcholinesterase after a single intraperitoneal dose of 200 mg/kg 2,4-D may play a role.

Caterpillars (*Eupackardia calleta*) fed 2,4-D (U46-D Fluid) at field application levels showed no elevated mortality, but there was evidence of ontogenic transfer to the imagines (adult moths) (Deml and Dettner 2001). Also, the adults emerged months earlier than the control *E. calleta* (Deml and Dettner 2001). The authors implied that adults descending from "treated" larvae could inbreed with unpredictable consequences for the offspring or that those with accelerated development may die without fertilizing eggs since other adults will not eclose until months later (Deml and Dettner 2001). The 48-h LD50 in honeybees (*Apis mellifera*) was 6.44 ug/bee for the acid formulation, and 3.70 ug/bee for the sodium salt of 2,4-D (Atkins *et al.* 1973).

Table 2.4 lists the LC and LD50s for 2,4-D in a number of terrestrial birds and mammals. It appears that, regardless of formulation, 2,4-D is relatively non-toxic to birds and mammals. The LD and LC50 for the bird species listed are 420 mg/L and over 5,000 ppm respectively (Heath *et al.* 1972, Morgulis *et al.* 1998). Oral gavage of pure 2,4-D formulations to rats resulted in a range of LD50s from 553-975 mg/kg for females, and 639-1,090 for males (Gorzinski *et al.* 1987). Similarly, dermal LD50s in rabbits were greater than 2,000 mg/kg (Gorzinski *et al.* 1987).

2.5.2 Mecoprop

2.5.2.1 Plants

Tests on *Lemna minor* (duckweed) found an average 10-d EC50 (based on frond number, fresh weight, and total chlorophyll) of 6,242 ug/L and tests on *Scenedesmus subspicatus* (freshwater green algae) found a 96-h EC50 of 102.7 mg/L (Kirby and Sheahan 1994). This great difference in toxicity is due to the fact that algal growth is not controlled by auxins.

2.5.2.2 Terrestrial Animals

Since MCPP is highly plant selective, the risk of acute toxicity to animals is low. However, kidney damage/failure is a characteristic of exposure to high doses.

MCPP is virtually non-toxic to mammals (Table 2.3). The acute oral LD50 for rats to the acid formulation of MCPP is 558-1,210 mg/kg b.wt. (U.S. EPA 1988, Verschuuren *et al.* 1975), and 1,060 mg/kg b.wt. for the diethanolamine salt (Gurd *et al.* 1965). The sodium salt and diethanolamine salt were of similar acute toxicity to mice with oral LD50s of 650 and 600 mg/kg b.wt. respectively (Gurd *et al.* 1965). Intraperitoneal tests with MCPP proved more toxic to mice and rats with LD50s of 350-600 mg/kg b.wt. for the acid, diethanolamine and sodium salt formulations (Gurd *et al.* 1965, Verschuuren *et al.* 1975). The acid form of MCPP is a persistent eye irritant that produces corneal opacities, iritis and conjuctival irritation (U.S. EPA 1988). Dermal studies of MCPP (acid) on rabbits show the LD50 is >2 g/kg and that exposure of abraded and unabraded skin (0.5 g for 24 h) did not result in dermal irritation (U.S. EPA 1988). However, dermal exposure of rabbits to 0.5 g/kg for three weeks resulted in skin erythema and

significant growth retardation (Verschuuren 1975). The inhalation LD50 for rats is greater than 12.5 mg/L (Extoxnet 1995).

MCPP is toxic to honey bees (*Apis mellifera*) with a 48-h LD50 of 1.67 ug/bee (Atkins *et al.* 1973), and practically non-toxic to birds (U.S. EPA 1988). The LC50 for MCPP acid is greater than 5,620 and 5,000 ppm (diet) for mallard ducks and northern bobwhite respectively (U.S. EPA 1988). Dietary studies for MCPP dimethylamine salt in mallards and Japanese quail show LC50 values of >12 000 and >14 000 ppm, respectively (U.S. EPA 1988). The acute oral LD50 for MCPP acid in northern bobwhite is 700 mg/kg (U.S. EPA 1988).

2.5.2.3 Aquatic Animals

As seen in Table 2.3, MCPP acid is of low toxicity to fish with 24 and 48-h LC50s of 250 mg/L (Hartley and Kidd 1986). The 96-h LC50 for rainbow trout and bluegill sunfish is 124 and >100 ppm respectively (Extoxnet 1995).

Table 2.2 Acute toxicity of 2,4-D and its formulations to aquatic organisms.

Species	Common Name	Life Stage/Age/ Weight	Formulation[a]	Test Type[b]	Duration (h)	Water Hardness (mg/L)	pH	Effect (mg/L)[c]	Reference
Aquatic Invertebrates									
Brachionus calyciflorus	rotifer	NR	NR	S, N	24	NR	NR	LC50 = 5	George *et al.* 1982
Daphnia lumholtzi	water flea	NR	NR	S, N	21	NR	NR	LC50 = 20	George *et al.* 1982
Daphnia magna	water flea	neonate	acid DMA IOE	S, N	48	136-157	7.0-8.2 8.0-8.2 8.2-8.4	LC50 = 25-36.4 LC50 = 184 LC50 >0.006*	Alexander *et al.* 1985
Mesocyclops leuckarti	copepod	NR	NR	S, N	720	NR	NR	LC50 >50	George *et al.* 1982
Gammarus lacustris	amphipod	60 d	BEE PGBE IOE DMA	S, N	96	30	7.1	LC50 = 0.44 LC50 = 1.6 LC50 = 2.4 LC50 >100	Sanders 1969
Procambarus spp.	crawfish	NR	Weedestroy (DMA)	SR, N	96	128	7.5	LC50 = 1 870	Abdelghani *et al.* 1997
Aquatic Plants and Phytoplankton									
Salvinia natans	fern	14 leaves	Deherban A (DMA)	S, N	4 wks	NR	NR	EC50 = 6 (leaf growth) EC50 = 6.5 (wet weight) EC50 = 0.3 (amt. chlorophyll)	Goncz and Sencic 1994
Phyllospora comosa	brown algae	1 d zygotes	acid	S, N	96	NR	7	LC50 = 100	Burridge *et al.* 1995
Dunaliella tertiolecta	green algae	NR	acid	S, N	4 + 2 h	NR	NR	EC50 = 185 (growth)	Okay and Gaines 1996
Phaeodactylum tricornutum	diatom	NR	acid	S, N	4 + 2 h	NR	NR	EC50 = 362 (growth)	Okay and Gaines 1996
Fish									
Oncorhynchus clarki	cutthroat trout	0.4-0.8 g	BE PGBEE IOE	NR	96	NR	NR	LC50 = 0.78 LC50 = 0.77 LC50 >50	Woodward 1982
Oncorhynchus mykiss	rainbow trout	0.7 g	DEA DEA DEA IOE IOE IOE	S, M	96	4.8 38.9 80.5 4.8 30.9 80.5	6.3 7.5 8 6.3 7.5 8	LC50 = 409 LC50 = 511 LC50 = 744 LC50 = 167 LC50 = 164 LC50 = 79	Wan *et al.* 1991
Oncorhynchus mykiss	rainbow trout	0.7 g	BEE	S, M	96	4.2 43.4 86	6.1 7.5 8.1	LC50 = 0.8 LC50 = 1.3 LC50 = 2.2	Wan *et al.* 1990
Oncorhynchus tshawytscha	chinook salmon	fry 14.6-15.4 g 14.6-15.4 g	BEE BEE PGBEE	F, M	96	15-17	7.0-7.2	LC50 = 0.315 LC50 = 0.375 LC50 = 0.246	Finlayson and Verrue 1985
Oncorhynchus tshawytscha	chinook salmon	0.7 g	BEE	S, M	96	43.4	7.5	LC50 = 0.6	Wan *et al.* 1990
Oncorhynchus keta	chum salmon	0.7 g	BEE	S, M	96	43.4	7.5	LC50 = 0.7	Wan *et al.* 1990

Species	Common Name	Life Stage/Age/ Weight	Formulation[a]	Test Type[b]	Duration (h)	Water Hardness (mg/L)	pH	Effect (mg/L)[c]	Reference
Oncorhynchus keta	chum salmon	fry	BE acid IOE	S, N	96	10-33	NR	LC50 <1 LC50 >10 & <50 LC50 >1 & <5	Meehan *et al.* 1974
Salmo gairdneri	steelhead -rainbow trout	fry fry 6.4-7.8 g 6.4-7.8 g 7.6-7.8 g	BEE PGBEE BEE PGBEE BEE	F, M F, M F, M F, M S, M	96	15-17	7.0-7.2	LC50 = 0.518 LC50 = 0.329 LC50 = 0.468 LC50 = 0.342 LC50 = 1.2-3.7	Finlayson and Verrue 1985
Salmo gairdneri	steelhead -rainbow trout	fingerlings	acid BE IOE	S, N	96	10-33	NR	LC50 >50 LC50 <1 LC50 >10 & <50	Meehan *et al.* 1974
Salmo gairdneri	steelhead -rainbow trout	0.26-0.34 g	acid DMA IOE	S, N	96	78-108	3.3-7.8 7.3-8.1 7.7-8.3	LC50 = 358 LC50 = 250 LC50 >0.006*	Alexander *et al.* 1985
Oncorhynchus kisutch	chinook salmon	0.7 g	DEA DEA DEA IOE IOE IOE	S, M	96	4.8 38.9 80.5 4.8 30.9 80.5	6.3 7.5 8 6.3 7.5 8	LC50 = 472 LC50 = 493 LC50 = 662 LC50 = 156 LC50 = 158 LC50 = 63	Wan *et al.* 1991
Oncorhynchus kisutch	chinook salmon	0.7 g	BEE	S, M	96	4.2 43.4 86	6.1 7.5 8.1	LC50 = 1.1 LC50 = 1.5 LC50 = 4.3	Wan *et al.* 1990
Oncorhynchus kisutch	chinook salmon	fry fry fry fingerlings fingerlings fingerlings	BE acid IOE IOE acid BE	S, N	96	10-33	NR	LC50 <1 LC50 >10 & <50 LC50 >10 & <50 LC50 >50 LC50 >50 LC50 >1 & <5	Meehan *et al.* 1974
Oncorhynchus gorbuscha	pink salmon	0.7 g	DEA DEA DEA IOE IOE IOE	S, M	96	4.8 38.9 80.5 4.8 30.9 80.5	6.3 7.5 8 6.3 7.5 8	LC50 = 291 LC50 = 363 LC50 = 438 LC50 = 30 LC50 = 70 LC50 = 21	Wan *et al.* 1991
Oncorhynchus gorbuscha	pink salmon	0.7 g	BEE	S, M	96	4.2 43.4 86	6.1 7.5 8.1	LC50 = 0.4 LC50 = 0.9 LC50 = 1.1	Wan *et al.* 1990
Oncorhynchus gorbuscha	pink salmon	fry	BE acid IOE	S, N	96	10-33	NR	LC50 <1 LC50 >10 & <50 LC50 >1 & <5	Meehan *et al.* 1974
Oncorhynchus nerka	sockeye salmon	0.7 g	BEE	S, M	96	43.4	7.5	LC50 = 1.4	Wan *et al.* 1990
Oncorhynchus nerka	sockeye salmon	smolts	acid BE IOE	S, N	96	10-33	NR	LC50 >50 LC50 <1 LC50 >5 & <10	Meehan *et al.* 1974
Lepomis macrochirus	bluegill sunfish	juvenile	Weedestroy (DMA)	SR, N	96	128	7.5	LC50 = 664	Abdelghani *et al.* 1997
Lepomis macrochirus	bluegill sunfish	55 g	DMA	S, N	96	40	7.0	LC50 = 177 LC50 = 160	Schultz 1973
Lepomis macrochirus	bluegill sunfish	NR	acid DMA IOE	S, N	96	78-108	3.4-8 7.3-7.9 7.6-8.3	LC50 = 263 LC50 = 524 LC50 >0.006*	Alexander *et al.* 1985

Species	Common Name	Life Stage/Age/ Weight	Formulation[a]	Test Type[b]	Duration (h)	Water Hardness (mg/L)	pH	Effect (mg/L)[c]	Reference
Ictalurus punctatus	channel catfish	40-70 g	NR	SR, N	96	30	7.0-7.2	LC50 = 90	Gallagher and Di Giulio 1991
Ictalurus punctatus	channel catfish	juvenile	Weedestroy (DMA)	SR, N	96	128	7.5	LC50 = 452	Abdelghani *et al.* 1997
Ictalurus punctatus	channel catfish	52 g	DMA	S, N	96	40	7.0	LC50 = 125-193	Schultz 1973
Ictalurus punctatus	channel catfish	14 g	acid DMA	S, N	48	22	8.2	LC50 >10 LC50 >10	McCorkle *et al.* 1977
Oryzias latipes	Japanese medaka	18-71 mg	NR	F, M	96	38-52	7.88	LC50 = 2780	Holcombe *et al.* 1995
Pimephales promelas	fathead minnow	NR	DMA	S, N	96	40	7.0	LC50 = 335	Schultz 1973
Pimephales promelas	fathead minnow	0.14 g 0.11 g 0.10 g	acid DMA IOE	S, N	96	78-108	3.3-7.8 6.6-7.9 7.8-8.4	LC50 = 320 LC50 = 344 LC50 >0.006*	Alexander *et al.* 1985
Salvelinus malma	Dolly Varden	fingerlings	acid BE IOE	S, N	96	10-33	NR	LC50 >50 LC50 >1 & <5 LC50 >10 & <50	Meehan *et al.* 1974
Marone saxatilis	striped bass	NR	NR	S, N	96	50	7.2	LC50 = 70.1	Rehwoldt *et al.* 1977
Fundulus diaphanus	banded killifish	NR	NR	S, N	96	50	7.2	LC50 = 26.7	Rehwoldt *et al.* 1977
Lepomis gibbosus	pumpkinseed	NR	NR	S, N	96	50	7.2	LC50 = 94.6	Rehwoldt *et al.* 1977
Alburnus alburnus	bleak	embryos larvae	Dikonirt (Na salt)	S, N	48	NR	NR	LC50 = 12.9 LC50 = 51.6	Biro 1979
Roccus americanus	white perch	NR	NR	S, N	96	50	7.2	LC50 = 40	Rehwoldt *et al.* 1977
Anguilla rostrata	American eel	NR	NR	S, N	96	50	7.2	LC50 = 300.6	Rehwoldt *et al.* 1977
Libistes reticulatus	guppy	NR	NR	S, N	96	50	7.2	LC50 = 70.7	Rehwoldt *et al.* 1977
Cyprinus carpio	common carp	4.9 g	acid	SR, N	96	141-223	7.0-7.5	LC50 = 270	Neskovic *et al.* 1994
Cyprinus carpio	common carp	NR	NR	S, N	96	50	7.2	LC50 = 96.5	Rehwoldt *et al.* 1977
Amphibian									
Triturus cristatus carnifex	crested newt	5.7 g (male) 9.4 g (female)	Agroxone 5 (37% IOE)	SR, N	96	NR	NR	LC50 = 102 LC50 = 132	Pacces Zaffaroni *et al.* 1986
Bufo melanostictus	Indian toad	tadpole	NR	S, N	96	220	8.3	LC50 = 8.05	Vardia *et al.* 1984

NR - not reported

[a] BEE - butoxyethanol ester; BE - butyl ester; DMA- dimethylamine salt; PGBE - propylene glycol butyl ether ester; IOE - isooctyl ester (also called 2-ethylhexyl ester (EHE)), DEA - diethanolamine salt

[b] S - static test; SR - static renewal test; F - flowthrough test; N - nominal concentration; M - measured concentration

[c] LC50 = concentration lethal to 50% of population; EC50 = concentration affecting 50% of population

* water solubility level

Table 2.3 Acute toxicity of mecoprop to mammals, birds and fish.

Species	Common Name	Size/Age	Formulation [a]	Test Type	Duration (h)	Effect [b]	Reference
Mammals							
Rattus norvegicus	rat	NR	acid	inhalation	4	LC50 >12.5 mg/L	Extoxnet 1995
Oryctolagus cuniculus	rabbit	NR	acid	dermal	-	LD50 >2 g/kg	U.S. EPA 1988
Rattus norvegicus	rat	NR	acid	oral	-	LD50 = 558	U.S. EPA 1988
Rattus norvegicus	rat	100-120 g	acid	oral intraperitoneal	- -	LD50 = 1 210 LD50 = 402	Verschuuren *et al.* 1975
Rattus norvegicus	rat	NR	DEA	oral intraperitoneal	- -	LD50 = 1 060 LD50 = 350	Gurd *et al.* 1965
Mus musculus	mouse	NR	DEA	oral intraperitoneal	- -	LD50 = 600 LD50 = 400	Gurd *et al.* 1965
Rattus norvegicus	rat	NR	Na salt	intraperitoneal	-	LD50 = 500	Gurd *et al.* 1965
Mus musculus	mouse	NR	Na salt	oral intraperitoneal	- -	LD50 = 650 LD50 = 600	Gurd *et al.* 1965
Birds							
Anas platyrhynchos	mallard duck	NR	acid	dietary	NR	LC50 >5 620	U.S. EPA 1988
Anas platyrhynchos	mallard duck	NR	DMA	dietary	NR	LC50 >12 000	U.S. EPA 1988
Colinus virginianus	northern bobwhite	NR	acid	oral dietary	- NR	LD50 = 700 LC50 >5 000	U.S. EPA 1988
Coturnix coturnix japonica	Japanese quail	NR	DMA	dietary	NR	LC50 >14 000	U.S. EPA 1988
Fish							
Oncorhynchus mykiss	rainbow trout	NR	acid		96	LC50 = 124	Extoxnet 1995
Lepomis macrochirus	bluegill sunfish	NR	acid		96	LC50 >100	Extoxnet 1995

NR - not reported

[a] DMA- dimethylamine salt; DEA - diethanolamine salt

[b] LD50 - dose lethal to 50% of the population (mg/kg body weight); LC50 - concentration lethal to 50% of the population (ppm)

Table 2.4 Acute toxicity of 2,4-D and its formulations to birds and mammals.

Species	Common Name	Life Stage/ Age/Weight	Formulation[a]	Test Type	Duration	Effect (mg/kg)[b]	Reference
Birds							
Gallus domesticus	broiler chick	21 d	U-46 D-Fluid (DMA)	oral gavage	-	LD50 = 420[m]	Morgulis *et al.* 1998
Colinus virginianus	northern bobwhite	2-3 wks	BEE DMA	dietary	5 d	LC50 >5 000 ppm LC50 >5 000 ppm	Heath *et al.* 1972
Coturnix coturnix japonica	Japanese quail	2-3 wks	BEE DMA	dietary	5 d	LC50 >5 000 ppm LC50 >5 000 ppm	Heath *et al.* 1972
Phasianus colchicus	ring-necked pheasant	2-3 wks	BEE DMA	dietary	5 d	LC50 >5 000 ppm LC50 >5 000 ppm	Heath *et al.* 1972
Anas platyrhynchos	mallard duck	2-3 wks	BEE DMA	dietary	5 d	LC50 >5 000 ppm LC50 >5 000 ppm	Heath *et al.* 1972
Mammals							
Rattus norvegicus	Wistar rats	204-277 g	Dikamin D (DMA)	oral gavage	-	LD50 = 1 145.5[f], 940[m] ul/kg	Varnagy *et al.* 1995
Rattus norvegicus	Fischer 344 rats	young adult	acid IOE DMA IBE Na salt BEE BE	oral gavage	-	LD50 = 639[m], 764[f] LD50 = 982[m], >720-<864[f] LD50 = 1 090[m], 863[f] LD50 = 700[m], 553[f] LD50 = 876[m], 975[f] LD50 = 887[m], 831[f] LD50 = 732[m], 600[f]	Gorzinski *et al.* 1987
Oryctolagus cuniculus	New Zealand white rabbits	young adult	acid IOE DMA IBE Na salt BEE BE	dermal	14 d (observation)	LD50 >2 000 LD50 >2 000 LD50 = 2 244 LD50 >2 000 LD50 >2 000 LD50 >2 000 LD50 >2 000	Gorzinski *et al.* 1987

[a] DMA - dimethylamine salt; IBE - iso-butyl ester; BEE - butoxyethanol ester; BE - butyl ester; IOE - isooctyl ester (also called 2-ethylhexyl ester [EHE])

[b] f - female; m - male

2.6 CHRONIC TOXICITY AND ECOLOGICAL EFFECTS

2.6.1 2,4-D

2.6.1.1 General Effects

Exposure of several freshwater fish species (striped bass, banded killifish, white perch, American eel, carp and guppy) to 0.1 ppm 2,4-D for 10 months resulted in no observable physiological symptoms and no significant differences in weight/time relationships between exposed and control fish (Rehwoldt *et al.* 1977). However, exposure of larval Japanese medaka to 2,4-D (39.2-42.5 mg/L) for 28 d resulted in significantly decreased mean weights (Holcombe *et al.* 1995). As with acute studies, subacute exposure of aquatic organisms to 2,4-D results in histopathological and biochemical alterations. Carp exposed to 2,4-D for 14 d developed histopathological alterations of the gills, liver and kidney at 250 mg/L, and altered enzyme activities (alkaline phosphatase and glutamicoxaloacetic phosphatase) at 200 mg/L (Neskovic *et al.* 1994).

Adaptation of organisms to prolonged, continuous 2,4-D exposure has been observed in the marine microalgae *Phaeodactylum tricornutum* where the cell population initially decreased upon addition of 500 mg/L 2,4-D and then recovered (Okay and Gaines 1996). Pond studies of 2,4-D DMA at concentrations of approximately 0.5 or 1.0 mg/L (rate of five or 10 kg/ha) found that arrowhead (*Sagittaria montividensis*) was the only plant species killed by the herbicide (Boyle 1980). Within four to six weeks after treatment, however, arrowhead reappeared (Boyle 1980). Boyle (1980) also noted that 2,4-D treatment appeared to stimulate the growth of plankton and rooted macrophytes, and that bluegill sunfish taken from treated ponds were significantly larger than controls (Boyle 1980). This growth stimulation at low levels of 2,4-D was also observed in a similar study by Sarkar *et al.* (1991) where treatment with 4.5-10.5 kg/ha/year for one year resulted in significant increases in bottom fauna.

Repeated dermal exposure of Fischer 344 rats to a 24 percent aqueous solution of 2,4-D dimethylamine (DMA) for two weeks caused severe dermatitis (Mattson *et al.* 1986). Broiler chicks fed over 2 000 mg/kg 2,4-D for three weeks exhibited reduced food consumption and growth rates (Whitehead and Pettigrew 1972). Subchronic and chronic dietary studies of 2,4-D DMA in Wistar rats (200 ppm for 30 d and 180 d, respectively) resulted in no overt clinical signs of toxicity but there were serum enzyme level alterations including increased serum aspartate aminotransferase (AST) activity and albumin levels (Paulino *et al.* 1996). A subchronic dietary study of 2,4-D acid in Fischer 344 rats (0-150 mg/kg/d for 13 weeks) resulted in decreased body weight gain, minor alterations in serum enzyme levels, and kidney damage (Gorzinski *et al.* 1987). Further 13-week subchronic studies of 2,4-D acid, 2,4-D DMA and 2,4-D 2-ethylhexyl ester (also called IOE) using Fischer 344 rats and beagles were conducted at doses of 1-300 and 1-7.5 mg/kg/d (acid equivalents), respectively (Charles *et al.* 1996a; 1996b). Observed effects in the rat were comparable for all formulations and included reduced body weight gain, decreases in red blood cell mass, decreases in serum T3 and T4 (triiodothyronine and thyroxine) levels in females, and increases in kidney, liver and thyroid weights (Charles *et al.* 1996a). Effects in the dog were also comparable for all formulations and included reduced body weight gain, increased serum creatinine and alanine aminotransferase (ALT) levels, and decreased alkaline phosphatase (AP) levels (Charles *et al.* 1996b). A one-year chronic study (0-7.5 mg/kg/d) in beagles found no clinical symptoms of 2,4-D toxicity except a slightly reduced female body weight gain, and serum biochemistry alterations at the highest dose (Charles *et al.* 1996b). Rats fed 2,4-D for two years exhibited altered serum biochemistry parameters at doses above 75 mg/kg (Charles *et al.* 1996c). Rat pups nursing from treated dams (50-100 mg/kg 2,4-D) until post-natal day nine or 15, exhibited reduced body weight gain and diminished tissue weights and contained detectable

2,4-D residues in the stomach, blood, kidney and brain (Sturtz *et al.* 2000). Based on the above studies, a subchronic NOEL of 15 mg/kg/d in rats and 1.0 mg/kg/d in beagles was suggested for the acid, dimethylamine and ethylhexyl/ isooctyl ester formulations of 2,4-D (Charles *et al.* 1996a; 1996b, Gorzinski *et al.* 1987).

Adaptation was observed after repeated oral administration of subtoxic doses of 2,4-D in pigs (50 mg/kg/d for up to one month) and chickens (300 mg/kg/d for 12-24 d) as plasma levels in pigs and chickens started to decline after about a week (Erne 1966). Varnagy *et al.* (1995) found that repeated oral treatments of Dikamin D (Na salt) to rats over 28 d resulted in an observed tolerance to the compound accounted for by an increased metabolic capacity.

2.6.1.2 Carcinogenic Activity and Genotoxicity

In vivo and *in vitro* tests in aquatic and terrestrial organisms indicate that 2,4-D is a hepatic peroxisome inducer/proliferator (Kawashima *et al.* 1984, Lundgren *et al.* 1987, Mustonen *et al.* 1989, Pineau *et al.* 1996, Vainio *et al.* 1983). A peroxisome proliferator causes an increase in the size and number of hepatic peroxisomes – cellular organelles characterized by the presence of catalase and fatty acid B-oxidizing activity (Mustonen *et al.* 1989). Peroxisome proliferators in general have been linked to the formation of hepatic tumours in rats (Abdellatif *et al.* 1990, Gallagher and DiGiulio 1991). However, while 2,4-D (0.05 percent) was found to double the peroxisomal B-oxidation of fatty acids, no cancers were observed in treated rats (Abdellatif *et al.* 1990).

Blakley *et al.* (1992) found that while exposure to 0-50 mg/kg/d 2,4-D amine (Weed-no-More) for 15-52 weeks had a mild enhancing effect on chemically induced pulmonary adenoma production in mice, there was no effect on the incidence of murine lymphocytic leukemia. Hansen *et al.* (1971) conducted a chronic dietary study in beagles (two years: 0-500 ppm) and found no treatment related lesions. Dietary administration in mice of 2,4-D (149 and 333 ppm), 2,4-D IPE (isopropyl ester), 2,4-D BE, and 2,4-D IOE (111-149 ppm) gave no significant indication of tumorigenicity (Innes *et al.* 1969). Two two-year chronic dietary studies of 2,4-D acid in rodents generated contradictory results (Charles *et al.* 1996c, Hansen *et al.* 1971). Hansen *et al.* (1971) found a dose-related increase in the incidence of total tumours in female rats and an increase in malignant tumours in male rats fed 0-1 250 ppm 2,4-D for two years, while Charles *et al.* (1996c) found no oncogenic effect of 0-300 mg/kg 2,4-D in mice or 0-150 mg/kg in rats.

There have been many human epidemiological studies on phenoxy herbicide exposure and non-Hodgkin's lymphoma (NHL), but few animal studies. In a case-control study, Hayes *et al.* (1991) found only a modest association (odds ratio of 1.3) between canine malignant lymphoma (non-Hodgkin's) in the dogs of owners who treated their lawns with a 2,4-D formulation, with the risk increasing with the number of applications per year. Cohort and case-control studies were conducted in humans to examine a possible link between 2,4-D exposure and NHL. Bond *et al.* (1988) found two cases of NHL in a cohort of 878 chemical workers potentially exposed to 2,4-D; however, both cases were among the subset of workers with the potential for additional exposure to TCDD and/or H/OCDD. A case-control study conducted in Washington State found no increased risk of NHL among farmers who reported regularly working with any specific phenoxy herbicide product (including 2,4-D) or phenoxy herbicides in general (Woods and Polissar 1989). However, a statistically significant risk of NHL was identified when phenoxy herbicides were considered in the presence of organic solvents – a joint exposure odds ratio of 1.50 (Woods and Polissar 1989). Another case-control study of farmers in Nebraska found a 50 percent excess of NHL among farmers who mixed or applied 2,4-D and this risk increased with the average frequency of use for those exposed for 20 or more days per year (Zahm *et al.* 1990). Adjustment for organophosphate and fungicide use resulted in an odds ratio of 3.1 for farmers who used 2,4-D for more than 20 d per year (Zahm *et al.* 1990). Both case-control studies were based on interviews with cases or next of kin.

A number of genotoxicity studies have been conducted and are presented in Table 2.5. Tests of 2,4-D acid as well as its salt and ester formulations with plant species indicate that 2,4-D is both clastogenic and mutagenic to plants (Al-Najjar and Soliman 1982, Khalatkar and Bhargava 1982, Khalatkar and Bhargava 1985, Mohandes and Grant 1972, Pavlica 1991, Surya Kumari and Vaidyanath 1989). Clastogenic is defined as a substance or process which causes breaks in chromosomes or other chromosomal aberrations (including chromatin bridges, fragments, lagging chromosomes, stickiness, chromatin bodies) (Adhikari and Grover 1988). These findings are not unexpected since, being an herbicide, 2,4-D is highly toxic to plants.
In general, 2,4-D was negative in the Ames test (Charles *et al.* 1999a, Klopman *et al.* 1985, Zetterberg *et al.* 1977), except for strain TA97 in which 2,4-D was mutagenic with S9 activation (Kappas 1988). 2,4-D testing in the prokaryotic DNA repair assay indicated positive activity in *B. subtilis* but not in *E. coli* (Klopman *et al.* 1985), and exposure of fungi (*Aspergillus nidulans*) and yeast (*Saccharomyces cerevisiae*) to 2,4-D was weakly associated with mitotic recombination and gene conversion (Kappas 1988, Klopman *et al.* 1985, Siebert and Lemperle 1974, Zetterberg *et al.* 1977).

Sex-linked recessive lethal tests with *Drosophila melanogaster* have varied results, with three of the four tests in Table 2.5 indicating that 2,4-D does cause lethal mutations (Kale *et al.* 1995, Magnusson *et al.* 1977, Tripathy *et al.* 1993). The *Drosophila melanogaster* wingspot test, where induced genetic changes are phenotypically expressed as mosaic spots on the wings of eclosing adults, was also positive (Tripathy *et al.* 1993).
2,4-D was not clastogenic to bullfrog tadpole erythrocytes as determined by the alkaline single cell DNA electrophoresis assay (Clements *et al.* 1997).

In mammalian cell lines, it appears that 2,4-D does not cause sister chromatid exchanges (Linnainmaa 1984, Mustonen *et al.* 1989), except in two studies with human lymphocytes exposed to more than 10 ug/ml for 48-72 hours (Korte and Jalal 1982, Turkula and Jalal 1985). Amine formulations of 2,4-D, except for the trimethyl amine (Clausen *et al.* 1990), were found to be clastogenic in *in vitro* studies with human lymphocytes and fibroblasts (Clausen *et al.* 1990, Mustonen *et al.* 1986), but negative in rat lymphocytes (Gollapudi *et al.* 1999). *In vivo* studies in rats found that 2,4-D was clastogenic in bone marrow cells (Adhikari and Grover 1988, Turkula and Jalal 1987), but *in vivo* studies of all 2,4-D formulations in mice indicate that 2,4-D is not clastogenic at any level in the bone narrow micronucleus test (Charles *et al.* 1999b). Pavlica *et al.* (1991) and Ahmed *et al.* (1977a) found that 2,4-D caused thioguanine resistant mutations and ouabain resistant mutations in Chinese hamster V79 fibroblasts, but Gollapudi *et al.* (1999) found that the BEE, IPA and TIPA formulations did not cause thioguanine mutants. *In vivo* and *in vitro* tests with rat hepatocytes found that 2,4-D did not cause unscheduled DNA synthesis (Charles *et al.* 1999a) in contrast to an *in vitro* study of 2,4-D in human SV40 transformed cells (Ahmed *et al.* 1977a).

Since 2,4-D is not metabolized to an active ingredient, is eliminated from the body within days and does not accumulate in body tissues, did not produce tumours in rat, mice and beagle studies, and gives generally negative results in genotoxicity assays, the carcinogenic or tumorigenic risk of 2,4-D does not appear to be substantial. Epidemiological findings of an increased risk of NHL in humans with 2,4-D exposure was not well supported by the animal study by Hayes *et al.* (1991), so findings on this relationship are still inconclusive.

Table 2.5 Genotoxicity/mutagenicity studies of 2,4-D and its formulations.

Test	Organism	Formulation [a]	Dose Range	Mutagenic Potential [b]	Reference
Plants					
clastogenicity	onion (*Allium cepa*)	2,4-D acid	25-100 ppm (4 h)	positive	Surya, Kumari and Vaidyanath 1989
clastogenicity	onion (*Allium cepa*)	Estasol 128 (BE)	50 & 100 ppm (2 & 6 h)	positive (6 h)	Mohandes and Grant 1972
clastogenicity	barley seeds (*Hordeum vulgare*)	NR NR	100 ppm (9 h) 100 ug/g (9-18 h)	positive (M1 generation)	Khalatkar and Bhargava 1982
clastogenicity	shallot roots (*Allium ascalonicum*)	NR	45 & 450 uM	positive	Pavlica *et al.* 1991
clastogenicity	wheat (*Triticum* sp.)	2,4-D Na salt	0.1% soil application	positive (M1 generation)	Al-Najjar and Soliman 1982
mutation	barley seeds (*Hordeum vulgare*)	NR NR	100 ppm (9 h) 100 ug/g (9-18 h)	positive (M2 generation) positive (M2 generation)	Khalatkar and Bhargava 1982 Khalatkar and Bhargava 1985
mutation	barley seeds (*Hordeum vulgare*)	Estasol 128 (BE)	100-500 ppm (6 h)	positive (200 ppm - M2 generation)	Mohandes and Grant 1972
mutation	rice (*Oryza sativa*)	2,4-D acid	100-300 ppm (4 h)	positive	Surya, Kumari and Vaidyanath 1989
Prokaryotes/ Eukaryotes					
DNA repair (rec-assay)	*Bacillus subtilis*	NR	NR	negative (-)	Shirasu *et al.* 1976
mitotic recombination	*Aspergillus nidulans*	NR	4-48 uM	positive (+)	Kappas 1988
mitotic recombination	*Saccharomyces cerevisiae*	2,4-D acid	NR	negative	Klopman *et al.* 1985
mitotic recombination	*Saccharomyces cerevisiae*	2,4-D Na	0.2 & 0.3 mg/mL (3 h)	positive (0.3 mg/mL)	Zetterberg *et al.* 1977
mitotic gene conversion	*Saccharomyces cerevisiae*	U46D Fluid (DMA)	1 000 ppm (16 h)	weak positive	Siebert and Lemperle 1974
DNA repair assay	*Escherichia coli* *Bacillus subtilis*	2,4-D acid	NR	negative positive	Klopman *et al.* 1985
gene conversion	*Escherichia coli* WP2	NR	0-5 000 ug/plate	negative (+ and -)	Moriya *et al.* 1983
gene reversion (Ames test)	*Salmonella typhimurium* (5 strains)	NR	0-5 000 ug/plate	negative (+ and -)	Moriya *et al.* 1983
gene reversion (Ames test)	*Salmonella typhimurium*	2,4-D acid	NR	negative	Klopman *et al.* 1985
gene reversion (Ames test)	*Salmonella typhimurium*	2,4-D Na	0.5 mg/mL	negative	Zetterberg *et al.* 1977
gene reversion (Ames test)	*Salmonella typhimurium* (5 strains)	NR	250-750 ug/g	weak positive in 1 strain (+)	Kappas 1988
gene reversion (Ames test)	*Salmonella typhimurium* (5 strains)	2,4-D acid, 2,4-D TIPA, 2,4-D DEA, 2,4-D DMA, 2,4-D EHE, 2,4-D IPA, 2,4-D BEE, 2,4-D IPE	10-14 000 ug/plate	negative (+ and -)	Charles *et al.* 1999a
Drosophila					
sex-linked recessive lethal	*Drosophila melanogaster*	NR	4.5 & 9.0 mM (3 d)	negative	Vogel and Chandler 1974

Test	Organism	Formulation [a]	Dose Range	Mutagenic Potential [b]	Reference
sex-linked recessive lethal	*Drosophila melanogaster*	2,4-D acid	1 000 ppm (2 wk)	weak positive	Magnusson *et al.* 1977
sex-linked recessive lethal	*Drosophila melanogaster*	NR	2.5-10 mM (48-72 h)	positive (>2.5 mM)	Tripathy *et al.* 1993
sex-linked recessive lethal	*Drosophila melanogaster*	2,4-D Amine	10 000 ppm	positive	Kale *et al.* 1995
wing spot test	*Drosophila melanogaster*	NR	2.5-10 mM (48-72 h)	positive (>5 mM)	Tripathy *et al.* 1993
Amphibian					
clastogenicity: alkaline single-cell DNA electrophoresis assay (*in vivo*)	tadpole erythrocytes (bullfrog)	Amsol (2,4-D amine)	4.06-65 mg/L (24h)	negative	Clements *et al.* 1997
Mammalian					
sister chromatid exchange	human lymphocytes	NR	50-250 ug/mL (72h)	positive (50 ug/mL)	Turkula and Jalal 1985
sister chromatid exchange	human lymphocytes	NR	0.2-60 ug/mL (48h)	positive (>10 ug/mL)	Korte and Jalal 1982
sister chromatid exchange	Chinese hamster ovary cells	Vesakontuho Tasku (amine salt)	10^{-6} - 10^{-3} M (1h)	weak positive	Linnainmaa 1984
sister chromatid exchange (*in vivo*)	rat lymphocytes	Vesakontuho Tasku (amine salt)	100-200 mg/kg/d (1wk)	negative	Linnainmaa 1984
sister chromatid exchange (*in vivo*)	rat lymphocytes	2,4-D acid	100 mg/kg/d (2wk)	negative	Mustonen *et al.* 1989
sister chromatid exchange (*in vivo*)	Chinese hamster bone marrow cells	Vesakontuho Tasku (amine salt)	100 mg/kg/d (9d)	negative	Linnainmaa 1984
clastogenicity	rat lymphocytes	2,4-D BEE, 2,4-D IPA, 2,4-D TIPA	175-5 000 ug/mL (4h)	negative (+ and -)	Gollapudi *et al.* 1999
clastogenicity	human lymphocytes	2,4-D acid Vesakontuho Tasku (amine salt)	0.125- 0.35 mM (24h) 0.50-1.25 mM (24h)	negative positive	Mustonen *et al.* 1986
clastogenicity	human lymphocytes	NR	0.2-60 ug/ml (48h)	positive (>50 ug/mL)	Korte and Jalal 1982
clastogenicity	human fibroblasts	2,4-D acid U46D Fluid (DMA) 2,4-D DMA 2,4-D TMA	10-100 mM (45min)	negative positive positive negative	Clausen *et al.* 1990
clastogenicity: bone marrow micronucleus test (*in vivo*)	rat	NR	25-250 ug/kg (4 & 24h)	positive (>75 ug/kg)	Turkula and Jalal 1987
clastogenicity: bone marrow micronucleus test (*in vivo*)	rat	2,4-D acid	17.5-70 mg/kg b.wt. (48h)	positive (>25 mg/kg)	Adhikari and Grover 1988
clastogenicity: bone marrow micronucleus test (*in vivo*)	mouse	2,4-D acid, 2,4-D DEA, 2,4-D DMA, 2,4-D IPA, 2,4-D TIPA, 2,4-D BEE, 2,4-D EHE, 2,4-D IPE	250-2 000 mg/kg	negative	Charles *et al.* 1999b
mutation: thioguanine resistance	Chinese hamster V79 fibroblasts	NR	10-100 ug/mL (1h)	positive	Pavlica *et al.* 1991

Test	Organism	Formulation [a]	Dose Range	Mutagenic Potential [b]	Reference
mutation: thioguanine resistance	Chinese hamster ovary cells	2,4-D BEE, 2,4-D IPA, 2,4-D TIPA	150-5 000 ug/mL (4-5h)	negative (+ and -)	Gollapudi *et al.* 1999
mutation: ouabain resistance	Chinese hamster V79 cells	2,4-D Fluid	0.01 mM (48h)	positive	Ahmed *et al.* 1977b
unscheduled DNA synthesis (*in vivo*)	rat primary hepatocytes	2,4-D acid, 2,4-D DEA, 2,4-D DMA, 2,4-D IPA, 2,4-D TIPA, 2,4-D BEE, 2,4-D EHE, 2,4-D IPE	400 & 1 000 mg/kg	negative	Charles *et al.* 1999a
unscheduled DNA synthesis	rat primary hepatocytes	2,4-D acid, 2,4-D DEA, 2,4-D DMA, 2,4-D IPA, 2,4-D TIPA, 2,4-D BEE, 2,4-D EHE, 2,4-D IPE	0.5-478 ug/mL (18-20h)	negative	Charles *et al.* 1999a
unscheduled DNA synthesis	human SV-40 transformed cells	2,4-D Fluid	1-1 000 uM (8h)	positive	Ahmed *et al.* 1977a

NR - information not reported

[a] 2,4-D Na - sodium salt; 2,4-D DMA - dimethylamine salt; 2,4-D DEA - diethanolamine salt; 2,4-D IPA - isopropylamine; 2,4-D TIPA - triisopropanolamine; 2,4-D BEE - butoxyethyl ester; 2,4-D EHE - ethylhexyl ester; 2,4-D IPE - isopropyl ester

[b] (+) with S9 activation; (-) without S9 activation

2.6.1.3 Neurotoxicity

As observed in acute studies, subchronic exposure to 2,4-D, specifically 2,4-D BE, caused alterations in the levels of neurotransmitters (including serotonin and 5-hydroxyindoleacetic acid) in the brains of adult rats exposed to 69 mg/kg/d for 15 or 45 d, and in pups exposed in utero during lactation (Evangelista de Duffard *et al.* 1990a). These changes were reversible except in pups born and fed by treated mothers (Evangelista de Duffard *et al.* 1990a). Evangelista de Duffard (1990b) demonstrated a relationship between testosterone and 2,4-D BE behavioural effects in male rats following dietary administration of 69 mg/kg/d 2,4-D BE for 15 d where intact males and gonadectomized males treated with testosterone exhibited poorer open field and rotarod performance.

Mattson *et al.* (1997) found no treatment-related changes in the central or peripheral nervous system of rats fed up to 150 mg/kg/d for one year, except for retinal degeneration. Treatment-related effects of the eyes (including lens opacity, retinal degeneration and cataracts) were also observed by Charles *et al.* (1996c) in rats fed 150 mg/kg/d 2,4-D acid for two years. Rats exposed to repeat dermal applications of 12 percent and 24 percent 2,4-D dimethylamine (DMA) for three and two weeks respectively, showed no evidence of treatment-related histopathological changes in the central or peripheral nervous systems (Mattson *et al.* 1986). Injection of rats with 80-100 mg/kg 2,4-D for three to 12 weeks resulted in no evidence of motor or sensory dysfunction (Toyoshima *et al.* 1985).

Post-natal studies in rats (Duffard *et al.* 1996, Bortolozzi *et al.* 1999, Rosso *et al.* 1997, Rosso *et al.* 2000) and reproductive studies in broiler chicks discussed in the next section (Duffard *et al.* 1982, Duffard *et al.* 1987, Mori de Moro *et al.* 1985) indicate that pup and foetal exposure to 2,4-D results in a variety of neurological and CNS effects. The developing nervous system of rat pups was found to be sensitive to 2,4-D exposure from postnatal days (PND)15-25 and nine to 25 through mother's milk (100 mg/kg treated dams) resulting in decreased brain total lipids (phospholipids, free fatty acid content), the appearance of cholesterol esters, and a deficit in brain myelin (Duffard *et al.* 1996). Rosso *et al.* (1997) also found that exposure of rat pups to 2,4-D (70-100 mg/kg) resulted in delayed CNS development between seven to 25 PND. Pre- and postnatal exposure of rats to approximately 70 mg/kg/d modified serotonergic behavioural patterns in developing and young rats with no accompanying overt signs of toxicity (Bortolozzi *et al.* 1999). Rat pups exposed to 70 or 100 mg/kg 2,4-D from PND 7-25 showed behavioural alterations, decreased myelin deposition, and changes in ganglioside levels indicative of delayed CNS development (Rosso *et al.* 2000).

In conclusion, neurotoxic effects, including neurobehavioral changes, damage to the blood/brain barrier, alterations in the membrane-related organic ion transport systems, and changes in the levels of neurotransmitters were observed in the above mammalian studies at doses above the threshold for renal tubular secretion (approximately 50 mg/kg). Above this threshold, general acute toxic effects would be expected.

2.6.1.4 Reproductive, Immune and Other Responses

A bioassay was performed where propagules of the aero-aquatic fungus *Pseudoaegerita matsushimae* were exposed to 2,4-D (dissolved in an emulsifiable oil concentrate carrier) for 16 hours to assess the impact on germination (Premdas and Kendrick 1991). Germination was stimulated at low concentrations (0.1 - 3.0 mg/L) and inhibited to varying degrees at higher levels (3.0 mg/L -100 g/L) (Premdas and Kendrick 1991). Experiments with 2,4-D in soybean embryos found that teratogenic effects on embryo morphology and development resulted from 22.5 uM 2,4-D (Shoemaker *et al.* 1991).

2,4-D does not appear to have reproductive effects in aquatic organisms. Fathead minnows exposed to nominal concentrations of 0.07-1.8 mg/L 2,4-D butoxyethanol ester (BEE) for ten months experienced no mortality, little spawning difficulty, and no hatching or fry survival difficulties (Mount and Stephan 1967). Chinook salmon exposed to 2,4-D BEE in continuous egg-to-fry studies experienced a reduction in growth, slower yolk-sac absorption, and increased mortality at higher concentrations resulting in a no-effect concentration of 40 ug/L (Finlayson and Verrue 1985). The teratogenic potential of 2,4-D as evaluated by the frog embryo teratogenic assay on *Xenopus laevis* (FETAX) was found to be low as both the EC50 and LC50 were greater than 270 mg/L in a natural water sample (Morgan *et al.* 1996).

Mammal studies indicate that 2,4-D causes reproductive, foetal and thyroid effects. There is some evidence of placental transfer of 2,4-D in rabbits (Sandberg *et al.* 1996) and pigs (Erne 1966). Maternal rabbit administration of 2,4-D (1 - 40 mg/kg) on d 28-30 of gestation resulted in rapid placental transfer (30 min with total tissue content <20 percent of dam) to foetal plasma and brain, with high levels found in maternal reproductive organs, blood, kidney and liver (Sandberg *et al.* 1996). A pregnant sow fed 2,4-D amine during the entire pregnancy produced piglets who contained considerable amounts of 2,4-D (Erne 1966). However, administration of 2,4-D to mice in late stage pregnancy showed only slight, temporary accumulation in the visceral yolk sac and elimination from all maternal and foetal tissues within 24 hours (Lindquist and Ullberg 1971). A three-generation rat feeding study found that although the highest dose of 1,500 ppm 2,4-D had no effect on fertility or litter size, there was a reduced survival rate of pups and reduced pup weight (Hansen *et al.* 1971). Pre- and postnatal studies in rats found that more than 100 mg/kg/d 2,4-D (along with its derivatives BE, BEE, DMA and IOE at the same level) administered to dams during d 6-15 of gestation resulted in fetopathy and an increased incidence of foetal skeletal abnormalities (Khera and McKinley 1972). A study on the endocrine effects of 2,4-D on neonatal American alligators found no significant effects on the observed parameters (plasma hormone concentrations, gonadal-adrenal mesonephros aromatase activity, and gonadal histopathology) measured (Crain *et al.* 1997). A 13-week study of 1-7.5 mg/kg/d 2,4-D in beagles saw decreased testicular weights (Charles *et al.* 1996b). A study on the metabolic and reproductive endocrine systems in ewes tested 10.0 mg/kg 2,4-D orally administered three times a week for 43 d (Rawlings *et al.* 1998). A marked decrease in serum thyroxine concentration (which may be partially explained by competition for binding) was observed (Rawlings *et al.* 1998). Thyroxine is the major secretory product of the thyroid gland and its principal function is to stimulate the consumption of oxygen and thus the metabolism of all cells and tissues in the body. However, 2,4-D had no significant effect on the mean serum concentrations of cortisol, insulin, luteinizing hormone, follicle stimulating hormone, progesterone, or estradiol and no significant increase in the severity of oviducal intraepithelial cysts (Rawlings *et al.* 1998). Charles *et al.* (1996a) also observed decreased thyroxine and triiodothyronine levels in female rats administered 1-300 mg/kg/d of various 2,4-D formulations for 13 weeks.

Injection of 2,4-D into fertile hen eggs at a rate of about 2.0 mg/60 g egg was found to be embryotoxic and teratogenic, while immersion in a five percent solution had only a slight effect on hatching and chick viability (Gyrd-Hansen and Dalgaard-Mikkelsen 1974). A later study indicated no adverse effects on embryonic development, hatching success or weight gain of white leghorn chicks from 2,4-D sprayed eggs (11.2 kg/ha) (Somers *et al.* 1978). 2,4-D did not have an effect on the eggshell thickness of eggs laid by 250 and 1,500 mg/kg 2,4-D treated Japanese quail and mallard duck females respectively (Haegele and Tucker 1974). Several studies were conducted on the effects of 2,4-D butyl ester (BE) on chicks hatched from topically treated hen eggs on d 0 of incubation (Duffard *et al.* 1982, Duffard *et al.* 1987, Castro de Cantarini *et al.* 1989, Duffard *et al.* 1990, Arguello *et al.* 1990, Castro de Cantarini *et al.* 1992, Evangelista de Duffard *et al.* 1993, Mori de Moro *et al.* 1985). Duffard *et al.* (1987) found no evidence of hydrolysis of the butyl ester to the acid form *in vivo*, but Castro de Cantarini *et al.* (1992) found that 73 percent of 2,4-D recovered *in vitro* from chick livers was in the acid form. At 0.8-6.3 mg/egg 2,4-D BE there was a dose-related decrease in cholesterol and glycolipids

indicating an alteration in the normal process of myelination in chicks (Duffard *et al.* 1982). Another study by Mori de Moro *et al.* (1985) also found that the application of 2.4 mg/egg 2,4-D BE produced hypomyelination in the brains of chicks. Application of 3.1 mg/egg of 2,4-D BE resulted in a half-time of penetration of three d and 50 percent mortality (Castro de Cantarini *et al.* 1989). This dose resulted in variations in DNA and protein levels in chick brains and skeletal muscles (Duffard *et al.* 1987), decreased lipid levels (phospholipids) in the liver but no effect on GSH or GST (Evangelista de Duffard *et al.* 1993), induced myotonia and altered the biochemical composition of skeletal muscles, most notably a high increase in free fatty acids (Duffard *et al.* 1990), as well as myopathy (Arguello *et al.* 1990).

2.6.1.5 Immunotoxicity

In general, 2,4-D does not appear to be immunotoxic. Fischer rats exposed to 10 mg/kg 2,4-D (amine salt) twice weekly for 28 d showed no signs of clinical toxicity, no mitogenic activity (did not alter lymphocyte blastogenesis) and no impairment of antibody response (Blakley *et al.* 1998). Studies investigating the effect of topically applied 2,4-D BE (3 wks, 0-300 mg/kg @ 3x/wk) and gastrically intubated 2,4-D BE (3 wks, 0-100 mg/kg @ 3x/wk) found no effect on antibody production in mice, but did find an enhancement of B- and T- lymphocyte proliferative responses (Blakley 1986, Blakley and Schiefer 1986). After a one-year chronic study of 2,4-D (0-7.5 mg/kg) in beagles, Charles *et al.* (1996b) concluded that 2,4-D did not induce an immunotoxic response. The female offspring of 2,4-D n-butylester treated rats (0-200 mg/kg on d 11 of gestation) appeared clinically unaffected and the lymphoid tissues (thymus and spleen) were also unaffected (Blakley and Blakley 1986). There was also no evidence of impairment of the humoral immune response but there was a generalized reduction in lymphocyte mitogen responses seen at the highest dose of 200 mg/kg (Blakley and Blakley 1986).

2.6.2 Mecoprop

Long-term feeding studies were conducted in rats. Rats fed diethanolamine MCPP for seven months at up to 2,500 ppm diet experienced unspecified mortality as a result of infection at the highest dose level, reduced weight gain at >1,000 ppm, and increased kidney weight at all doses tested (Gurd *et al.* 1965). A 90-d study of rats fed MCPP acid up to 3,200 mg/kg in their diet found reduced weight gain and food consumption, decreased haemoglobin content and erythrocyte counts, as well as increased kidney weights at the highest dose level (Verschuuren *et al.* 1975). The highest tolerable dose in cattle and sheep for the diethanolamine salt was 100 mg/kg and 175 mg/kg respectively, over 10 d (Hartley and Kidd 1986).

Pregnant rats fed 125 mg/kg/d of MCPP acid on d 6-15 of gestation experienced an increase of intra-uterine death, decreased offspring body length, and an increased incidence of delayed or absent bone formation in offspring (Irvine 1980). MCPP was not found to be teratogenic or fetotoxic in rabbits in doses up to 75 mg/kg/d (U.S. EPA 1988).

Studies show that MCPP may be mutagenic at only very high, toxic doses. MCPP was not found to damage DNA in the *Bacillus subtilis* rec-assay (Shirasu *et al.* 1976), did not induce mutation or somatic recombination in *Aspergillus nidulans* (Aulicino *et al.* 1976), nor did it induce streptomycin resistance in *Streptomyces coelicolor* (Carere *et al.* 1976) or mutants in *Salmonella typhimurium* (Carere *et al.* 1976, Moriya *et al.* 1983). There was no evidence of reverse or forward mutations in spot tests of *Salmonella* (2,000 ug MCPP) and *S. colelicolor* (1,000 ug MCPP) respectively, with or without activation (U.S. EPA 1988). MCPP caused an increase in sister chromatid exchange after single oral doses of 470 and 3,800 mg/kg in Chinese hamsters (U.S. EPA 1990a).

A 90-d study feeding Wistar rats up to 450 ppm (26 mg/kg/d) MCPP found increased absolute and relative kidney weights at 150 ppm, and at 450 ppm there was increased creatinine

levels in females and decreased glucose levels in male rats (Kirsch 1985). A rise in serum creatinine levels is indicative of kidney failure.

2.7 POTENTIAL FOR PHENOXY HERBICIDES TO ACT AS ENDOCRINE DISRUPTORS

Our working definition of an endocrine disrupting compound is:

> *An exogenous agent that directly interferes with the synthesis, secretion, transport, binding action, or elimination of endogenous hormones and neurohormones, resulting in physiological manifestations of the neuroendocrine, reproductive or immune systems in an intact organism.*

2,4-D is an analogue of the growth stimulating plant hormone indole acetic acid (IAA) and is thus designed specifically to disrupt the hormonal system of plants. However, there is some evidence from rat studies that at levels above 10 mg/kg, 2,4-D accumulates in the brain and alters the levels of neurotransmitters like serotonin, resulting in behavioural changes (Bortolozzi *et al.* 1998, Bortolozzi *et al.* 1999, Elo and MacDonald 1989, Evangelista de Duffard *et al.* 1990a, Oliveira and Palermo-Neto 1993). Reproductive effects including fetotoxicity and teratogenicity were observed in rats fed 100 mg/kg/d (Khera and McKinley 1972) and hen eggs injected with 2.0 mg/60g egg (Gyrd-Hansen and Dalgaard-Mikkelsen 1974). The FETAX assay using *Xenopus laevis* indicated that 2,4-D had a low teratogenic potential (Morgan *et al.* 1996). Studies in adult rats and ewes found that 2,4-D decreased the serum thyroxine concentration at levels of 1-300 mg/kg/d and 10 mg/kg respectively (Charles *et al.* 1996a, Rawlings *et al.* 1998).

According to the above definition, 2,4-D meets the criteria of an endocrine disruptor as noted by the observed effects on the central nervous system and neurotransmitters, as well as the thyroid.

MCPP is a hormone mimic, albeit selective for plants. There is no information to indicate that MCPP has the potential to disrupt hormone function in animals; however, there is a clear deficiency of essential studies examining the chronic toxicity to wildlife. Such studies are necessary to determine whether there is the potential for endocrine disruption in wildlife.

2.8 RISK TO ONTARIO ENVIRONMENTS AND RECOMMENDATIONS

In general, while aquatic organisms are much more acutely sensitive to phenoxy herbicides than mammals, effects were seen at dose levels well above those found in the environment, and above the Canadian Water Quality Guideline for the protection of aquatic life (4.0 ug/L). Phenoxy herbicides degrade quickly in the environment and are rapidly excreted from organisms. This being said however, the data on the toxicity of 2,4-D and mecoprop are difficult to classify due to the different characteristics of the solvents and emulsifying agents, as well as the rapid dissociation/hydrolyzation of amine and ester formulations to the free acid form.

The action of 2,4-D on the central nervous system and the thyroid indicates the potential for endocrine disruption at levels of 10 mg/kg or more and given this, the apparent low risk associated with 2,4-D use and exposure must be qualified. The information needed to determine if low level exposure to mecoprop will affect wildlife is not available since there is a lack of studies examining the acute and chronic toxicity and effects of mecoprop, particularly its salt formulations. The U.S. EPA (1988) has stated that chronic toxicity, oncogenicity, reproduction and general metabolism studies were not required for their assessment since MCPP and its salts and ester do not have food uses. They also consider the potassium salt formulation as equivalent to MCPP acid and therefore do not require a separate toxicity data set for it (U.S. EPA 1988). Mecoprop may not have a direct food use, but in Ontario it is applied to food as well as lawns for

weed control. As it is moderately persistent, animals such as squirrels and birds who traverse and feed on treated urban landscapes will be exposed to low levels throughout their active season.

The following issues should be priorities for further investigation of the degree of risk posed by 2,4-D and mecoprop:

- more precise use information to help characterize exposure to high risk organisms
- continued environmental monitoring of phenoxy acids, particularly mecoprop and its degradates, particularly in urban watersheds
- acute and chronic studies on the salt and ester formulations of mecoprop as well as its degradates, including studies using environmentally relevant concentrations
- the potential for endocrine disruption should be investigated in more detail and in other organisms, particularly aquatic organisms, using realistic exposure scenarios; MCPP in particular is deficient in information.

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3.0 CHLOROTHALONIL

3.1 DESCRIPTION AND USE

Chlorothalonil ([2,4,5,6- tetrachloro-1,3 benzenedicarbonitrile]; CAS No. 1897-45-6) is a non-systemic, broad-spectrum organochlorine fungicide. Chlorothalonil was first registered in 1966 by the Diamond Shamrock Corporation; however, the current manufacturer is ISK Biotech Corporation of Mentor, Ohio (Caux *et al.* 1996). In Ontario, chlorothalonil is registered for use on turfgrass, ornamental plants and trees, berries, and some fruit and vegetable crops to protect against diseases like leaf spot, blight and mould (Table 3.1). Chlorothalonil may be formulated as dust, suspension, or as wettable powders under various trade names, such as Daconil and Bravo. Chlorothalonil is among the most commonly used fungicides in Ontario agriculture. In 1998, a total of 649 805 kg of fungicides were used on surveyed crops, with 19 percent of that total (120 751 kg) being chlorothalonil (Hunter and McGee 1999). Its high usage in Ontario agriculture has remained relatively constant since 1993, when 115 613 kg of chlorothalonil were applied (Hunter and McGee 1994). Compared to agriculture, urban usage is much less with only 5,831 kg used by MOEE licensed pesticide applicators on residential lawns, industrial lawns, parks, and golf courses in 1993 (Hunter and McGee 1994).

3.2 ENVIRONMENTAL FATE AND CONCENTRATIONS

Chlorothalonil is typically applied by ground equipment as a broadcast, band, or foliar spray and is also registered for aerial application in agriculture (Caux *et al.* 1996). It may also be registered for domestic purposes (OMAFRA 2000d). It enters the natural environment directly through application and indirectly via spray drift, foliar wash-off, run-off, soil erosion and volatilization.

Chlorothalonil is non-polar, has moderate water solubility (900 ug/L at 25^{o}C), and is non-volatile (5.72 x 10-7 mm Hg at 25^{o}C) (Caux *et al.* 1996). It is moderately sorbed to soil and is resistant to hydrolysis and photolysis (U.S. EPA 1999). Chlorothalonil is moderately mobile and has been detected in the tile drain outflow and groundwater of sandy soils at levels of 0.06-272.6 ug/L within 30 days of application (Krawchuk and Webster 1987), but has not often been detected in the leachate from other soils (Leger *et al.* 1994, O'Neill *et al.* 1992).

Chlorothalonil is stable at neutral and acidic pH but hydrolyses at higher pH (Szalkowski and Stallard 1977). Persistence in the environment varies from a few hours to over 60 days, and is dependent on organic matter, application rate, adsorption to sediment, environmental conditions (moisture and temperature) and previous use history at the site of application (Caux *et al.* 1996, U.S. EPA 1999, Katayama *et al.* 1995). Repeat applications of chlorothalonil suppress soil degradation (Katayama *et al.* 1995).

Dissipation occurs primarily through aerobic and anaerobic microbial metabolism (Caux *et al.* 1996, U.S. EPA 1999). The rate of dissipation is increased under conditions of increased temperature, soil moisture and organic content, and dissolved oxygen (Davies 1988, Ernst *et al.* 1991, U.S. EPA 1999, Mori *et al.* 1996). Chlorothalonil has a half-life of two hours to eight days in aquatic systems, with greater than 65 percent associating with particulate matter (Caux *et al.* 1996, Davies 1988), and 10 to 40 days in soil (Szalkowski 1976). The addition of farmyard manure accelerates the dissipation of chlorothalonil in soil by maintaining a near neutral pH and by increasing the degrading capacity of fungi (Mori *et al.* 1996; 1998).

The major degradation product of chlorothalonil is 4-hydroxy-2,5,6-trichloroisophthalonitrile (DS-3701). This and other metabolites are mobile in sandy soils and have the potential to leach (U.S. EPA 1999), although DS-3701 has not been detected in Canadian groundwater samples (Leger *et al.* 1994).

The Canadian water quality guideline for chlorothalonil based on the protection of aquatic life is 0.18 ug/L (CCME 1999). O'Neill *et al.* (1992) measured chlorothalonil concentrations at levels below this guideline, ranging from 0.006-0.011 ug/L in surface waters and 0.005-0.034 ug/L in precipitation, in the Maritimes. Two other Canadian studies surveying urban streams from the Toronto area from 1998-2000 (Struger *et al.* 2002) and tributaries of Lake Erie during 1998-1999 (Struger *et al.* 1999) did not detect chlorothalonil in water samples with a detection limit of
0.1 ug/L.

Table 3.1 Recommended applications of chlorothalonil formulations for use in Ontario agriculture, turf and ornamentals (OMAFRA 2000a, OMAFRA 2000b, OMAFRA 2000c, OMAFRA 2000d).

Plant(s) Protected	Disease(s) Controlled	Formulation	Application Rate [a]
Agricultural Crops			
Blueberry	- Phomopsis stem canker, alternaria, anthracnose fruit rot	Bravo 500 (500g/L)	7.2
Strawberry	- Botrytis gray mould	Bravo 500	3.5
Sour cherry	- Brown rot, leaf spot, black knot	Bravo 500	7.0
Sweet cherry	- Brown rot	Bravo 500	7.0
Peach, nectarine	- Leaf curl, brown rot	Bravo 500	7.0
Broccoli, Brussels sprouts, cabbage, cauliflower	- Downy mildew, black leaf spot	Bravo 500	2.5-4.8
Carrots	- Leaf blight	Bravo 500	2.4-3.2
Celery	- Blight	Bravo 500	1.6-4.0
Cucumber, muskmelon, watermelon, pumpkin, squash	- Cucumber scab, anthracnose, powdery mildew	Bravo 500	4.8
Onion, leek	- Botrytis leaf blight, purple blotch	Bravo 500	2.4-4.8
Parsnip	- Phoma canker, leaf spot	Bravo 500	2.8
Potato	- Blight	Bravo 500	1.75-2.25
Corn	- Rust	Bravo 500	3.2
Tomato	- Anthracnose and blight - Septoria leaf spot	Bravo 500	2.8-3.2 3.8
Turfgrass			
Turf	- Anthracnose (*Colletotrichum graminicola*) - Brown patch, Rhizoctonia blight (*Rhizoctonia solani*), Dollar spot (*Sclerotinia homeocarpa*), Helminthosporium leaf spot/Melting Out (*Drechslera* spp.) - Fusarium patch (*Microdochium nivale*) - Grey snow mould, Typhula blight (*Typhula* spp.) - Pink snow mould (*Microdochium nivale*)	Daconil 2787F	170-190 95-190 370-450 250-500 460-700
Ornamentals			
Fir	- Needlecast	Daconil 2787F	2.5
Rose	- Black spot (*Diplocarpon rosae*), Botrytis	Daconil 2787F	2.5
Rhododendron	- Dieback (*Phytophthora*)	Daconil 2787F	2.5
Oak	- Anthracnose (*Gnomonia quercina*), leaf spot	Daconil 2787F	2.5
Firethorn	- Scab (*Venturia pyracanthae*)	Daconil 2787F	2.5
Black cherry, chokecherry, flowering cherry, pin cherry, peach, plum	- Blossom and twig blight (*Monilina fructicola*)	Daconil 2787F	2.5
Poplar	- Leaf spot (several fungi)	Daconil 2787F	2.5
Pine	- Canker (*Scleroderris abietina*), Lophodermium needlecast (*Lophodermium seditiosum*) - Tip blight (*Sirococcus*) - Brown spot (*Scirrhia*)	Daconil 2787F	2.4-4.8 3.6-6.0 9.5
Spruce	- Needlecast (*Rhizosphaera kalkhoffii*) - Tip blight (*Sirococcus conigenus*)	Daconil 2787F	9.5 3.6-6
Apple	- Cedar-apple rust (*Gymnosporangium juniperi-virginianae*), apple scab (*Venturia inequalis*)	Daconil 2787F	2.5
Privet, Dogwood	- Leaf spot	Daconil 2787F	2.5
Ash	- Leaf spot (*Mycosphaerella* sp.)	Daconil 2787F	2.5
Euonymus	- Anthracnose (*Glomerella cingulata*)	Daconil 2787F	2.5
Hawthorn	- Hawthorn rust (*Gymnosporagnium globosum*), quince rust (*G. clavipes*), leaf spot (*Fabraea* sp.)	Daconil 2787F	2.5
Horse chestnut	- Anthracnose (*Glomerella cingulata*), leaf blotch (*Guignardia aesculi*)	Daconil 2787F	2.5

[a] L/ha for agricultural applications; mL/100m^2 for turfgrass applications; L/1 000 L water for ornamental applications

3.3 BIOCONCENTRATION AND METABOLISM

3.3.1 Fish

Freshwater organisms have a limited capacity to bioconcentrate chlorothalonil. Two 96-hour flowthrough studies using steelhead-rainbow trout found that chlorothalonil uptake was rapid and significant with levels in the muscle indicating a concentration factor of 740 and 940 for each replicate (Davies and White 1985). The depuration phase was also rapid with blood levels of chlorothalonil dropping exponentially after removal to fresh water (Davies and White 1985). However, Tsuda *et al.* (1992) found that the bioconcentration of chlorothalonil by freshwater fish was low with whole body bioconcentration factors (BCFs) of 18 and 25 in the willow shiner and common carp respectively. There are no studies on the ability of soil-dwelling insects or plants to bioconcentrate chlorothalonil but based on the limited bioconcentration in aquatic organisms, the risk is likely minimal. Metabolism is rapid with excretion of chlorothalonil from the willow shiner at a rate of 0.04/h (Tsuda *et al.* 1992). Studies in channel catfish indicate that chlorothalonil metabolism and detoxification is accomplished in the liver and gills through a glutathione-S-transferase (GST)-catalyzed conjugation with glutathione (GSH) creating polar metabolites, which may be excreted in bile or urine (Gallagher and DiGiulio 1992a, Davies 1985).

3.3.2 Mammals

Studies in rats and monkeys show that mammalian absorption of chlorothalonil, whether oral, dermal or endotracheal, is low (Chin *et al.* 1981, Magee *et al.* 1990, Marciniszyn *et al.* 1984a; 1986). After doses of 1.5, 5, 50, and 200 mg/kg b.wt., male Sprague-Dawley rats absorbed 15.5-32 percent of the administered dose of chlorothalonil (Marciniszyn *et al.* 1986). Dermal absorption of chlorothalonil is also low. Chin *et al.* (1981) examined absorption in male Sprague-Dawley rats by oral, dermal and endotracheal routes and found less than 6 percent of the administered dose of one mg/kg b.wt. was recovered in blood and urine within 48 h, suggesting that tissue deposition or fecal excretion accounts for the remainder of the dose. Approximately 28 percent of dermally applied chlorothalonil at five mg/kg was absorbed by rats over a 120 h exposure period (Marciniszyn *et al.* 1984a). Dermal absorption in monkeys was also slow with 90 percent of an administered dose of five mg/kg b.wt. chlorothalonil recovered from the skin surface after 48 h (Magee *et al.* 1990). Mammalian metabolism of chlorothalonil is via GSH conjugation (Rosner *et al.* 1996, Bessi *et al.* 1999) and then enzymatic cleavage in the digestive tract to yield thiolated compounds, which may then be excreted in bile or urine (Hillenweck *et al.* 1997; 1998; 1999). Excretion of chlorothalonil mainly occurs via the feces, with 52-98 percent of the administered dose excreted via this route (Marciniszyn *et al.* 1984b; 1985, Rosner *et al.* 1996, Savides *et al.* 1989; 1990a; 1990b).

3.3.3 Plants

Chlorothalonil does not translocate in plants from the site of application and is metabolized to a limited extent on plants with the 4-hydroxy metabolite (DS-3701) less than 5 percent of the residue (WHO 1995).

3.4 TOXIC MECHANISM OF ACTION

The target organism of chlorothalonil is fungi. The fungitoxic mode of action is to bind and deplete cellular glutathione (GSH), effectively inhibiting glucose oxidation (Bessi *et al.* 1999, Gallagher *et al.* 1992c). Glutathione is a peptide that occurs widely in plant and animal tissues and plays an important role in biological oxidation-reduction processes and the activation of some enzymes (U.S. EPA 1999).

Tests on channel catfish found enhanced chlorothalonil toxicity resulted from GSH depletion (Gallagher *et al.* 1992b). This phenomenon has been observed in other species and although the exact mechanism of action is not known (U.S. EPA 1999), it has been suggested that once GSH stores were depleted, chlorothalonil was free to react with sulfhydryl groups of critical functional proteins (Gallagher *et al.* 1992b). Several studies have shown that chlorothalonil inhibits essential thiol-dependent cellular enzymes including glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and nicotinamide adenine dinucleotide phosphate hydrogenase (NADPH) oxidase, which are involved in glycolysis and superoxide production respectively (Baier-Anderson and Anderson 1998; 2000a; 2000b, Long and Siegel 1975).

3.5 ACUTE TOXICITY

Acute toxicity tests for aquatic organisms, insects and birds are summarized in Tables 3.2 and 3.3. The majority of tests were conducted on aquatic organisms, but some information was available for amphibians and mammals.

3.5.1 Mammals

Chlorothalonil is not acutely toxic to mammals at concentrations likely to be found in the environment (Table 3.2). A Holstein cow fed Daconil at five ppm in grain for four d showed no adverse effects and neither the parent compound nor metabolite was found in the milk, urine or manure after exposure ended (Gutenmann and Lisk 1966). Acute oral LD50s for chlorothalonil in rats, mice and dogs was >10 g/kg b.wt., 6 g/kg b.wt., and >5 g/kg b.wt., respectively (Paynter 1965a, Powers 1965, Yoshikawa and Kawai 1966). The metabolite DS-3701 appears to be more acutely toxic to rats with an oral LD50 of 332 mg/kg b.wt. (Wazeter 1971). The 24-h dermal LD50 for rabbits was greater than 10 g/kg but skin irritation resulted from exposure greater than one g/kg (Doyle and Elsea 1963). Inhalation studies showed the 1-h LC50 for technical chlorothalonil in rats was 310 ug/L while the 4-h LC50s for various commercial formulations were >7,160 ug/L (ISK Biotech 1985).

3.5.2 Insects

Honeybees (*Apis mellifera*) are relatively tolerant to chlorothalonil with a 48-h LD50 of 14.28 ug/bee (Atkins *et al.* 1973).

3.5.3 Birds

Chlorothalonil is also not acutely toxic to birds at concentrations likely to be found in the environment but they seem more sensitive than mammals (Table 3.2). Mallard ducks fed a single dose of 4,640 mg/kg b.wt. of technical chlorothalonil showed lethargy, lower limb weakness, and reduced body weight gain, but not mortality (Fink *et al.* 1977). A 5-d exposure to chlorothalonil resulted in a LOED (based on weight gain) and NOED for mallards of 1,500 and 843 mg/kg b.wt./d, respectively (Shults *et al.* 1979b). The metabolite DS-3701 appears more toxic to

mallards with an acute oral LD50 of 158 mg/kg/d (Beavers *et al.* 1978) and a 5-d LD50 and LOED of 300 and 84.3 mg/kg/d, respectively (Shults *et al.* 1981b). Five-day exposure of northern bobwhite to technical chlorothalonil resulted in a NOED of 1,000 (Shults *et al.* 1979a), and 5-d exposure to DS-3701 resulted in an LD50 and LOEL of 174.6 and 100 mg/kg/d, respectively (Shults *et al.* 1981a). The NOED for technical chlorothalonil to Japanese quail was 2,000 mg/kg b.wt. (Shults *et al.* 1987).

3.5.4 Aquatic Organisms and Amphibians

In contrast to mammals and birds, fish are more sensitive to chlorothalonil exposure, with dissolved oxygen influencing toxicity (Table 3.3). In 96-h flowthrough tests with rainbow trout, a decrease in dissolved oxygen from 8.0 to 5.1 ug/L resulted in an increase in chlorothalonil toxicity from an LC50 of 17.1 to 10.5 ug/L (Davies and White 1985). This trend was seen with the metabolite DS-3701 where a reduction in dissolved oxygen from 6.4-7.6 to 3.4-5.2 ug/L resulted in a decrease in the 28-d LC50 from 57 to 32 ug/L (Ernst *et al.* 1993). Davies and White (1985) attribute this to the drop in blood haematocrit experienced during chlorothalonil exposure, which may inhibit key respiratory enzymes and thus increase toxicity under conditions of low dissolved oxygen. Studies in rainbow trout indicate that the 96-h LC50 for technical chlorothalonil ranges from 10.5 ug/L (Davies and White 1985) to 76 ug/L (Ernst *et al.* 1991) depending on study conditions like dissolved oxygen. The metabolite DS-3701 was less toxic to rainbow trout fry than technical chlorothalonil in a 28-d study under similar conditions of dissolved oxygen, with a LC50 of 34 000 ug/L for DS-3701 compared to 54 ug/L for chlorothalonil (Ernst *et al.* 1993). Other fish listed in the table appear to be moderately sensitive to chlorothalonil exposure with 96-h LC50s between 16.3 for the common jollytail (Davies and White 1985) and 62 ug/L in the bluegill sunfish (SDS Biotech Corporation 1979). Increased duration of exposure to technical chlorothalonil resulted in increased toxicity to rainbow trout, common jollytail, and spotted and golden galaxias (Davies and White 1985).

There are few studies on the acute toxicity of chlorothalonil to amphibians but the 48-h LC50 for a tadpole was 160 ug/L (Hashimoto and Nishiuchi 1981).

Aquatic invertebrates seem less sensitive to chlorothalonil exposure than fish. *Daphnia magna* are moderately sensitive to technical chlorothalonil exposure with 48-h LC50s of 70 and 120 ug/L (LeBlanc 1977a, ISK Biotech 1982), and are less sensitive to DS-3701 with a 48-h LC50 of 26 000 ug/L (LeBlanc 1977b). Other species of water flea (*Daphnia pulex*, *Moina macrocopa*) were less sensitive to chlorothalonil with 3-h LC50s of $\geq$7,800 ug/L (Hashimoto and Nishiuchi 1981). Freshwater shrimp species (*Paratya australiensis*, *Astacopsis gouldi*) were most sensitive to chlorothalonil with 96-h LC50s ranging from 12 to 16 ug/L (Davies and Cook 1990). Longer exposure increased toxicity (Davies and Cook 1990). Isopod and amphipod species had LC50s greater than 40 ug/L (Davies and Cook 1990).

Table 3.2 Acute toxicity of chlorothalonil and its metabolite, DS-3701, to mammals, insects and birds.

Species	Common name	Age	Chemical formulation	Dose method	Duration	Dose (mg/kg/d) [a]	Effect	Reference
Mammals								
Bos taurus	Holstein cow	NR	NR	dietary	4 d	NOED = 5 ppm	- no residues detected in manure, milk or urine	Gutenmann and Lisk 1966
Rattus norvegicus	rat	NR	NR	oral gavage	-	LD50 >10 000		Powers 1965
Rattus norvegicus	rat	NR	technical commercial formulations	inhalation	1 h 4 h	LC50 = 310 ug/L LC50 >7 160 ug/L		ISK Biotech 1985
Rattus norvegicus	rat	NR	DS-3701	oral gavage	-	LD50 = 332		Wazeter 1971
Canis lupus familiaris	dog	NR	NR	oral gavage	-	LD50 >5 000		Paynter 1965a
Mus musculus	mouse	NR	NR	oral gavage	-	LD50 = 6 000		Yoshikawa and Kawai 1966
Oryctolagus cuniculus	rabbit	NR	NR	dermal	24 h	LD50 >10 000	- skin irritation observed at doses >1 000 mg/kg	Doyle and Elsea 1963
Insects								
Apis mellifera	honey bee	NR	technical	dusted	48 h	LD50 = 14.28 ug/bee		Atkins *et al.* 1973
Birds								
Anas platyrhynchos	mallard duck	14 d	technical	oral gavage	-	LOED = 4 640 mg/kg	- lethargy, lower limb weakness, reduced body weight gain	Fink *et al.* 1977
Anas platyrhynchos	mallard duck	14 d	technical	dietary	5 d	LOED = 1 500 NOED = 843	- weight gain	Shults *et al.* 1979b
Anas platyrhynchos	mallard duck	14 d	DS-3701	oral gavage		LD50 = 158		Beavers *et al.* 1978
Anas platyrhynchos	mallard duck	14 d	DS-3701	dietary	5 d	LD50 = 300 LOED = 84.3	- reversible lethargy, coordination loss and lower limb weakness	Shults *et al.* 1981b
Colinus virginianus	northern bobwhite	14 d	technical	dietary	5 d	NOED = 1 000		Shults *et al.* 1979a
Colinus virginianus	northern bobwhite	14 d	DS-3701	dietary	5 d	LD50 = 174.6 LOEL = 100	- lethargy, loss of righting reflex	Shults *et al.* 1981a
Coturnix coturnix japonica	Japanese quail	20 weeks	technical	oral gavage	-	NOED = 2 000		Shults *et al.* 1987

NR - information not reported

[a] LOED - lowest observed effect dose; NOED - no observed effect dose; LD50 - median lethal dose; mg/kg body weight/d unless indicated otherwise

Table 3.3 Acute toxicity of chlorothalonil and its metabolite, DS-3701, to freshwater invertebrates, fish and amphibians.

Species	Common name	Life Stage (age or weight)	Chemical formulation	Dose method [a]	Duration	Dissolved oxygen (mg/L)	LC50 (ug/L) [b]	Reference
Invertebrates								
Daphnia magna	water flea	<24 h	technical	S, N	48 h	7.8-8.0	120	ISK Biotech 1982
Daphnia magna	water flea	<24 h	technical	S, N	48 h	7.9-8.8	70	LeBlanc 1977a
Daphnia magna	water flea	<24 h	commercial	S, M	48 h	9.1-9.3	128-202	Ernst *et al.* 1991
Daphnia magna	water flea	<24 h	commercial	S, N	48 h	8.5-8.6	882*	ISK Biotech 1989
Daphnia magna	water flea	<24 h	DS-3701	S, N	48 h	7.0-7.4	26 000	LeBlanc 1977b
Daphnia pulex	water flea	NR	NR	S, N	3 h	NR	7 800	Hashimoto and Nishiuchi 1981
Moina macrocopa	water flea	NR	NR	S, N	3 h	NR	≥10 000	Hashimoto and Nishiuchi 1981
Cloeon dipterum	mayfly	NR	NR	S, N	48 h	NR	1 800	Hashimoto and Nishiuchi 1981
Paratya australiensis	freshwater shrimp	0.05-0.15 g	technical	F, M	96 h 7 d	NR	16 10.9	Davies and Cook 1990
Astacopsis gouldi	freshwater shrimp	0.13 g	technical	F, M	96 h 7 d	NR	12 3.6	Davies and Cook 1990
Colubotelson chiltoni minor	isopod	NR	technical	F, M	7 d	NR	>40	Davies and Cook 1990
Neoniphargus sp.	amphipod	NR	technical	F, M	7 d	NR	>40	Davies and Cook 1990
Fish								
Oncorhynchus mykiss	rainbow trout	3.5-4.0 g	technical commercial	S, M	96 h	8.4-11.2	76 69	Ernst *et al.* 1991 Ernst *et al.* 1991
Oncorhynchus mykiss	rainbow trout	9.5 g	technical	F, N	96 h	5.1 8.0	10.5 17.1	Davies 1987
Oncorhynchus mykiss	rainbow trout	6-11 g	technical	F, M	24 h 96 h 96 h	8.03 8.03 5.12	40.2 17.1 10.5	Davies and White 1985
Oncorhynchus mykiss	rainbow trout	fry	technical technical DS-3701 DS-3701 DS-3701	S, N SR, N SR, N SR, N SR, N	96 h 28 d 28 d 28 d 28 d	9.2-10.4 5.6-10.6 5.9-10.6 6.4-7.6 3.4-5.2	57 54 3400 57 32	Ernst *et al.* 1993
Lepomis macrochirus	bluegill sunfish	NR	technical	S, NR	48 h 96 h	NR	46-77 62	SDS Biotech Corporation 1979
Gasterosteus aculeatus	threespine sticklebac k	0.3 g	commercial	S, M	96 h	9.2-9.5	<73	Ernst *et al.* 1991
Galaxias maculatus	common jollytail	7-10 g	technical	F, M	48 h 96 h	8-9 8-9	18.2 16.3	Davies and White 1985
Galaxias truttaceus	spotted galaxias	8-20 g	technical	F, M	48 h 96 h	8-9 8-9	25.8 18.9	Davies and White 1985
Galaxias auratus	golden galaxias	7-11 g	technical	F, M	48 h 96 h	8-9 8-9	46.6 29.2	Davies and White 1985
Oryzias latipes	Japanese medaka	NR	NR	S, N	48 h	NR	88	Hashimoto and Nishiuchi 1981
Misgurnus anguilicaudatus	pond loach	NR	NR	S, N	48 h	NR	150	Hashimoto and Nishiuchi 1981
Ictalurus punctatus	channel catfish	NR	technical	S, N	96 h	5-6	52	Gallagher *et al.* 1992c

Species	Common name	Life Stage (age or weight)	Chemical formulation	Dose method [a]	Duration	Dissolved oxygen (mg/L)	LC50 (ug/L) [b]	Reference
Amphibians								
Bufo bufo japonicus	Japanese common toad	Tadpole	NR	S, N	48 h	NR	160	Hashimoto and Nishiuchi 1981

NR - information not reported

[a] S - static; SR - static renewal; F - flowthrough; M - measured concentration; N - nominal concentration

[b] LC50 - median lethal concentration; *EC50 - concentration causing an effect in 50 percent of population (immobilization)

3.6 CHRONIC TOXICITY AND ECOLOGICAL EFFECTS

3.6.1 Mammals

Most studies in mammals have examined chronic effects since chlorothalonil is not very acutely toxic. Rats fed up to 1,500 mg/kg chlorothalonil in the diet for 22 weeks, exhibited reduced growth, as well as increased kidney and liver weights at the highest doses (Blackmore and Schott 1968). There were accompanying kidney alterations, including swelling of the tubular epithelium, epithelial degeneration and tubular dilation, at all dose levels (Blackmore and Schott 1968). Similar effects on the kidney and stomach were seen in rats fed chlorothalonil 0-1,500 mg/kg b.wt./d for 90 d (Wilson 1981, Wilson *et al.* 1985b), rats fed up to 40 mg/kg b.wt./d for 13 weeks (Wilson *et al.* 1983a; 1985a), rats fed 175 mg/kg b.wt./d for 91 days (Ford *et al.* 1987), and rats fed 1,500, 5,000 or 15 000 mg/kg diet for two years (Paynter 1967, Paynter and Crews 1967). However, a 17-week study found no treatment-related effects in the kidneys of rats fed up to 120 mg/kg (Busey 1975). Another two-year study in rats at dose levels between four and 60 mg/kg diet found no effects on survival, growth haematological or biochemical parameters and thus a NOEL of three mg/kg b.wt./d was suggested (Holsing and Shott 1970). A 30-d feeding study in beagles found no compound-related histopathologic changes in brain, liver, kidney, heart or reproductive tissues at levels of up to 500 mg/kg b.wt./d (Fullmore and Laveglia 1992). Dermal studies in rabbits found only treatment-related irritation when applied at up to 1,000 mg/kg/d, five d/week for three weeks (Paynter 1965b), and when applied at up to 50 mg/kg/d for 21 d (Shults *et al.* 1986).

A dietary study in rats evaluated the carcinogenicity of chlorothalonil at doses of 1.8, 3.8 and 15 mg/kg b.wt./d (Wilson *et al.* 1989a). The higher doses resulted in renal tumours and papillomas and carcinomas of the forestomach, giving a NOEL of 3.8 and 1.8 mg/kg/d respectively (Wilson *et al.* 1989a). A two-year study in Charles River CD-1 mice at 750, 1,500 or 3,000 mg/kg diet showed a treatment-related increase in the incidence of gastric and renal tumours (Wilson *et al.* 1983b, Wilson *et al.* 1985c). The induction of forestomach tumours is believed to result from sustained cell proliferation that occurs, in turn, through a direct histopathological response to the local irritant effects of chlorothalonil itself on the squamous cell epithelium (Wilkinson and Killeen 1996). Cell proliferation increases the probability of converting spontaneous DNA damage to heritable change (Wilkinson and Killeen 1996). Based on studies in rats, the nephrotoxic agent results from the metabolization of the glutathione conjugate to the cysteine metabolite and then to the thiol metabolite by the action of B-lyase in the kidney (Hillenweck *et al.* 1999, Wilkinson and Killeen 1996, Wilson *et al.* 1990). Thus, chlorothalonil is not an initiator (does not directly interact with DNA) but a promoter of tumours (Bessi *et al.* 1999, Wilkinson and Killeen 1996).

3.6.2 Fish and Aquatic Invertebrates

Exposure of channel catfish to a sublethal concentration of chlorothalonil (42 ug/L) for 144 h, resulted in acute necrosis of the intestinal epithelium in 57 percent of exposed fish (Gallagher *et al.* 1992c).

3.6.3 Mutagenicity

Table 3.4 lists a number of studies assessing the mutagenic potential of chlorothalonil. Chlorothalonil did not cause mutations or chromosomal aberrations in plants (Kahlon and Banerjee 1979, Tomkins and Grant 1972) and a positive result in *Aspergillus nidulans* (Martinez-

Rossi and Azevedo 1987) was not reproduced in any other prokaryotic or eukaryotic point mutation test (de Bertoldi *et al.* 1980, Moriya *et al.* 1983, Quinto *et al.* 1981, Shirasu *et al.* 1977, Wei 1982). Positive results were also observed in Chinese hamster ovary cells where chlorothalonil caused sister chromatid exchanges and chromosome aberrations at levels between 0.2-3 ug/mL (Galloway *et al.* 1987). Legator (1974) conducted a number of *in vivo* tests in mice and did not find chlorothalonil, at 6.5 mg/kg/d for 5 d, to be mutagenic, but McGregor *et al.* (1988) found it to cause forward mutations in mouse lymphoma cells. Lebailly *et al.* (1997) found that DNA damage in human peripheral blood lymphoctyes following chlorothalonil exposure only occurred when preceding cell death and concluded that chlorothalonil could not be considered to be truly genotoxic. Similarly, Godard *et al.* (1999) determined that although Chinese hamster ovary cells exhibited a clear dose-dependent increase in DNA strand breakage *in vitro*, when rats were administered single oral doses of chlorothalonil (200 or 2,000 mg/kg b.wt.) no DNA strand breakage could be detected in whole blood, bone marrow, thymus, liver or kidney. However, Lodovici *et al.* (1997) reported that rats dosed for 10 d at doses of 0.13-1 mg/kg/d exhibited a dose-dependent increase in oxidative liver DNA damage. Considering the tests listed below (Table 3.4) and a report from Caux *et al.* (1996), Health Canada concluded that chlorothalonil is not a mammalian genotoxic agent based on 18 mutagenicity assays, it is unlikely that chlorothalonil will show mutagenic activity in most systems.

Table 3.4 Genotoxicity/mutagenicity studies of chlorothalonil and its formulations.

Test	Organism	Formulation	Dose Range	Mutagenic Potential [a]	Reference
Plants					
somatic mutation (chimeras)	*Tradescantia* flowers	commercial (75% WP)	1 500 ppm	negative	Tomkins and Grant 1972
chromosomal aberrations	barley (*Hordeum vulgare)*	commercial (75% WP)	0-1 500 ppm	negative	Tomkins and Grant 1972
chromosomal aberrations	barley (*Hordeum vulgare)*	commercial (75% a.i.)	0-1 000 ppm	negative	Kahlon and Banerjee 1979
Prokaryotes/Eukaryotes					
gene reversion (Ames test)	*Salmonella typhimurium* (5 strains)	NR	NR	negative (+ and -)	Quinto *et al.* 1981
gene reversion (Ames test)	*Salmonella typhimurium* (5 strains)	technical	0.76, 7.6, 76, 764 ug/plate	negative (+ and -)	Wei 1982
gene reversion (Ames test)	*Salmonella typhimurium* (5 strains)	NR	1-10 ug/plate	negative (+ and -)	Shirasu *et al.* 1977
gene reversion	*Escherichia coli* WP2	NR	10-500 ug/plate	negative (+ and -)	Shirasu *et al.* 1977
gene reversion (Ames test)	*Salmonella typhimurium* (5 strains)	NR	0-5 000 ug/plate	negative (+ and -)	Moriya *et al.* 1983
gene conversion	*Escherichia coli* WP2	NR	0-5 000 ug/plate	negative (+ and -)	Moriya *et al.* 1983
gene conversion	*Aspergillus nidulans* (strains biA1 methG1 and 118)	commercial	0.4-0.8 ug/mL	positive (-)	Martinez-Rossi and Azevedo 1987
mitotic gene conversion	*Saccharomyces cerevisiae*	commercial	0-2 500 ppm	negative (+ and -)	de Bertoldi *et al.* 1980
mitotic gene conversion	*Aspergillus nidulans*	commercial	0-200 ppm	negative	de Bertoldi *et al.* 1980
DNA repair (rec-assay)	*Bacillus subtilis*	NR	2-200 ug	negative (-)	Shirasu *et al.* 1977
Mammalian Cells					
in vivo cytogenetic abnormalities	mouse	NR	6.5 mg/kg/d (5 d)	negative	Legator 1974
in vivo host-mediated assay (8 strains *S. typhimurium*)	mouse	NR	6.5 mg/kg/d (5 d)	negative	Legator 1974
in vivo dominant lethal assay (5 d dosing and 8 weeks mating)	mouse	NR	6.5 mg/kg/d (5 d)	negative	Legator 1974
forward mutation	mouse lymphoma cells	technical	>0.12 ug/mL	positive (-)	McGregor *et al.* 1988
sister chromatid exchange	Chinese hamster ovary cells	technical	0.2-2.5 ug/mL	positive (+)	Galloway *et al.* 1987
chromosome aberration	Chinese hamster ovary cells	technical	0.5-3 ug/mL	positive (+ and -)	Galloway *et al.* 1987

NR - information not provided

[a] (+) with S9 activation; (-) without S9 activation

3.6.4 Reproductive, Immune and Other Responses

3.6.4.1 Mammals

Teratological evaluations have been conducted in the rat and rabbit. Mizens *et al.* (1983) concluded that chlorothalonil was not teratogenic in Sprague-Dawley rats following the administration of 0, 25, 100 or 400 mg/kg b.wt./d to pregnant rats on d 6-15 of gestation and the resulting lack of compound-related incidences of foetal external, internal or skeletal malformations. A study involving rabbits dosed with 0, 5 or 50 mg/kg/d chlorothalonil on d 6-18 of pregnancy resulted in 44 percent abortion rate, decreased maternal and foetal body weights and a reduction in the number of implants and live fetuses per pregnancy at the highest dose; however, there were no treatment-related external, internal or skeletal malformations. It was therefore concluded that chlorothalonil was not teratogenic to rabbits (Shirasu and Teramoto 1975). A one-generation study in rats at treatment levels of 0-3,000 mg/kg diet 10 weeks prior to mating found no effect on reproductive parameters such as mating, fertility and gestation length and no treatment-related abnormalities in the offspring (Wilson *et al.* 1989b). A two-generation study in Charles River CD rats involved the treatment of parental animals from each generation with 0, 500, 1,500 and 3,000 mg/kg diet (Lucas and Benz 1990). No mortalities or signs of clinical toxicity, and no effect on mating, fertility or gestation was observed in the F0 or F1 parent animals; no treatment-related gross malformations, effect on the number of live pups or pup survival was observed in the offspring from treated parents (Lucas and Benz 1990).

3.6.4.2 Birds

An 18-week reproductive study of technical chlorothalonil on adult mallard ducks found that 10 000 mg/kg diet reduced egg production and hatching success but a dose of 5,000 mg/kg diet had no observable effects (Shults *et al.* 1988a). A 21-week reproductive study on northern bobwhite found that the high doses of 5,000 and 10 000 mg/kg diet resulted in decreased body weight gain, general health and reproductive impairments such as reduced hatching survival (10 000 mg/kg diet) and reduced survival of offspring (5,000 mg/kg diet) (Shults *et al.* 1988b). Based observation that the 1,000 mg/kg diet dose had no apparent effects on parent or offspring, a NOEC of 1,000 mg/kg diet was established (Shults *et al.* 1988b). A 19-week study in adult mallard ducks with DS-3701 resulted in a LOED for reduced eggshell thickness of 15.4 and a NOEL of 7.54 mg/kg/d (ISK Biotech 1988).

3.6.4.3 Aquatic Organisms

A two-generation study of technical chlorothalonil (6.2-100 ug/g) in *Daphnia magna* for 21 d during each generation found a NOEC of 35 ug/L based on survival and reproduction (Shults *et al.* 1982). *Daphnia magna* neonates exposed to BRAVO 500 in static tests experienced delayed reproduction at 32 ug/L but no effect on either growth or number of young produced at concentrations of up to 180 ug/L (Ernst *et al.* 1991). Exposure of fathead minnows to technical chlorothalonil over one full life cycle (egg to egg) resulted in no significant effects in either the F0 or F1 generation at <3.0 ug/L, but there was significantly reduced hatchability and fry survival of the F0 eggs at 16 ug/L, and reduced reproductive success of F0 fish by exposure to >6.5 ug/L (Shults *et al.* 1980). The second-generation F1 eggs experienced reduced hatchability at 6.5 ug/L but fry survival at this concentration was not affected (Shults *et al.* 1980).

3.7 ENDOCRINE DISRUPTION POTENTIAL

Our working definition of an endocrine disrupting compound is:

> *An exogenous agent that directly interferes with the synthesis, secretion, transport, binding action, or elimination of endogenous hormones and neurohormones, resulting in physiological manifestations of the neuroendocrine, reproductive or immune systems in an intact organism.*

Studies in mammals (Lucas and Benz 1990, Mizens *et al.* 1983, Shirasu and Teramoto 1975, Wilson *et al.* 1989b) conclude that chlorothalonil does not affect reproduction, and is not teratogenic. However, reductions in eggshell thickness and fecundity in birds were observed from exposure to technical chlorothalonil at levels of 5,000 mg/kg diet or more (Shults *et al.* 1988a; 1988b) and the metabolite DS-3701 at levels above 15.4 mg/kg/d (ISK Biotech 1988). *Daphnia magna* experienced delayed reproduction upon exposure to 32 ug/L chlorothalonil (Ernst *et al.* 1991), and fathead minnows had a decreased number of eggs per spawn, egg hatchability, and fry survival at doses of 6.5 ug/L chlorothalonil and greater (Shults *et al.* 1980).

Chlorothalonil does not appear to have a direct effect on the endocrine system. However, it does have the ability to react with sulfhydryl groups of proteins and enzymes like GAPDH and NADPH oxidase (Baier-Anderson and Anderson 2000a) and so may interfere with other enzymes or hormones that have free sulfhydryl groups.

Chlorothalonil is a proven non-genotoxic carcinogen in mammals and fish, and a suspected immunomodulator. The inhibition of NADPH oxidase results in the suppression of reactive oxygen species (O_2^-, H_2O_2, and HOCl) which are necessary to promote the efficient destruction of microbes (Baier-Anderson and Anderson 1998; 2000a; 2000b). This may result in severe, recurrent infections. According to the above definition, chlorothalonil may qualify as an endocrine disruptor since it has the potential to interfere with endogenous hormones/ neurohormones and enzymes, and is an immunomodulator.

3.8 RISK TO ONTARIO ENVIRONMENTS AND RECOMMENDATIONS

The low acute toxicity of chlorothalonil to birds and laboratory mammals, coupled with its low rate of detection (all detections being below the Canadian water quality guideline of 0.18 ug/L) and short persistence in the environment suggests minimal risk to most wild avian and mammalian species. However, recent studies on the potential of chlorothalonil to interfere with endogenous hormones/neurohormones and its potential for immunomodulation indicate that this may be an area of concern in addressing risk to wildlife.

The following issues should be priorities for further evaluation of the extent and degree of risk posed by chlorothalonil and its metabolites:

- more precise use of information for monitoring in urban areas as well as agricultural watersheds in high-risk counties
- an improvement in extraction efficiency and possible preservation techniques should be investigated to obtain reliable results since two studies (Harman-Fetcho *et al.* 2000 and O'Neill *et al.* 1992) noted difficulties in recovering chlorothalonil from natural water samples
- studies on how other pesticides occurring in the environment affect the toxicity of chlorothalonil, since chemicals which utilize or deplete tissue glutathione may predispose organisms to acute toxic effects
- further study on the potential for endocrine disruption in wildlife to chronic chlorothalonil/DS-3701 exposure, and also on the potential for immunomodulation.

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4.0 CHLORPYRIFOS

4.1 DESCRIPTION AND USE

Chlorpyrifos ([O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate]; CAS No. 2921-88-2) is a broad-spectrum organophosphate (OP) insecticide used to control a wide range of insect and arthropod pests in agriculture and turf (Giesy *et al.* 1999). In Ontario, these pests include grubs, ants and aphids in turfgrass and ornamental plants, and maggots and cutworms in agricultural crops like corn and fruit (Table 2.1). Chlorpyrifos is also applied to the soil surrounding or beneath buildings as protection against termites (Barron and Woodburn 1995). Produced by the Dow Chemical Company in Midland, Michigan since 1962, chlorpyrifos is still a highly used OP insecticides in North America.

Past uses of chlorpyrifos (no longer registered) included direct application to water bodies as a larvicide and pest control on cattle and sheep, which involved spray-dip or pour-on applications (Racke 1993). In June 2000, the U.S. EPA banned most home uses of the pesticide Dursban (chlorpyrifos) due to human health concerns. Canada also discontinued registration of domestic class residential uses, both indoors and outdoors, as of December 2001. Other commercial class uses, such as use in playgrounds and schools, have also been discontinued.

Chlorpyrifos is formulated as a number of different commercial products, including granular forms (G), encapsulated forms, emulsifiable concentrates (EC) and wettable powders (WP). Formulations available for use in Canada include the trade names Dursban, Lorsban and Pyrate insecticide (Table 4.1). These include Lorsban 15G (15 percent G); Lorsban 4E (479 g/L EC); Dursban 2E (288 g/L EC), and Pyrate 480EC (480 g/L EC).

A 1998 survey of agricultural pesticide use in Ontario found that out of a total 153 049 kg insecticide used, an estimated five percent (7,311 kg) of that was chlorpyrifos (Hunter and McGee 1999). Information on urban use that year was not available; however, the previous survey done in 1993 found that 27 times more chlorpyrifos (125 766 kg) was applied by Ministry of Environment and Energy (MOEE) licensed applicators to residential and industrial lawns, parks, golf courses, cemeteries, roadsides, and schools that year than to agricultural crops (Hunter and McGee 1994). This indication of urban usage does not include homeowner usage of over-the-counter formulations for lawn and garden care.

Table 4.1 Recommended applications of chlorpyrifos formulations for use in Ontario turf, agriculture and ornamentals (OMAFRA 1999, OMAFRA 2000a, OMAFRA 2000b, OMAFRA 2000c, OMAFRA 2000d).

Plant(s) Protected	Insect(s) Controlled	Formulation	Application Rate [a]
Turfgrass			
Turf	- Ant, cutworm, hairy chinch bug, sod webworm - Annual bluegrass weevil, hyperodes weevil, European chafer, Japanese beetle, white grub	Dursban 2E Dursban Turf Chlorpyrifos 1G Dursban 2E Dursban Turf Chlorpyrifos 1G	45 22-23 1.0 90 45 2.0
Agriculture			
Onion	- Onion maggot	Lorsban 15G	8-16 kg/ha
Garlic	- Onion maggot	Lorsban 4E	3.5 L in 1 000 L water
Broccoli, Brussels sprout, cabbage, cauliflower, rutabaga	- Root maggot	Lorsban 15G Lorsban 4E	0.6-1.0 kg/1 000 m row 210 mL/130 L water per 1 000 m row at 1 000 L water/ha
Broccoli, Brussels sprout, bulb onion, cabbage, carrot, cauliflower, celery, cucumber, garlic, pak choy, radish, pepper, potato, rutabaga, sugar beet, sweet corn, tomato	- Cutworm	Pyrinex 480 EC Lorsban 4E	1.2-2.4 L/ha
Peach and nectarine	- 1st generation oriental fruit moth	Lorsban 50W	3.40 kg/ha
Corn	- Corn rootworm	Lorsban 15G	75 g/100 m
Potato	- Flea beetle, tarnished plant bug	Lorsban 4E	1.0 L/ha
Ornamentals			
Maple	- Aphid - Cottony maple scale (*Pulvinaria innumerabilis*), Lecanium or European fruit lecanium (*Lecanium corni*) - Forest tent caterpillar (*Malacosoma disstria*)	Dursban 2E Pyrate 480EC Dursban 2E Pyrate 480EC Dursban 2E Pyrate 480EC	0.750 0.375 4.0 2.0 1.0 0.500
Birch	- Bronze birch borer (*Agrilus anxium*)	Dursban 2E Pyrate 480EC	1.0 0.500
Hickory	- Hickory gall aphid (*Phylloxera caryaecaulis*)	Dursban 2E Pyrate 480EC	0.750 0.375
Beech	- Aphids (*Fagiphagus imbricator*, *Phyllaphis fagi*)	Dursban 2E Pyrate 480EC	0.750 0.375
Ash	- Ash borer (*Podosesia syringae*) - Fall webworm (*Hyphantria cunea*) - Oystershell scale (*Lepidosaphes ulmi*)	Dursban 2E Pyrate 480EC Dursban 2E Dursban 2E Pyrate 480EC	1.0 0.500 1.0 4.0 2.0
Honey Locust	- Leafhopper (*Macropsis fumipennis*)	Dursban 2E Pyrate 480EC	2.0 1.0
Privet	- Privet thrips (*Dendrothrips ornatus*)	Dursban 2E Pyrate 480EC	1.0 0.500
Magnolia	- Magnolia scale (*Neolecanium cornuparvum*)	Dursban 2E	4.0
Spruce	- Cooley spruce gall adelgid (*Adelges cooleyi*), spruce spider mite (*Oligonychus ununguis*)	Dursban 2E Pyrate 480EC	0.750-1.0 0.375-0.500

Plant(s) Protected	Insect(s) Controlled	Formulation	Application Rate [a]
Pine	- Pine bark adelgid (*Pineus strobi*) - Pine sawflies (*Neodiprion sertifer, N. lecontei*) - Pine spittle bug (*Aphrophora cribrata*) - White pine aphid (*Cinara strobi*)	Dursban 2E Dursban 2E Pyrate 480EC Dursban 2E Pyrate 480EC Dursban 2E Pyrate 480EC	0.750 1.0 0.50 0.175-0.300 0.088-0.150 0.750 0.375
Poplar	- Poplar and willow borer	Dursban 2E Pyrate 480EC	1.0 0.500
Black cherry, chokecherry, flowering cherry, pin cherry, peach, plum	- Aphid - Eastern tent caterpillar	Dursban 2E Pyrate 480EC Dursban 2E Pyrate 480EC	0.750 0.375 1.0 0.375
Oak	- Golden oak scale (*Asterolecanium variolosum*)	Dursban 2E Pyrate 480EC	4.0 2.0
Rose	- Japanese beetle larvae (*Popillia japonica*)	Dursban Turf	4.5
Willow	- Aphid	Dursban 2E Pyrate 480EC	0.750 0.375
Mountain Ash	- Mountain ash sawfly (*Pristiphora geniculata*)	Dursban 2E	1.0
Lilac	- Lilac borer (*Podesia syringae* var. *syringae*)	Dursban 2E Pyrate 480EC	1.0 0.500
Elm	- Elm bark beetle (*Scolytus multistriatus, Hylurgopinus rufipes*)	Dursban 2E Pyrate 480EC	10.0 10.0

[a] Turfgrass application rate - mL/100m^2 for EC, and kg/100m^2 for G formulations; ornamental application rate - L/1,000 L water

Table 4.2 Physical and chemical properties of chlorpyrifos and its major metabolites (Giesy *et al*. 1999, Racke 1993, Richards and Baker 1993).

	Chlorpyrifos	TCP (metabolite)	TMP (metabolite)
Chemical name	O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate	3,5,6-trichloro-2-pyridinol	3,5,6-trichloro-2-methoxypyridine
CAS number	2921-88-2	6515-38-4	31557-34-3
Chemical formula	$C_9H_{11}Cl_3NO_3PS$	$C_5H_2Cl_3NO$	$C_6H_4Cl_3NO$
Molecular weight	350.6	198.4	212.4
Melting point	41 - 44 ^oC	174 - 175 ^oC	
Vapour pressure (25^oC)	2.0 x 10 $^{-5}$ mmHg	2.48 x 10^{-5} mmHg (neutral form)	9.68 x 10^{-3} mmHg
Henry's law constant	6.64 x 10^{-3} atm-L mol^{-1}		
Water solubility (25^oC)	1.39 mg/L (distilled)	49 000 mg/L (anionic form pH7)	20.9 mg/L
Log K_{ow}	4.7 - 5.3	1.35, 3.21 (ionized pH7, unionized pH3)	4.3
K_{oc}	8 500		

4.2 ENVIRONMENTAL FATE AND CONCENTRATIONS

Chlorpyrifos is typically applied as a spray or granules to soil and water bodies to control both larval and adult pests (i.e., mosquitoes). It enters the environment directly through application and indirectly via spray drift, foliar wash-off, run-off, soil erosion and volatilization.

Chlorpyrifos is non-polar, not very water soluble and possesses moderate volatility (Table 4.2). It is readily sorbed to soil, particularly peat moss and soils high in organic matter, with limited potential to migrate, either through the soil profile (leaching) or soil surface (run-off), as well as limited bioavailability (Racke 1993). Field scale studies of chlorpyrifos runoff revealed a loss of <0.30 percent applied ingredient under conditions of a simulated worst-in-100-year event of 13.6 cm rainfall in one h (Sauer and Daniel 1987). Chlorpyrifos is stable in neutral and acidic aqueous environments with stability decreasing with increasing pH and temperature (U.S. EPA 1999). Persistence in the environment varies over two orders of magnitude, from a few days to well over 100 d, depending on organic matter, application method and rate, formulation, environmental conditions like moisture and temperature, and previous use history at the site of application (U.S. EPA 1999, Harris *et al.* 1968; 1969; 1971; 1973a; 1973b).

Dissipation occurs through volatilization, biodegradation, hydrolysis, photodegradation, and adsorption to sediment (Giesy *et al.* 1999, Getzin 1985). The rate of dissipation is increased under conditions of increased temperature, decreased soil moisture and organic content, and a surface instead of soil incorporated application (Harris *et al.* 1968; 1971; 1973b). Chlorpyrifos has a dissipation half-life of <1-7 d in the water column (Hurlbert *et al.* 1970, Smith *et al.* 1966), >14 d in sediment (Hurlbert *et al.* 1970), and 7-<30 days depending on formulation and application to either the soil surface or incorporation into the soil profile (Harris *et al.* 1968; 1969; 1971; 1973a; 1973b, Tashiro and Kuhr 1978).

In all systems (soil, water, plants) the major pathway of chlorpyrifos breakdown begins with cleavage of the phosphorus ester bond to yield 3,5,6-trichloro-2-pyridinol (TCP). The other major metabolite of chlorpyrifos is 3,5,6-trichloro-2-methoxypyridine (TMP). TCP is more polar than chlorpyrifos (Table 4.2) and so has the potential to move through the environment; while TMP is less stable and is more likely to volatilize off surface soil and waters (Racke 1993).

The Canadian Water Quality Guideline for the Protection of Aquatic Life for chlorpyrifos in freshwater is 0.0035 ug/L and was established in 1997 (CCME 1999). Struger *et al.* (1994) detected chlorpyrifos in two out of eight water samples taken from two urban detention ponds in Guelph, Ontario. The average concentration was 0.148 ug/L. The Niagara fruit belt of Ontario had detectable levels of chlorpyrifos in 12 out of 76 samples taken, with a maximum concentration of 0.417 ug/L (Struger 1999), and chlorpyrifos was also detected in water and sediment of the Holland River watershed where intensive vegetable crop production occurs (Bishop *et al.* 1999). However, in a recent survey of urban streams in Toronto, Ontario, Struger *et al.* (2002) did not detect chlorpyrifos in any of 133 surface water samples collected at a detection limit of 0.05 ug/L. Given this detection limits, all detections of chlorpyrifos are exceedences of the Canadian Water Quality Guideline.

4.3 BIOCONCENTRATION AND METABOLISM

Chlorpyrifos is readily absorbed from food with absorption efficiencies of 41-72 percent among most terrestrial species, while dermal uptake varies considerably with uptake minimal in plants, as low as one percent for man, 60 percent for the rat and 90 percent for target (and some non-target) insects (Dow AgroSciences LLC, Racke 1993).

Chlorpyrifos could have the potential to bioconcentrate in fish and other aquatic organisms, plants and soil dwelling insects due to its low water solubility and moderate level of hydrophobicity (log K_{ow} 4.7-5.3) (Giesy *et al.* 1999). Within freshwater fish species, whole fish bioconcentration factors (BCFs) range from 100 for the bluegill sunfish (Eaton *et al.* 1985), 460 in the common carp (Tsuda *et al.* 1992), to 1,680 for fathead minnows (Jarvinen *et al.* 1983). Plants do not bioconcentrate chlorpyrifos since negligible levels enter plant roots or penetrate the cuticle (Racke 1993), and BCFs in soil insects are low; <1 for European chafer grubs (*Amphimallon majalis*) (Tashiro and Kuhr 1978). The major metabolite TCP is polar and therefore less likely to bioconcentrate than chlorpyrifos with BCFs for mosquitofish, *Daphnia magna* and snails (*Physa* sp.) of 16.34, 21.77, and 13.67 respectively (Lu and Metcalf 1975).

Fish, mammals and some insects quickly transform chlorpyrifos to TCP, TMP and other metabolites. Fish rapidly absorb and de-phosphorylate chlorpyrifos to TCP for conjugation with glucuronic acid and excretion in urine and bile (Barron *et al.* 1993), with a half-life in common carp and threespine stickleback of 34.7 and 13.4 h respectively (Tsuda *et al.* 1992, Deneer 1994). In mammals, chlorpyrifos is metabolically detoxified in the liver by cytochrome P-450 mono-oxygenases to TCP (Atterbury *et al.* 1997), with studies in ruminants indicating excretion of >63 percent chlorpyrifos as metabolites (Gutenmann *et al.* 1968). Rat studies indicate that the majority of a single dose of radio labelled chlorpyrifos appeared in the urine within the first day (Smith *et al.* 1967). Insect metabolism is rapid with 25-30 percent of a topically applied dose excreted within 24 h (Racke 1993), and studies in chlorpyrifos-resistant German cockroaches (*Blattella germanica*) found a higher level of water soluble metabolites and lower levels of chlorpyrifos-oxon than susceptible roaches, suggesting that resistance is due to both a rapid transformation to chlorpyrifos-oxon by NADPH-dependent monooxygenases and then hydrolysis (Siegfried *et al.* 1990).

4.4 TOXIC MECHANISM OF ACTION

Despite the rapid detoxification of chlorpyrifos, chlorpyrifos enters general circulation and is locally activated to its neurotoxic form chlorpyrifos-oxon by P-450 dependent mono-oxygenases in target tissues (Mattsson *et al.* 1996, Racke 1993, Russell and Chambers 1996, Sultatos *et al.* 1984). Chlorpyrifos oxon interferes with the normal functioning of cholinesterase (ChE) enzymes responsible for the binding and termination of the biological activity of the neurotransmitter acetylcholine (ACh) (Carr and Chambers 1996). With the accumulation of free, unbound ACh at the nerve endings of all cholinergic nerves, there is a continual stimulation of electrical activity causing mortality (Katz *et al.* 1997). Chlorpyrifos was found to inhibit ChE activity in brain and blood samples taken from treated rats in a dose-dependent manner for up to 53 days (Bushnell *et al.* 1993). A high level of cholinesterase inhibition (75 percent) was also observed within one week in white leghorn hens fed up to 200 ppm Dursban for one year (Sherman and Herrick 1973). Chlorpyrifos affects the central nervous system, producing incoordination and loss of reflexes, involuntary muscle contractions, and autonomic and respiratory dysfunction (Zheng *et al.* 2000).

4.5 ACUTE TOXICITY

Acute toxicity tests for aquatic organisms and birds are summarized in Tables 4.3 and 4.4. The majority of tests were conducted on aquatic organisms, but some information was available for birds, amphibians and mammals. Differences in behaviour, feeding ecology, receptor sensitivity and pharmacokinetics account for the more than one million-fold variation in chlorpyrifos sensitivity among species (Giesy *et al.* 1999). Specifically, individual and species susceptibility to chlorpyrifos is related to the binding affinity of chlorpyrifos oxon to AChE and

to its subsequent rate of inactivation (Giesy *et al.* 1999). Although study comparison is difficult, fish and aquatic invertebrates appear to be more acutely sensitive to chlorpyrifos than birds, amphibians or mammals.

4.5.1 Mammals and Birds

Chlorpyrifos is more toxic to mammals following oral administration than after dermal exposure (Table 4.4). Dermal exposure of rats and rabbits to chlorpyrifos resulted in mild skin and eye irritation (U.S. EPA 1999) and LD50s ranging from 202-6,730 mg/kg b.wt. (Gaines 1969, Marshall and Roberts 1978). The acute oral LD50s for mice, rats, guinea pigs and rabbits are 111, 118-245, 504 and >1,000 mg/kg b.wt. respectively (Gollapudi *et al.* 1996, McCollister *et al.* 1974). While age is thought to be a factor in the acute toxicity of chlorpyrifos to rats (Zheng *et al.* 2000), a concurrent study examining neurochemical parameters in rats at lower doses (0.15-15 mg/kg/d for 14 d) found age differences in chlorpyrifos sensitivity much reduced (Zheng *et al.* 2000).

The single oral dose LD50 for mallard ducks to technical chlorpyrifos ranged from 29.4 - 145 mg/kg b.wt. depending on age (Hudson *et al.* 1972, Tucker and Haegele 1971), while the 5-d dietary LC50 ranged from 671-1,080 ppm (Gile *et al.* 1983). The oral LD50 and 5-d dietary LC50 for northern bobwhites were $\geq$32 mg/kg (formulation dependent) and >353 ppm, respectively (Hill and Camardese 1984, Gile *et al.* 1983). The oral LD50 in chickens was greater than 25.4 mg/kg b.wt. (McCollister *et al.* 1974, Sherman *et al.* 1967), and the 14-d dietary LC50 was >400 ppm (Sherman *et al.* 1967). The LD50s (single dose oral gavage) for all the species shown in Table 4.4 are lower than 157 mg/kg b.wt. with red-winged blackbirds, grackles, European starlings, ring-necked pheasants and house sparrows being the most sensitive to chlorpyrifos exposure. A study by Hill and Camardese (1984) found that granular chlorpyrifos (Lorsban 15G) was three times less toxic than technical grade chlorpyrifos to northern bobwhites.

4.5.2 Aquatic Invertebrates and Fish

Algae appear to be relatively tolerant to chlorpyrifos exposure. Exposure of blue-green algae (*Anabaena flos-aquae, Coccochloris peniocystis* and *Oscillatoria* sp.*)*, green algae (*Chlamydomonas reinhardii* and *Chlorella pyrenoidosa*) and diatoms (*Navicula pelliculosa* and *Navicula minima*) to 100 ug/L Dursban for 5-7 d resulted in the stimulation of growth (as measured by absorbance at 678 nm) of *Anabaena flos-aquae* (on nitrogen-free media) and *Chlamydomonas reinhardii*, and decreased growth of *Chlorella pyrenoidosa* (Burmingham and Colman 1977).

Aquatic invertebrates have a wide range of sensitivities to chlorpyrifos exposure (Table 4.3). *Brachionus calyciflorus*, *Aplexa hypnorum* and *Limnodrilus hoffmeisteri* appear to be least sensitive to acute chlorpyrifos exposure with 24-72 h LC(EC)50s greater than the highest concentrations tested, 11 850 ug/L, 806 ug/L, and 36 ug/L, respectively (Ferrando and Andreu-Moliner 1991, Phipps and Holcombe 1985, van Wijngaarden *et al.* 1993). Increased duration of exposure had little effect on acute toxicity of chlorpyrifos to snails (*Anisus vortex*, *Bithynia tentaculata*, *Lymnaea stagnalis*, *Bromophalaria alexandra*) (Ibrahim *et al.* 1992, van Wijngaarden and Leeuwangh 1989). Planaria (*Dugesia dorotocephala*) are also not very sensitive to chlorpyrifos exposure (Villar *et al.* 1993). Amphipods (*Gammarus lacustris*, *G. pseudolimnaeus* and *G. pulex*), and water fleas (*Ceriodaphnia dubia*, *Daphnia longispina* and *D. magna*), and cladoceran (*Simocephalus vetulus*) were found to be very sensitive with 24-96 h LC50s between 0.07-1.7 ug/L (Kersting and van Wijngaarden 1992, Sanders 1969, van Wijngaarden *et al.* 1993, van Wijngaarden and Leeuwangh 1989).

The insect species listed in Table 4.3 are sensitive to chlorpyrifos exposure with LC50s ranging for the most part from <1-30 ug/L, with the exception of the diving beetle (*Hydrophylus* sp. with an LC50 of 100) and some midge species. Increased exposure time resulted in increased toxicity for the stonefly (*Claassenia sabulosa*) (Mayer and Ellersieck 1986).

Chlorpyrifos is quite acutely toxic to fish although they appear to be more tolerant than invertebrates (Table 4.3). Channel catfish, fathead minnows and lake trout are the least sensitive species of fish with 96-h LC50s greater than 98 ug/L (Holcombe *et al.* 1982, Jarvinen and Tanner 1982, Mayer and Ellersieck 1986, Phipps and Holcombe 1985).

4.5.3 Amphibians

What little information there is suggests that amphibians have acute tolerances to chlorpyrifos similar to that of fish with LC50s ranging from one to >1,000 ug/L. The LC50s for the American toad and leopard frog tadpoles were one and 3,000 ug/L respectively, while adult leopard frogs had an LC50 of 30 000 ug/L (Whitney 1965). The 96-h LC50 of chlorpyrifos to tiger frog tadpoles was 0.019 mg/L (Abbasi and Soni 1991). The adult smooth newt exposed to Dursban 4E exhibited a change in locomotional behaviour with a 96-h EC10 >96 ug/L (van Wijngaarden *et al.* 1993).

4.5.4 Non-target Terrestrial Invertebrates

Chlorpyrifos is fairly non-toxic to earthworms with 14-d LC50s in six species (*Aporrectodea caliginosa*, *A. longa*, *Lumbricus rubellus*, *L. terrestris*, *Eisenia fetida*, and *E. veneta*) ranging from 104-1,174 mg/kg soil with *L. rubellis* being the most sensitive and *E. veneta* the least sensitive (Ma and Bodt 1993). Chlorpyrifos is more toxic to other non-target organisms such as the stablefly (*Stomoxys calcitrans*), greenbug (*Schizaphis graminum)* and flour moth (*Anagasta kuehniella*) with reported 24-h LC50s (mg/kg substrate) of 0.017, 1.6 and >500 respectively (Kenaga *et al.* 1965). Chlorpyrifos exposure (960 g/ha) to three species of carabid beetles resulted in 100 percent mortality to larvae and adults within 16 and 100 d respectively (Kegel 1989). The European corn borer (*Ostrinia nubilalis*), the lady beetle (*Hippodamia convergens*) and the housefly (*Musca domesticus*) had 24-h LD50s of 13-32, 14-22, and 44-93 ng a.i./mg b.wt. respectively (Siegfried 1993). Atkins *et al.* (1973) observed a 48-h LD50 of 0.114 ug/bee in honeybees (*Apis mellifera*) and further study found LD50s of 0.051ug/bee for larvae and 0.076 ug/insect for adults (Atkins and Kellum 1986).

Table 4.3 Acute toxicity of chlorpyrifos to aquatic species.

Species	Common name	Life stage (age or size)	Dose method [a]	Chemical formulation	Duration	Effect (ug/L) [b]	Reference
Invertebrates							
Dugesia dorotocephala	planaria	20-25 mg	SR, N	technical	7 d	LC50 = 2 000-4 300	Villar *et al.* 1993
Brachionus calyciflorus	rotifer	<1 d	S, N	NR	24 h	LC50 = 11 850	Ferrando and Andreu-Moliner 1991
Anisus vortex, Bithynia tentaculata, Lymnaea stagnalis	snails	NR	F, N	commercial	10 d	LC50 >94	van Wijngaarden and Leeuwangh 1989
Aplexa hypnorum	snail	adult	F, M	NR	96 h	LC50 >806	Phipps and Holcombe 1985
Bromophalaria alexandra	snail	adult juvenile	SR, N	commercial	56 d 84 d	LC50 >500 LC50 >250	Ibrahim *et al.* 1992
Helisoma trivolvis	snail	adult	S, N	technical	72 h	LC50 >2 000	Kenaga *et al.* 1965
Lanistes carinatus	snail	NR	S, N	technical	24 h	LC50 = 2.71	Abdel Karim *et al.* 1985
Nephelopsis obsura	leech	50-400 mg	S, N	technical	42 d	LC50 >144	Singhal *et al.* 1989
Limnodrilus hoffmeisteri	oligocheate	adult	SR, N	commercial	96 h	EC10 >36 (movement and breakage)	van Wijngaarden *et al.* 1993
Gammarus lacustris	amphipod	adult adult	S, N S, N	technical technical	96 h 24 h	LC50 = 0.11 LC50 = 0.76	Sanders 1969
Gammarus pulex	amphipod	6.5-16.8 mm	F, N	commercial	96 h	LC50 = 0.07	van Wijngaarden *et al.* 1993
Daphnia longispina	water flea	adult	SR, N	commercial	96 h	LC50 = 0.3	van Wijngaarden *et al.* 1993
Daphnia magna	water flea	<1d	S, M	technical	48 h	LC50 = 1	Kersting and van Wijngaarden 1992
Daphnia sp.	water flea	adult and nymph	S, N	NR	48 h	LC50 = 0.016	Kenaga *et al.* 1965
Daphnia pulex	water flea	NR	F, N	commercial	48 h	LC50 = 0.21	van Wijngaarden and Leeuwangh 1989
Simocephalus vetulus	cladoceran	juvenile - adult	S, N	commercial	96 h	LC50 = 0.5	van Wijngaarden *et al.* 1993
Orconectes immunis	crayfish	1.8 g	F, M	technical	96 h	LC50 = 6	Phipps and Holcombe 1985
Proasellus coxalis	isopod	adult	F, N	commercial	96 h	EC10 >20 (response to tactile stimulus)	van Wijngaarden *et al.* 1993
Asellus aquaticus	isopod	adult	SR, N	technical	96 h	EC50 = 2.7 (response to tactile stimulus)	van Wijngaarden *et al.* 1993
Triops longicaudatus	tadpole shrimp	4-5 d	S, N	commercial	24 h	LC50 = 4	Walton *et al.* 1990
Insects							
Simulium vitattum	blackfly	larval	S, N	technical	24 h	LC50 = 27	Siegfried 1993
Hydropschy/Cheumatopsyche sp.	caddisfly	larval	S, N	technical	24 h	LC50 =30.6	Siegfried 1993
Pseudagrim spp.	dragonfly	adult	S, N	technical	24 h	LC50 = 0.0001	Abdel Karim *et al.* 1985
Crocothemis erthryaea	dragonfly	adult	S, N	technical	24 h	LC50 = 0.0058	Abdel Karim *et al.* 1985
Enellagma/Ishnura spp.	damselfly	nymph	S, N	technical	24 h	LC50 = 11.4	Siegfried 1993

Species	Common name	Life stage (age or size)	Dose method [a]	Chemical formulation	Duration	Effect (ug/L) [b]	Reference
Chaoborus obscuripes	diptera	larval	F, N	commercial	96 h	LC50 = 6.6	van Wijngaarden *et al.* 1993
Hydrophylus spp.	diving beetle	nymph	S, N	technical	24 h	LC50 = 100	Siegfried 1993
Caenis horaria	mayfly	nymph	F, N	commercial	96 h	LC10 >3	van Wijngaarden *et al.* 1993
Cleon dipterum	mayfly	NR	F, N	commercial	72 h	LC50 = 0.25	van Wijngaarden and Leeuwangh 1989
Claassenia sabulosa	stonefly	nymph	S, N	technical	96 h 24 h	LC50 = 0.57 LC50 = 8.2	Mayer and Ellersieck 1986
Corixa puctata	water strider	NR	F, N	commercial	96 h	LC50 = 1.94	van Wijngaarden and Leeuwangh 1989
Fish							
Oreochromis niloticus	Nile tilapia	19.5-22 cm	S, N	technical	24 h	LC50 = 0.31	Abdel Karim *et al.* 1985
Oreochromis niloticus	Nile tilapia	1.5 g 13.8 g	S, N	commercial	48 h	LC50 = 62 LC50 = 114	El-Refai *et al.* 1976
Oreochromis mossambica	Mozambique tilapia	1.3-1.5 g	S, N	technical	24 h 48 h	LC50 = 0.029-0.03 LC50 = 0.027-0.029	Dutt and Guha 1988
Gambusia affinis	mosquitofish	4.5-6 cm	S, N	technical	24 h	LC50 = 0.34	Abdel Karim *et al.* 1985
Carassius auratus	goldfish	immature	S, N	NR	48 h	LC50 = 0.18	Kenaga *et al.* 1965
Lepomis macrochirus	bluegill sunfish	0.5 g 0.6 g	S, N	technical	96 h 24 h	LC50 =1.7-4.2 LC50 >10	Mayer and Ellersieck 1986
Lepomis macrochirus	bluegill sunfish	0.8 g	F, M	NR	96 h	LC50 = 10	Phipps and Holcombe 1985
Cyprinus carpio	common carp	1.1-1.4 g 1.1-1.4 g	S, N	technical	24 h 48 h	LC50 = 0.0018-0.0036 LC50 = 0.0014-0.0028	Dutt and Guha 1988
Cyprinus carpio	common carp	1.75 g 31.5 g	S, N	commercial	48 h	LC50 = 280 LC50 = 59	El-Refai *et al.* 1976
Ictalurus punctatus	channel catfish	7.9 g	F, M	technical	96 h	LC50 = 806	Phipps and Holcombe 1985
Ictalurus punctatus	channel catfish	0.8 g	S, N	technical	96 h 24 h	LC50 = 280 LC50 = 410	Mayer and Ellersieck 1986
Oncorhynchus clarki	cutthroat trout	0.9 g 2.3 g 1.4 g	S, N	technical	96 h	LC50 = 5.4-26 LC50 = 13.4 LC50 = 18.4	Mayer and Ellersieck 1986
Salmo gairdneri	steelhead-rainbow trout	0.6-1.5 g	S, N	technical	96 h	LC50 = 51 (1.6°C) LC50 = 15 (7.2°C) LC50 = 7.1 (12.7°C)	Macek *et al.* 1969
Pimephales promelas	fathead minnow	31 d	F, M	technical	96 h	LC50 = 203	Holcombe *et al.* 1982
Pimephales promelas	fathead minnow	1 d	S, M F, M F, M	technical technical commercial	96 h	LC50 = 150-170 LC50 = 140 LC50 = 120	Jarvinen and Tanner 1982
Pimephales promelas	fathead minnow	0.5 g	F, M	technical	96 h	LC50 = 542	Phipps and Holcombe 1985

Species	Common name	Life stage (age or size)	Dose method [a]	Chemical formulation	Duration	Effect (ug/L) [b]	Reference
Oncorhynchus mykiss	rainbow trout	juvenile	F, M	technical	96 h	LC50 = 8	Holcombe *et al.* 1982
Oncorhynchus mykiss	rainbow trout	1.4 g	S, N	technical	96 h	LC50 = 7.1	Mayer and Ellersieck 1986
Oncorhynchus mykiss	rainbow trout	3.0 g	F, M	technical	96 h	LC50 = 9	Phipps and Holcombe 1985
Oncorhynchus mykiss	rainbow trout	600-900 g	F, M	technical	72 h	LC100 = 208	Bradbury *et al.* 1991
Gasterosteus aculeatus	threespine stickleback	NR	F, N	technical	7 d	LC50 = 8.5	van Wijngaarden and Leeuwangh 1989
Pungitius pungitius	ninespine stickleback	adult	F, N	technical	96 h	LC50 = 4.7	van Wijngaarden *et al.* 1993
Salvelinus namaycush	lake trout	2.3 g 0.3 g	S, N	technical	96 h 24 h	LC50 = 98-205 LC50 = 419	Mayer and Ellersieck 1986

NR - information not reported

[a] S - static; F - flowthrough; SR - static renewal; M - measured concentration; N - nominal concentration

[b] LC(x) - concentration lethal to (x)% of the population; EC(x) - concentration causing an effect in (x)% of the population

Table 4.4 Acute toxicity of chlorpyrifos to mammals and birds.

Species	Common name	Age	Chemical formulation	Dose method	Duration (d)	Effect [a]	Reference
Mammals							
Rattus norvegicus	Sprague-Dawley rat	7 d 21 d 90 d	technical	oral gavage	-	LD10 = 15 LD10 = 47 LD10 = 136	Zheng *et al.* 2000
Rattus norvegicus	Dow-Wistar rat	NR	technical	oral gavage	-	LD50 = 135-245	McCollister *et al.* 1974
Rattus norvegicus	Sherman rat	NR	technical	oral gavage	-	LD50 = 118-155	McCollister *et al.* 1974
Rattus norvegicus	Sherman rat	adult (male) adult (female)	technical	oral gavage	-	LD50 = 155 mg/kg LD50 = 82 mg/kg	Gaines 1969
Cavia porcellus	guinea pig	NR	technical	oral gavage	-	LD50 = 504	McCollister *et al.* 1974
Oryctolagus cuniculus	New Zealand white rabbit	NR	technical	oral gavage	-	LD50 >1 000	McCollister *et al.* 1974
Mus musculus	CD-1 mouse	10-11 weeks	technical	oral gavage	-	LD50 = 111	Gollapudi *et al.* 1996
Rattus norvegicus	Sherman rat	adult (male)	technical	dermal	3	LD50 = 202 mg/kg	Gaines 1969
Oryctolagus cuniculus	rabbit	NR	various commercial formulations	dermal	NR	LD50 = 504-6730	Marshall and Roberts 1978
Birds							
Anas platyrhynchos	mallard duck	14 d	NR	dietary	5	LC50 = 671-1080 [b]	Gile *et al.* 1983
Anas platyrhynchos	mallard duck	1.5 d 7 d 30 d 180 d	technical	oral gavage	-	LD50 = 145 LD50 = 29.4 LD50 = 50.4 LD50 = 83.3	Hudson *et al.* 1972
Anas platyrhynchos	mallard duck	2-4 months	technical	oral gavage	-	LD50 = 75.6	Tucker and Haegele 1971
Colinus virginianus	northern bobwhite	14 d	NR	dietary	5	LC50 = 353-421 [b]	Gile *et al.* 1983
Colinus virginianus	northern bobwhite	adult	technical 15% granular	oral gavage	-	LD50 = 32 LD50 = 108	Hill and Camardese 1984 Hill and Camardese 1984
Streptopelia risoria	ringed turtledove	adult	15% granular	oral gavage	-	LD50 = 157	Hill and Camardese 1984
Gallus domesticus	white leghorn chicken	10-12 d	technical	dietary oral gavage	14 -	LC50 >400 [b] LD50 = 25.4	Sherman *et al.* 1967
Gallus domesticus	white leghorn chicken	chick (male)	technical	oral gavage	-	LD50 = 32	McCollister *et al.* 1974
Sturnus vulgaris	European starling	adult	NR	oral gavage	-	LD50 = 5	Schafer 1972
Sturnus vulgaris	European starling	4 d	technical	oral gavage	-	LD50 >2	Meyers *et al.* 1992
Agelaius phoeniceus	red-winged blackbird	adult	NR	oral gavage	-	LD50 = 13	Schafer 1972
Agelaius phoeniceus	red-winged blackbird	3 d	technical	oral gavage	-	LD50 = 2	Meyers *et al.* 1992
Quiscalus quiscula	common grackle	adult	NR	oral gavage	-	LD50 = 13	Schafer and Brunton 1971

Species	Common name	Age	Chemical formulation	Dose method	Duration (d)	Effect [a]	Reference
Alectoris graeca	chukar partridge	2-4 months	technical	oral gavage	-	LD50 = 60.7	Tucker and Haegele 1971
Columba livia	rock dove	adult	technical	oral gavage	-	LD50 = 26.9	Tucker and Haegele 1971
Phasianus colchicus	ring-necked pheasant	2-4 months	technical	oral gavage	-	LD50 = 8.41	Tucker and Haegele 1971
Coturnix coturnix japonica	quail	7-8 weeks	technical	oral gavage	-	LD50 = 15.9	Tucker and Haegele 1971
Passer domesticus	house sparrow	adult	NR	oral gavage	-	LD50 = 10	Schafer and Brunton 1971
Passer domesticus	house sparrow	adult	technical	oral gavage	-	LD50 = 21	Tucker and Haegele 1971

NR - information not reported

[a] LC50 (mg/kg/d) - median lethal concentration (*ad libitum* feeding with chlorpyrifos incorporated into diet); LD50 (mg/kg body weight) - median lethal dose (single-dose oral gavage)

[b] ppm

4.6 CHRONIC TOXICITY AND ECOLOGICAL EFFECTS

4.6.1 Mammals

In a 90-d study, the systemic NOEL and LOEL (based on reduced body weight) for rats fed chlorpyrifos were 10 ppm and 200 ppm, respectively (U.S. EPA 1999). A 13-week daily dietary exposure (0-15 mg/kg b.wt./d) study in Fischer 344 rats saw minimal clinical symptoms of toxicity, no evidence of treatment-related neuropathological changes, and no gross or histopathological lesions (Mattsson *et al.* 1996). A 13-week inhalation study in Fischer rats of chlorpyrifos between approximately 5-20 ppb (0.07 mg/kg/d assuming 100 percent absorption) saw no exposure-related changes in urinalysis, haematology, clinical chemistry, body or organ weights, pathology, or cholinesterase activities (Corley *et al.* 1989). Seven-week old rats were fed chlorpyrifos at doses of 0-3 mg/kg b.wt./d for two years (McCollister *et al.* 1974). There were no observed effects on body weight gain, food intake, mortality rates or histopathological alterations of organs in treated rats, however doses of one and three mg/kg/d depressed plasma and red blood cell ChE and the highest dose of three mg/kg/d depressed brain ChE activity (McCollister *et al.* 1974). This study was also conducted on dogs at dose levels of 0-3 mg/kg b.wt./d for either one or two years with similar findings (McCollister *et al.* 1974).

Several studies conducted with rats indicate that chlorpyrifos is a developmental neurotoxin, directly affecting developing cells (Crumpton *et al.* 2000, Jett and Navoa 2000, Whitney *et al.* 1995, Song *et al.* 1997). The neurochemical and physiological basis is still being investigated but hypotheses include: the non-cholinergic targeting of the expression and function of major nuclear transcription factors involved in brain cell replication and differentiation (Crumpton *et al.* 2000), induced oxidative stress in the developing brains of rats (Jett and Navoa 2000), the inhibition of DNA and protein synthesis in the brain (Whitney *et al.* 1995), and action on adenyl cyclase that functions in the coordination of cell development (Song *et al.* 1997). As a developmental neurotoxin, chlorpyrifos may have subsequent effects on axonogenesis, synaptic development and apoptosis and therefore the potential to evoke behavioural teratogenesis at subtoxic doses (Crumpton *et al.* 2000). Chlorpyrifos does not appear to be neurotoxic in adult animals (Maurissen *et al.* 2000). A subchronic study in adult Long-Evans rats with doses of up to 10 mg/kg/d at five d/week for four weeks found no effect on long-term memory or on motivation/attention (Maurissen *et al.* 2000).

Pregnant Long-Evans rats exposed to up to seven mg/kg/d chlorpyrifos on gestational d 14-18 experienced no overt signs of toxicity but there was evidence that chlorpyrifos (as TCP) crossed the placental barrier to the foetus (Hunter *et al.* 1999). While the maternal liver contained five times more TCP than the foetal liver at the time of peak effect (5 h after last dose), TCP levels were over twice as high in the foetal brain than the maternal brain (Hunter *et al.* 1999). A study by Deacon *et al.* (1980) on pregnant CF-1 mice found no evidence of teratogenicity at dose levels up to 25 mg/kg/d. Foetal body weight and crown-rump length were decreased among litters of mice given 25 mg/kg chlorpyrifos and minor skeletal variants were significantly increased, but these effects were associated with the severe maternal toxicity observed at that level (Deacon *et al.* 1980). A later study by Breslin *et al.* (1996) in Fischer 344 rats treated on gestational d 6-15 concurred that oral administration of chlorpyrifos at parentally toxic levels (up to 15 mg/kg/d) was not embryolethal, embryo/fetotoxic, or teratogenic. A concurrent two-generation study in Fischer 344 rats found that chlorpyrifos did not adversely affect fertility indices, length of gestation, time to mating, pup sex ratio or litter size in any dose group (Breslin *et al.* 1996). A two-generation reproduction study with rats resulted in a developmental NOEL of one mg/kg/d and a LOEL of five mg/kg/d (LOEL based on reduced pup body weight and increased pup mortality) (U.S. EPA 1999). The effects of chlorpyrifos were studied on the

growth of reproductive organs and serum testosterone and estradiol concentrations in neonatal male and female rats where seven-day-old rats received 7.0 and 14.0 mg/kg chlorpyrifos subcutaneously for 15 d (Ahmad *et al.* 1993). Ahmad *et al.* (1993) reported no effect on body weight or mortality, but found significantly decreased weights of the reproductive organs (testis, epididymis, vas deferens, prostate, uterus, ovaries and oviducts) and suppressed serum testosterone and estradiol concentrations in both male and female rats. A more recent study on the metabolic endocrine and reproductive endocrine systems in ewes tested 12.5 mg/kg chlorpyrifos two times a week for 43 d (Rawlings *et al.* 1998). A marked decrease in thyroxine concentration (which may be partially explained by competition for binding) and an increase in serum concentrations of cortisol were observed (Rawlings *et al.* 1998). Thyroxine is the major secretory product of the thyroid and its principal function is to stimulate the consumption of oxygen and thus the metabolism of all cells and tissues in the body. Cortisol (hydrocortisone) is secreted by the outer layer of the adrenal gland and affects the metabolism of carbohydrate, protein, and fat; the maturation of white blood cells; the retention of salt and water in the body; the activity of the nervous system; and the regulation of blood pressure. Cortisol plays a major role in the body's response to stress so the mechanism of the effect of chlorpyrifos is unclear (Rawlings *et al.* 1998). However, chlorpyrifos had no significant effect on the mean serum concentrations of insulin, luteinizing hormone, follicle stimulating hormone, progesterone, or estradiol and no significant increase in the severity of oviducal intraepithelial cysts (Rawlings *et al.* 1998). Developmental toxicity studies in Fisher 344 rats and New Zealand white rabbits show that TCP, even at dose levels that produce maternal toxicity, is neither fetotoxic nor teratogenic (Hanley *et al.* 2000).

Two-year carcinogenic studies in rats resulted in a NOEL of five ppm and a LOEL of 100 ppm (based upon reduced body weight) (U.S. EPA 1999). In a 78-week oncogenicity study with mice, the systemic NOEL was 50 ppm and the LOEL (based on reduced body weight) was 250 ppm (U.S. EPA 1999). As described above, a two-year feeding study of Dow-Wistar rats at levels of up to three mg/kg/d found that tissue examination did not reveal any treatment-related alterations in the organs of treated rats, and there was no significant increase in the number or type of tumours (McCollister *et al.* 1974). A more recent two-year feeding study in Fischer 344 rats at subtoxic doses of up to 10 mg/kg/d also concluded that chlorpyrifos exposure did not cause a treatment-related increase in the incidence of neoplasm in any tissue examined (Yano *et al.* 2000).

Table 4.5 Genotoxicity/mutagenicity studies of chlorpyrifos and its formulations.

Test	Organism	Formulation	Dose Range	Mutagenic Potential [b]	Reference
Plants					
gene reversion assay	*Zea mays*	commercial	NR	negative (+ and -)	Gentile *et al.* 1982
chromosomal aberration (meiosis)	barley (*Hordeum vulgare*)	NR	0.2-1.0%	weak positive (4.3-15.5% of cells)	Kaur and Grover 1985
abnormal mitosis	*Vivia faba* (roots and seeds)	technical	0.125 saturated solution - 100% saturated solution	positive at $\geq$0.25 saturated solution	Amer and Farah 1983a
chromosomal aberration (meiosis)	*Vicia faba* (seedlings)	NR	100% (4 d)	positive	Amer and Farah 1983b
chromosomal aberration (meiosis)	*Vicia faba* (flowering stage)	NR	100% (1-4 d)	positive	Amer and Farah 1983b
Insects					
sex-linked recessive lethal test	*Drosophila melanogaster*	Durmet (20% a.i.)	$1x10^{-6}$-$5x10^{-5}$ %	positive at $1x10^{-5}$ and $5x10^{-5}$ (72h)	Patnaik and Tripathy 1992
wing mosaic test	*Drosophila melanogaster*	Durmet (20% a.i.)	$1x10^{-6}$-$5x10^{-5}$ %	positive at $\geq 5x10^{-6}$ (72h)	Patnaik and Tripathy 1992
Prokaryotes/Eukaryotes					
DNA repair (rec-assay)	*Bacillus subtilis*	NR	NR	negative (-)	Shirasu *et al.* 1976
gene reversion (Ames test)	*Salmonella typhimurium* (5 strains)	technical	0-100 mg/plate	negative (+ and -)	Gollapudi *et al.* 1995
gene reversion (Ames test)	*Salmonella typhimurium*	technical and commercial	NR	negative (+ and -)	Gentile *et al.* 1982
gene reversion (Ames test)	*Salmonella typhimurium* (5 strains)	NR	0-5 000 ug/plate	negative (+ and -)	Moriya *et al.* 1983
gene conversion	*Escherichia coli* WP2	NR	0-5 000 ug/plate	negative (+ and -)	Moriya *et al.* 1983
gene conversion	*Saccharomyces cerevisiae*	technical and commercial	NR	negative (+ and -)	Gentile *et al.* 1982
Mammalian/Avian Cells					
in vivo sister chromatid exchange	chick embryo	technical	1.11-1 110 ug injection	negative	Muscarella *et al.* 1984
sister chromatid exchange	Chinese hamster ovary cells	technical	1-100 ug/ml	negative	Muscarella *et al.* 1984
gene mutation	Chinese hamster ovary cells	technical	3.5-17.5 ug/mL	negative (+ and -)	Gollapudi *et al.* 1995
sister chromatid exchange	human lymphocytes	technical	0.02-20 ug/mL	positive at 2 and 20 ug/mL (+ and -)	Sobti *et al.* 1982
chromosomal aberration	rat lymphocytes	technical	5-167 ug/mL	negative (+ and -)	Gollapudi *et al.* 1995
unscheduled DNA synthesis assay	rat hepatocytes	technical	0.35-35 ug/mL	negative (+ and -)	Gollapudi *et al.* 1995
chromosome aberrations	mouse spleen cells	technical	0.5-4.0 ug/mL	positive	Amer and Alys 1992
sister chromatid exchange	mouse spleen cells	technical	0.5-4.0 ug/mL	positive (1-4 ug/mL)	Amer and Alys 1992
chromosomal aberrations or developmental abnormalities (bovine blastocyte study)	superovulated Holstein cow mated to treated bulls	Dursban 44 (43% a.i.)	NR	negative	Muscarella *et al.* 1984
in vivo bone marrow micronucleus test	mouse	NR	5-45 mg/kg b.wt. (intraperitoneal injection)	positive at $\geq$15 mg/kg b.wt. (multiple injections)	Amer and Fahmy 1982

Test	Organism	Formulation	Dose Range	Mutagenic Potential [b]	Reference
in vivo bone marrow micronucleus test	mouse	NR	80, 120 or 240 ppm (dietary: 1-10 weeks)	positive	Amer and Fahmy 1982
in vivo bone marrow micronucleus test	mouse	NR	99 mg/kg b.wt. (dermal: 1-14 d)	negative	Amer and Fahmy 1982
in vivo bone marrow micronucleus test	mouse	technical	7-90 mg/kg b.wt. (oral gavage)	negative (+ and -)	Gollapudi *et al.* 1995

NR - information not reported

[a] (+) with S9 activation; (-) without S9 activation

4.6.2 Birds

Numerous studies have examined chronic toxicity of chlorpyrifos to birds. White leghorn chickens fed 25-100 ppm Dursban in their diet for four weeks did not exhibit any clinical signs of poisoning, however there was significant inhibition of ChE, and increased food consumption in the 100 ppm treatment group (Schlinke 1970). Turkeys placed in pens surface-treated with 4.48 or 8.97 kg/ha Dursban for up to eight weeks contained residues of up to 0.157 and 0.066 ppm in the skin and body fat within one week of exposure (Claborn *et al.* 1970). However, these residues decreased to insignificant levels by eight weeks (Claborn *et al.* 1970).

As observed in mammals (Maurissen *et al.* 2000), chlorpyrifos does not appear to be neurotoxic in adult birds. Daily dosing of adult white leghorn hens with 10 mg/kg/d for 20 d resulted in a maximum inhibition of brain neurotoxic esterase (NTE) of 18 percent, no cumulative inhibition of lymphocyte or brain NTE and no clinical signs of organophosphate (OP) induced delayed neurotoxicity (Richardson *et al.* 1993).

Adult mallard ducks fed chlorpyrifos exhibited a reduction in body weight, brain AChE, egg production, eggshell thickness, egg weight and hatchling weight at 80 mg/kg feed for 18 weeks (Gile and Meyers 1986) but no adverse effects when fed eight mg/kg for almost two years (Meyers and Gile 1986). Other species of birds may be less sensitive to chlorpyrifos. Northern bobwhites exposed to 125 mg/kg chlorpyrifos in food for six months did not exhibit adverse reproductive effects as measured by the number of eggs laid, cracked eggs, eggshell thickness, viable embryos, hatching success, or body weight of hatchlings (Fink 1978). Adult chicken, chukar and northern bobwhite hens fed Dursban at 100 ppm for 16 weeks experienced no adverse effects on egg production or egg weight, but fertility was reduced by 15 percent, hatchability by 17 percent and residues of 0.126, 0.035, and 0.043 ppm were found in egg yolks (Schom *et al.* 1973). White leghorn hens fed Dursban at levels up to 200 ppm for one year experienced no treatment-related mortality, no detrimental effect on body weight, and no significant effect on overall egg production (Sherman and Herrick 1973). The negative correlation between nest productivity (number of fledged young) of American robins and the percentage of surrounding chlorpyrifos-treated lawns in an urban neighbourhood observed by Decarie *et al.* (1993) was thought to be attributed to the reduced numbers and biomass of earthworms due to repeat use of chlorpyrifos. The immersion of quail eggs in 420 g a.i./ha chlorpyrifos resulted in no significant effects on hatching success, deformity, incubation time, chick mass and tarsus length; however, immersion in double that concentration resulted in significantly increased incubation time and an increased percentage of deformed feet and legs, skeletal scoliosis, and bill deformities (Martin 1990). Dursban injected (0.35-5 mg/egg) into the yolks of four-day-old chicken, chukar, pheasant and northern bobwhite eggs resulted in embryo deformities including twisted necks and shortened/indented backs in all species (Abbott 1972, Schom *et al.* 1973).

4.6.3 Terrestrial Invertebrates

In a field study of a Dursban-treated agricultural plot (22.4 kg/ha), Thompson (1971) did not observe any treatment-related effect on the numbers or biomass of earthworms (*Lumbricus terrestris*). However, exposure of earthworms (*Eisenia veneta* and *Lumbricus rubellus*) to chlorpyrifos at sublethal levels for 14 d (121 and 9.5 mg/kg soil respectively) resulted in a 50 percent reduction in the number of cocoons produced (Ma and Bodt 1993).

4.6.4 Aquatic Biota

Early life stage and full life cycle studies have documented the chronic toxicity of chlorpyrifos to fish. Growth is generally the most sensitive endpoint in chronic toxicity fish studies. Other reported effects of chronic chlorpyrifos exposure to fish are behavioural avoidance, reduced feeding, changes in temperature preferences, and reduced brain acetyl-ChE (Barron and Woodburn 1995). A laboratory life-cycle chronic study saw first generation growth, mortality, or crippling effects in fathead minnows at concentrations greater than 1.21 ug/L, and reproductive effects (delayed sexual maturation, reduced egg production, fewer eggs per spawn) at 0.63 ug/L, as well as slight but significant growth effects on second-generation minnows at 0.12 ug/L (Jarvinen *et al.* 1983). Eaton *et al.* (1985) studied the effect of continuous exposure to chlorpyrifos (0.22 and 1.01 ug/L) versus pulsed exposure (3.1 and 11.5 ug/L for 24 h biweekly) over a period of 100 d. They reported no apparent adverse effects on fish survival, growth, or reproduction in fathead minnows, bluegill sunfish or white suckers continuously exposed, but crippling of fathead minnows and reversible acute toxicity symptoms (lethargy, tetany when startled) in blue-gills in the pulse-dosed stream (Eaton *et al.* 1985). Fish survived, reproduced and grew equally well in both streams (Eaton *et al.* 1985).

Eaton *et al.* (1985) also examined benthic invertebrates and observed major changes in the dominant species and a decline in the diversity of macroinvertebrate communities in both treated streams. There was a shift from amphipods to isopods and a reduction in populations of chironimids in both streams. Mesocosm studies with chlorpyrifos show population reductions of cladocerans, some copepod and chironomid species, population increases of rotifers and reduced growth of sunfish and fathead minnows (Racke 1993). Exposure of adult and juvenile snails (*Biomphalaria alexandrina*) to chlorpyrifos resulted in reduced egg production of 55 percent and 65 percent respectively at 0.25 ppm accompanied by reduced hatchability (Ibrahim *et al.* 1992). Planaria (*Dugesia dorotocephala*) exposed to concentrations greater than 0.75 mg/L chlorpyrifos exhibited behavioural effects such as coiling and ornamentation accompanied by unconsciousness (Villar *et al.* 1993). Chlorpyrifos also delayed head re-growth and resulted in eye and head abnormalities in planaria exposed to chlorpyrifos concentrations greater than 1.7 mg/L (Villar *et al.* 1993). The NOEC for mortality and reproduction in a 21-d chronic test of *Daphnia magna* was 0.1 ug/L (Kersting and vanWijngaarden 1992).

4.7 ENDOCRINE DISRUPTION POTENTIAL

Our working definition of an endocrine disrupting compound is:

> *An exogenous agent that directly interferes with the synthesis, secretion, transport, binding action, or elimination of endogenous hormones and neurohormones, resulting in physiological manifestations of the neuroendocrine, reproductive or immune systems in an intact organism.*

The previous chronic studies detailed reproductive effects in mallards fed 80 mg/kg chlorpyrifos, which falls within the range of LD50s for this species, and deformities from eggs immersed at environmentally irrelevant concentrations (7,600 mg/kg). There were no reproductive effects seen in fish studies, and mammalian studies in mice and rats found no evidence of embryolethality, teratogenicity or fetotoxicity. However, studies have shown chlorpyrifos to be a developmental neurotoxin that can subsequently affect axonogenesis, synaptic development and apoptosis. Chronic neural damage can mediate other effects in an organism through the endocrine pathways.

Based on the fact that chlorpyrifos is a developmental neurotoxin, and decreases and alters serum concentrations of thyroxine and cortisol, there is sufficient evidence to classify chlorpyrifos as an endocrine disrupting compound.

4.8 RISK TO ONTARIO ENVIRONMENTS AND RECOMMENDATIONS

Chlorpyrifos is not commonly detected in Ontario, particularly in urban watersheds, but those samples with chlorpyrifos detections have had concentrations up to 0.148 ug/L, which far exceeds the Canadian Water Quality Guideline for the protection of aquatic life of 0.0035 ug/L. Based on these levels of chlorpyrifos in Ontario, and the fact that chlorpyrifos is a developmental neurotoxin at low levels, there is a possible risk to some aquatic species, particularly those invertebrates with LD50s below one ug/L.

Mixtures of pesticides also pose a risk to wildlife. Other organophosphates are known to co-occur with chlorpyrifos and additive toxicity of chlorpyrifos and diazinon has been observed in *Ceriodaphnia dubia* (Giesy *et al.* 1999). Atrazine paired with chlorpyrifos has shown greater than additive toxicity in a study of 4th instar larvae of the aquatic midge (*C. tentans*) (Pape-Lindstrom and Lydy 1997), and a study by Jett *et al.* (1999) found that four common PAHs (pyrene, benzo(a)pyrene, anthracene, fluoranthene), together with chlorpyrifos, inhibited AChE in an additive manner.

The following issues should be priorities for further evaluation of the extent and degree of risk posed by chlorpyrifos:

- more precise use information to narrow the geographical focus for monitoring in high-risk counties
- further study on the ecological effects of mixtures of organophosphate pesticides and other pesticides (such as atrazine) on the various components of natural ecosystems
- further study of acute and chronic exposure to chlorpyrifos to evaluate the cellular, synaptic and behavioural consequences of low-level exposures
- further study on the potential for endocrine disruption in wildlife to chronic chlorpyrifos exposure.

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APPENDIX 1: GLOSSARY OF TERMS

Active ingredient – in a pesticide formulation, the chemical with biocidal properties, intended as the pest-targeting agent.

Acute – having a sudden onset, lasting a short time; of a stimulus, severe enough to induce a response rapidly.

Additivity – referring to the toxicity of a mixture of chemicals, which is approximately equal to a simple summation of known toxicities of individual elements of the mixture.

Antagonism – referring to the toxicity of a mixture of chemicals, which is less than a summation of known toxicities of individual elements of the mixture.

Autocrine – cellular communication in an organism in which cells secrete agents that have specific actions on the secreting cell, alone.

Bioaccumulation – process by which chemicals are taken up by organisms

Bioaccumulation factor – a value that is the ratio of tissue chemical residue to chemical concentration in an external environmental phase such as food or soil/sediment.

Bioconcentration – process by which there is a net accumulation of chemical from external environments into an organism; uptake greater than elimination.

Biomagnification – the trophic result of bioaccumulation and bioconcentration processes; in which tissue concentrations of chemicals increase as the chemical passes up through two or more trophic levels in a food chain.

Chronic – involving a stimulus that is lingering or continues for a long time.

Endocrine disrupter – an exogenous agent that interferes with the synthesis, secretion, transport, binding action or elimination of natural hormones in the body.

Endocrine system – the communication system in an organism that involves carrying messenger molecules in the bloodstream to distant target tissues; sister to autocrine and paracrine systems.

Estrogen – a family of female sex steroids responsible for producing estrus and the female secondary sex characteristics, and preparing the reproductive system for fertilization and implantation of the ovum.

Estrogenic – a chemical having qualities that allow it to function as estrogen in a body.

Formulation – the commercial form of a pesticide; includes active ingredient as well as inert ingredients (surfactants, process impurities, etc.).

Growth regulators – a chemical/hormone that directly influences metabolism of an organism and regulates growth rate.

Hormone – a chemical compound synthesized and secreted by an endocrine tissue into the bloodstream; influences the activity of a target tissue.

Inert ingredient – those chemical(s) in a pesticide formulation that are thought to have no adverse biocidal properties, intended as solubility agents etc. or with other proprietary functions.

In vitro – in an artificial environment outside the body; often used to describe toxicity tests performed using isolated cell cultures.

In vivo – within the living organism or tissue.

Mechanism of action – the pathway by which a toxicant produces an effect in an organism.

Neuroendocrine system – a major form of communication within the body, in which nerve cells release messenger molecules (hormones) into the bloodstream for transport to a distant target tissue.

Organochlorine – a family of chemicals that includes all chlorinated hydrocarbons.

Paracrine – cellular communication in an organism in which cells secrete agents that influence neighbouring cells.

Pesticide – a substance used to kill undesirable ... fungi, plants, insects, or other organisms ... [a] generic term ... used to describe fungicides, algaecides, herbicides, insecticides, rodenticides and other substances.

Surfactant – a surface–active substance that tends to reduce surface tension; used to describe synthetic and natural detergents added to pesticides to increase the solubility of the active ingredient.

Synergism – referring to the toxicity of a mixture of chemicals, which is greater than a summation of known toxicities of individual elements of the mixture.

Target site – the tissue or cell or receptor that a toxicant acts upon to produce a response in the organism.

Testosterone – a steroid androgen (hormone having masculinizing activity) synthesized by the testicular interstitial cells of the male, and responsible for the production and maintenance of male secondary sex characteristics.

Thyroid gland – an endocrine gland responsible for regulation of energy metabolism; the two major thyroid hormones are thyroxine and 3,5,3-triiodothyronine.

Toxicant – an agent capable of producing an adverse response (effect) in a biological system.

Toxicity – the inherent potential of a toxicant to cause adverse effects in a living organism when the organism is exposed to it.

APPENDIX 2: GLOSSARY OF ACRONYMS

ai – Active Ingredient

Ach – Acetylcholine

AChE – Acetylcholinesterase

AhR – Aryl Hydrocarbon Receptor

ATP – Adenosine Triphosphate

AT – Androgen Receptor

BCF – Bioconcentration Factor

CAS – Chemical Abstracts Number

ChE – Cholinesterase

CNS – Central Nervous System

DIDT – 5,6-dihydro-3h-imidazo(2,1-c)-1,2,4-dithiazole-3-thion

DNA – Deoxyribonucleic Acid

DT50 – Time until 50 percent disappearance of a compound from a media

EAC – Endocrine Active Chemical

EBDC – Ethylene Bisdithiocarbamate

EBIS – Ethylene Bisisothiocyanate Sulfide

EC – Emulsifiable Concentrate

EC50 – Effective concentration causing a response in 50 percent of a test population

ED50 – Effective Dose causing a response in 50 percent of a test population

EDA – Ethylene Diamine

EDC – Endocrine Disrupting Compound

EEC – Expected Environmental Concentration

ER – Estrogen Receptor

ETU – Ethylene Thiourea

EU – Ethylene Urea

FSH – Follicle Stimulating Hormone

GABA – Gamma-amino Butyric Acid

hER – human Estrogen Receptor

K_{ow} – Octanol/Water Partition Coefficient

LC50 – Concentration (In Water) Causing 50 percent Mortality

LD50 – Oral dose causing 50 percent mortality

LH – Luteinizing Hormone

LHRH – Luteinizing Hormone Releasing Hormone

LOEC – Lowest Observable Effect Concentration

MATC – Maximum Allowable Toxicant Concentration

MOE – Ministry of the Environment

NAS – National Academy of Sciences

NOAEC – No Observable Adverse Effect Concentration

NOEC – No Observable Effect Concentration

OMAFRA – Ontario Ministry of Agriculture, Food and Rural Affairs

OP – Organophosphorus Pesticide

PAH – Polycyclic Aromatic Hydrocarbon

PCB – Poly Chlorinated Biphenyl

PETD – Polymeric Ethylenethiuram Disulfide

PR – Progesterone Receptor

TBT – Tributyltin

TRH – Thyroid-Releasing Hormone

TSH – Thyroid-Stimulating Hormone

US EPA – United States Environmental Protection Agency

WHO – World Health Organization

WP – Wettable Powder

APPENDIX 3: LIST OF COMMON AND LATIN NAMES OF VERTEBRATE SPECIES

Fish:
American eel: *Anguilla rostrata*
Banded killifish: *Fundulus diaphanus*
Bluegill sunfish: *Lepomis macrochirus*
Channel catfish: *Ictalurus punctatus*
Common bleak: *Alburnus alburnus*
Common carp: *Cyprinus carpio*
Common jollytail: *Galaxias maculatus*
Cutthroat trout: *Oncorhynchus clarki*
Dolly Varden: *Salvelinus malma*
Fathead minnow: *Pimephales promelas*
Golden galaxias: *Galaxias auratus*
Goldfish: *Carassius auratus*
Guppy: *Libistes reticulatus*
Japanese medaka: *Oryzias latipes*
Lake trout: *Salvelinus namaycush*
Mosquito fish: *Gambusia affinis*
Mozambique tilapia: *Oreochromis mossambica*
Nile tilapia: *Oreochromis niloticus*
Pink salmon: *Oncorhynchus gorbuscha*
Pond loach: *Misgurnus anguilicaudatus*
Pumpkinseed sunfish: *Lepomis gibbosus*
Rainbow trout: *Oncorhynchus mykiss*
Redear sunfish: *Lepomis microlophus*
Salmon, chinook: *Oncorhynchus kisutch*
Salmon, chum: *Oncorhynchus keta*
Salmon, coho: *Oncorhynchus kisutch*
Salmon, sockeye: *Oncorhynchus nerka*
Spiny dogfish shark: *Squalus acanthias*
Spotted galaxias: *Galaxias truttaceus*
Spotted snakeheads: *Channa punctata*
Steelhead-rainbow trout: *Salmo gairdneri*
Striped bass: *Marone saxatilis*
Threespine stickleback: *Gasterosteus aculeatus*
White perch: *Roccus americanus*
White sucker: *Catostomus commersoni*
Willow shiner: *Gnathopogon elongatus caerulescens*
Winter flounder: *Pseudopleuronectes americanus*

Amphibians:
African Clawed Frog: *Xenopus laevis*
American toad: *Bufo americanus*
Bullfrog: *Rana catesbeiana*
Crested newt: *Triturus cristatus*
Common frog: *Rana temporaria*
Indian toad: *Bufo melanostictus*
Japanese common toad: *Bufo bufo japonicus*
Leopard frog: *Rana pipiens*
Smooth newt: *Triturus vulgaris*
Tiger frog: *Rana tigrina*

Reptiles:
American alligator: *Alligator mississippiensis*

Birds:
American robin: *Turdus migratorius*
Chicken: *Gallus domesticus*
Chukar: *Alectoris chukar*
Common grackle: *Quiscalus quiscula*
European starling: *Sturnus vulgaris*
House sparrow: *Passer domesticus*
Japanese quail: *Coturnix coturnix japonica*
Mallard duck: *Anas platyrhynchos*
Northern bobwhite: *Colinus virginianus*
Red-winged blackbird: *Agelaius phoeniceus*
Ringed turtledove: *Streptopelia risoria*
Ring-necked pheasant: *Phasianus colchicus*
Rock dove: *Columba livia*

Mammals:
Rodents
Chinese hamster: *Cricetus Griseus*
Guinea pig: *Cavia porcellus*
House mouse: *Mus musculus*
Laboratory strains:
CD-1 mice: *Mus musculus*
Norway rat: *Rattus norvegicus*
Laboratory strains:
Dow-Wistar rat: *Rattus norvegicus*
Fisher 344 rat: *Rattus norvegicus*
Sherman rat: *Rattus norvegicus*
Sprague-Dawley (SD) rat: *Rattus norvegicus*
Non-rodents
Domestic dog: *Canis lupus familiaris*
Domestic sheep: *Ovis aries*
Holstein cow: *Bos taurus*
New Zealand rabbit: *Oryctolagus cuniculus*
Rhesus monkey: *Macaca mulatta*