Pesticides In Ontario: A Critical Assessment Of Potential Toxicity Of Agricultural Products To Wildlife, With Consideration For Endocrine Disruption.

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Canadian Wildlife Service 2000
Environmental Conservation Branch
Ontario Region

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EXECUTIVE SUMMARY

This report 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 growing evidence that many environmental pollutants have mechanisms of action which directly or indirectly disrupt/modulate the endocrine system. Such pollutants have been termed **endocrine disrupting chemicals (EDC);** 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 use estimates. The report is based on published information from the period 1978 to 1998, with additional review literature published in 1999. Compounds were chosen for review based on comparative use and toxicity class information. Only those compounds whose estimated use within Ontario exceeded 10,000 kg were evaluated. Review results of the selected compounds will be reported in several volumes. Volume one includes the assessments of eight compounds within five pesticide familes: 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. Later volumes will examine the triazine herbicides, glyphosate, and metalochlor, as well as 2,4-dichlorophenoxyacetic acid (2,4-D) and other pesticides in extensive use in the urban landscape.

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.

Endosulfan:

- Surface waters in southern Ontario periodically contain concentrations of endosulfan that are lethal to fish.
- The possibility of fish and amphibian mortality in wetlands adjacent to sprayed fields is a serious environmental concern.
- There is potential for endocrine-modulated effects because endosulfan is an extremely potent neurotoxin and has moderately high bioaccumulative capacity.

Ethylenebisdithiocarbamate fungicides (EBDCs):

- Fish and amphibian studies using indigenous species describe chronic effects of EBDC exposure at environmentally relevant concentrations that are at or below currently applied analytical detection limits.
- Given the rapid degradation of the parent compounds and the substantial toxicity associated with several EBDC metabolites, the greatest environmental concern for this pesticide class likely lies with the rarely monitored breakdown products.
- There is insufficient evidence to confirm endocrine disruption in wildlife exposed to a principal breakdown product, ethylene thiourea (ETU) which produces high chronic toxicity in laboratory animals. Investigations have been hindered by the apparent species-specific nature of detoxification pathways.

Dinitroanilines:

- Existing information on environmental concentrations of trifluralin and pendimethalin suggest that neither compound is present in Ontario at levels capable of inducing acute or chronic effects in wildlife.
- Research is insufficient to confirm the reported endocrine disrupting capacity of trifluralin.

Dichloropropene:

- Inhalation of this compound may cause toxicity in wildlife inhabiting terrestrial ecosystems near areas of recent use.
- The literature does not indicate that dichloropropene acts as an endocrine disrupter, but few studies examined relevant endpoints.

Azinphos-methyl:

- Azinphos-methyl is frequently detected in surface water at concentrations that are acutely toxicity to aquatic wildlife. However, if azinphos-methyl is applied properly, away from open water, and measures are taken to decrease the amount entering the surface water through runoff, the acute risk to aquatic ecosystems can be diminished.
- Organophosphorus insecticides (OPs) have been shown to cause inhibition of cholinesterase activity in Ontario wildlife.
- There is evidence that OPs can affect reproduction and the endocrine system in birds but more research is needed to confirm these effects in the field.

Pesticide Mixtures:

- The practice of mixing pesticides during manufacturing and field application is common, yet the toxicological properties of such mixtures are generally unknown.

Priorities for further research:

1. More exposure data are critical for the accuracy of provincial risk assessments. Well designed monitoring of the environmental concentrations of these pesticides (water, sediment, soil, and animal tissues) must precede further assessments (i.e. keeping use patterns and chemical mixtures in mind).
2. Efforts should be made to investigate associated toxicity in indigenous species, particularly in those taxa that have traditionally been poorly represented (e.g. amphibians, reptiles, wild birds) with special attention paid to most sensitive life stages.
3. Endosulfan is a chemical of concern. Its use must be better documented, especially in the agricultural sectors that have not been well monitored to date (e.g. greenhouse production). Acute and chronic effects studies using indigenous species and environmentally-relevant exposures should be conducted.
4. Analytical chemistry methods used to detect EBDCs must be modified to improve detection limits to environmentally relevant concentrations. Further assessments should also include the primary toxic metabolites.
5. A number of field and laboratory inhalation toxicity studies on indigenous amphibian, reptile and small mammal species should be conducted.
6. Azinphos-methyl is a chemical of concern. Lethal concentrations occur in the environment. The endocrine-disruption effects found in wild birds need further evaluation to determine long-term impacts of OPs alone or in mixtures.
7. Toxicity assessments of pesticide mixtures must be initiated, and combinations should reflect those most likely to occur in the environment (outlined in Table 7.2).

SOMMAIRE EXÉCUTIF

Ce rapport fournit une revue en profondeur des concentrations dans l'environnement, de la toxicité environnementale, et du potentiel de perturbation endocrinienne de certains pesticides utilisés en Ontario. Nous avions pour objectif d'identifier le potentiel d'effets nocifs pour la faune, à des concentrations pouvant être rencontrées dans l'environnement, des composés largement utilisés dans les paysages agricoles et urbains.

On a de plus en plus d'indications que nombre de polluants environnementaux ont des mécanismes d'action qui peuvent, directement ou indirectement, perturber ou moduler le système endocrinien. Ces polluants ont été nommée **perturbateurs endocriniens**; aux fins de la présente évaluation, la définition de travail d'un perturbateur endocrinien est la suivante :

> *Un agent exogène qui interfère directement avec la synthèse, la sécrétion, le transport, la fixation ou l'élimination d'hormones et neurohormones endogènes, entraînant des manifestations physiologiques des systèmes neuro-endocrinien, reproducteur ou immunitaire dans un organisme intact.*

Des pesticides ou familles de pesticides ont été choisis à partir des estimations de 1993 et 1998 de leur utilisation agricole en Ontario. Ce rapport repose sur de l'information publiée entre 1978 et 1998, ainsi que sur une revue supplémentaire de la littérature publiée en 1999. Ces composés ont été retenus sur la base d'informations comparatives sur l'utilisation et la classe de toxicité. Seuls ceux dont l'utilisation estimative sur le territoire de l'Ontario dépassait 10 000 kg ont été évalués. Les résultats des revues sur les composés examinés seront publiés en plusieurs volumes. Le premier inclut les évaluations de huit composés de cinq familles de pesticides : un insecticide organochloré (endosulfan), trois fongicides à base d'éthylènebisdithiocarbamate (manèbe, mancozèbe, métirame), deux herbicides à base de dinitroaniline (trifluraline, pendiméthaline), un nématocide hydrocarboné halogéné (1,3-dichloropropène), et un insecticide organophosphoré (azinphos-méthyl). On a aussi étudié des mélanges de pesticides. Des volumes ultérieurs porteront sur les herbicides à base de triazine, de glyphosate et de métalochlore, ainsi que sur l'acide 2,4-dichlorophénoxyacétique (2,4-D) et d'autres pesticides largement utilisés en milieu urbain.

Les profils des pesticides ou familles de pesticides incluent les éléments suivants: une courte description des ingrédients actifs; les modalités d'utilisation en Ontario; l'occurrence dans les milieux naturels environnants; la toxicité aigüe et chronique, et le potentiel de perturbation endocrinienne associés à ces composés; une évaluation du risque pour la faune; et des recommandations en vue de travaux supplémentaires de recherche et de surveillance.

Endosulfan:

- Les eaux de surface du sud de l'Ontario contiennent périodiquement des concentrations d'endosulfan qui sont léthales pour le poisson.
- La possibilité de mortalité des poissons et amphibiens dans les milieux humides proches des champs traités est un grave problème environnemental.
- Il y a un risque de modulation du système endocrinien parce que l'endosulfan est une neurotoxine extrêmement puissante et a une capacité de bioaccumulation modérément élevée.

Fongicides à base d'éthylènebisdithiocarbamate (EBDC):

- Des études menées sur des espèces indigènes de poissons et d'amphibiens ont permis de décrire les effets chroniques de l'exposition au EBDC à des concentrations, susceptibles d'être rencontrées dans l'environnement, qui sont égales ou inférieures aux limites de détection analytique présentement appliquées.
- Étant donné la dégradation rapide des composés parents et la toxicité marquée de plusieurs métabolites de l'EBDC, le plus grand risque environnemental posé par cette classe de pesticides est probablement lié aux produits de dégradation rarement surveillés.

- On ne dispose pas encore de suffisamment d'indications pour confirmer la perturbation endocrinienne chez la faune exposée à un produit principal de dégradation, l'éthylènethiourée (ETU), qui a une forte toxicité chronique chez les animaux de laboratoire. Les études ont été entravées par la nature apparemment propre à l'espèce des voies de détoxification.

Dinitroanilines:

- Les informations dont on dispose actuellement sur les concentrations environnementales de trifluraline et de pendiméthaline suggèrent que ni l'un ni l'autre de ces composés n'est présent en Ontario à des niveaux susceptibles d'induire des effets aigüs ou chroniques chez la faune.
- Les recherches ne sont pas suffisantes pour confirmer la capacité de perturbation endocrinienne attribuée à la trifluraline.

Dichloropropène:

- L'inhalation de ce composé peut être toxique pour la faune habitant des écosystèmes terrestres situés à proximité d'une zone où le composé a été récemment utilisé.
- La littérature n'indique pas que le dichloropropène ait une action de perturbateur endocrinien, mais peu d'études ont examiné des paramètres pertinents à mesurer.

Azinphos-méthyl:

- L'azinphos-méthyle est souvent détecté dans les eaux de surface à des concentrations présentant une toxicité aigüe pour la faune aquatique. Cependant, si l'azinphos-méthyl est appliqué correctement, à distance de l'eau libre, et que des mesures sont prises pour abaisser la quantité atteignant les eaux de surface via le ruissèllement, le risque aigü pour les écosystèmes aquatiques peut être réduit.
- Il a été montré que les insecticides organophosphorés (OP) causaient une inhibition de l'activité de la cholinestérase chez la faune ontarienne.
- Il y a des indications que les OP peuvent affecter la reproduction et le système endocrinien chez les oiseaux, mais il faudra effectuer d'autres recherches pour confirmer ces effets *in situ*.

Mélanges de pesticides:

- Il est courant que des pesticides soient mélangés aux stades de la fabrication et de l'application au champ, mais les propriétés toxicologiques de ces mélanges sont généralement inconnues.

Priorités des recherches supplémentaires:

1. La précision des évaluations du risque dans la province exigera que l'on dispose de plus de données sur l'exposition. Toute évaluation ultérieure (c.-à-d. en gardant à l'esprit les modalités d'utilisation et les mélanges de substances chimiques) devra être précédée d'une surveillance bien conçue des concentrations environnementales de ces pesticides (eau, sédiments, sol, tissus animaux).
2. Il faudrait s'attacher à étudier la toxicité associée chez les espèces indigènes, surtout chez les taxons qui sont d'habitude mal représentés (p. ex. les amphibiens, les reptiles, les oiseaux sauvages), et en accordant une attention particulière aux stades biologiques les plus vulnérables.
3. L'endosulfan est une substance chimique préoccupante. Il convient de mieux documenter son utilisation, surtout dans les secteurs de l'agriculture qui n'ont pas encore fait l'objet d'une surveillance poussée (p. ex. la production en serre). Il faudrait mener des études des effets aigüs et chroniques faisant intervenir des espèces indigènes et des expositions susceptibles d'être rencontrées dans l'environnement.
4. Les méthodes de chimie analytique utilisées pour détecter les EBDC doivent être modifiées pour abaisser les limites de détection aux concentrations possibles dans l'environnement. Les évaluations supplémentaires doivent aussi inclure les métabolites primaires toxiques.

5. Il faudrait aussi effectuer un certain nombre d'étude de toxicité par inhalation *in situ* et en laboratoire sur des espèces indigènes d'amphibiens, de reptiles et de petits mammifères.
6. L'azinphos-méthyle est une substance chimique préoccupante. Des concentrations léthales sont présentes dans l'environnement. Les effets de perturbation endocrinienne observés chez les oiseaux sauvages doivent être mieux évalués afin de pouvoir déterminer les impacts à long terme des OP, seuls ou en mélanges.
7. Des évaluations de la toxicité des mélanges de pesticides doivent être lancées, et les combinaisons devraient refléter celles qui sont le plus susceptibles d'être rencontrées dans l'environnement (présentés au tableau 7.2).

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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, and, based on findings, 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 in-use pesticides. There has been a growing concern of contaminant-induced reproductive effects in wildlife and humans resident in the Great Lakes region [1-4].

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 had only materialized in the early 1990s. Previous to 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 [5,6,7], declining sperm counts in humans [8,9], induction of female specific protein production in male fish and the lack of gonad development in both sexes [10,11,12], masculinization of marine snails [13], as well as endocrine toxicity and vaginal cancer in the daughters of diethylstilbestrol (DES) treated women [14,15].

The heightened interest in endocrine related effects in recent years is evidenced by the vast number of articles and reviews published about various aspects of endocrine disruption (see [10,12,16-31]) and the numerous meetings and workshops that have been held [32,33].

In the US, congress passed the Food Quality Protection Act (1996) and the Safe Drinking Water Act (1996) which mandated the US 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 (by 1999) [27,8]. A more extensive exploration of environmental endocrine disruption will be completed by the National Academy of Science.

The US EPA's Science Policy Council's interim position [27] 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*".

Although the above US legislation focus primarily on human health consequences of endocrine active chemicals, the US EPA has included wildlife health as an endpoint of concern. In this context, wildlife includes vertebrate and invertebrate species [12].

1.2.1 Definitions and Terms of Endocrine Disruption

The US EPA [27] 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.

Also, 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 US EPA [27], 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.

As such, 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 behavior, female and male reproductive system development and function and thyroid function.

The European community [33] defines an EDC 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 (EACs), background information on endocrine physiology in the current context is provided by several recent reviews [25,14,9,21]. The modes of action of EACs can be categorized into five groups [14]; 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 [16].

The plasticizer diethylhydroxylphthalate, alters xenobiotic metabolism, activates the peroxisome proliferation activated receptor and affects testicular follicle stimulating hormone (FSH) signal transduction.

The estrogen and androgen receptors are nuclear receptors and 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 [19]. This results in mRNA production followed by protein expression.

Estrogen mimics act intra cellularly via three main mechanisms [19]:

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 [27,16] while others may be estrogenic or antiestogenic depending on the cellular environment [19]. Other EDCs act on the aryl hydrocarbon receptor (AhR) including dioxins and PCBs [10].

Non-receptor mediated mechanisms of action of EDCs are exemplified by the phytoestrogen -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 US EPA [27] 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 taxa/species specific endocrine function. The developing fetus is extremely sensitive to the hormonal environment in the uterus, for example, natural differences in hormone levels surrounding rat or mouse fetuses of only 10^{-12} M influence the timing of sexual development and the behavior of the animal in adult life [10].

Similarly for fish, exposure to estrogen mimics during a narrow window spanning just 10 days 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 [10]. In birds, sexual differentiation is estrogen dependent but this is not the case in mammals. Consequently, birds may be far 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 some integrated pest management programs have been implemented to reduce their use. Many green areas in urban environments are maintained with herbicides, and even the aquatic realm is 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 [35]. Since then, pesticides have gone through several transitions: from primarily inorganic and organometallic formulations to the organochlorine insecticide, triazine herbicides, organophosphorous, carbamate, and later, pyrethroid insecticides, and, more recently to sulfonyl urea 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, 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 acreages of each crop type per county as attained through OMAFRA's annual statistical crop surveys [36] to extrapolate county-wide estimates of pesticide use. The two most recent surveys conducted were in 1993 [37] and 1998 [38]. In 1993, pesticide use estimates were based on 1800 farm surveys out of a provincial total of 61,432 farms (2.9 %) whereas in 1998, only 1200 farms responded. Rapidly growing segments of the agricultural industry like 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 [37] estimated a total pesticide use of 6,246,442 kg, representing a reduction in the volume of pesticide use by 16.5%. The 1993 and 1998 surveys show a general decline in pesticide use from earlier surveys. However, some compounds, including glyphosate and metolachlor have increased overall through time, and markedly since 1993.

Newer, high efficacy pesticides are being used more widely. These are applied at grams per ha instead of kg per ha, which is reflected in the reduced total volume of pesticides used. For example, the sulfonyl urea and imidazolinone herbicides have acute phytotoxicity to non-target organisms at concentrations below the limits of analytical chemical detection [39,40]. The inability to detect these compounds in environmental samples is just one of the many factors that makes enviromental risk assessment very challenging.

Pesticide use in urban areas is more poorly assessed than in agriculture. The Ontario Ministry of the Environment (MOE) surveys individuals receiving commercial pesticide applicators licenses every five years [37]. However, totals obtained through these surveys represent an unknown proportion of the total urban use of pesticides, given that most pesticides may be purchased and applied by unlicensed homeowners for which there are no sales records.

Historical monitoring by Frank and associates [41-49] 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 been focussed on pesticides in agricultural ecosystems of concern (ie: fruit, tobacco, muck crops, row crops) and the urban environment [50-56].

1.4 Assessment methods

A subset of the pesticides used in Ontario agriculture [37] was selected for critical evaluation in this report. Selection was based on comparative use patterns and toxicity class data (Table 1.1). Only those compounds whose estimated use exceeded 10,000 kg [37] were evaluated. 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 US EPA [27] was considered. With these use and toxicity guidelines, seven pesticides or pesticide families were selected for review in Volume 1. They are underlined in Table 1.1. Volume 2 will include the triazine herbicides, glyphosate and metalochlor.

Each chapter profiles a pesticide or family of pesticides that was heavily applied in Ontario in 1993 and/or showed 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.
- Occurrence in surrounding natural environments
- Associated acute and chronic toxicity, and a critical assessment of its 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 use pattern maps were derived using 1993 data from Hunter and McGee [37] for Ontario, and from Gianessi and Anderson [57] for US states in the Great Lakes Basin. Environmental concentration maps were primarily derived from information in the US EPA STORET water quality database. Concentrations for Ontario were obtained from datasets from the Ontario Ministry of Environment and Environment Canada. 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 - 1998). Information from a 1999 issue of *Toxicology and Industrial Health* [29, 30] focussing on pesticides was incorporated.

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 [58]. Spray recommendations published by OMAFRA for local fruit, vegetable, and flower (greenhouse) production [59-62] 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 [58].

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 criticised in the literature recently [63], since several of these components have been shown to disrupt the endocrine system, for example, 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

Table 1.1 A selection of agricultural pesticides used in ontario. Compounds that are underlined were selected for review in this publication (Volume 1) and those in italics will be included in subsequent volumes.

Active Ingredient	1993 Use (kg ai.)[a]	1998 Use (kg ai.)[a]	% Change 1993-98	Potential Endocrine--Disrupter[b]	Soil Half-life (days)[c]	Solubility (mg/L)[c]	Mammalian Toxicity[c,d]	Fish LC50 (mg/L)[c,e]
Metolachlor (H)	1 327 315	1 376 570	+4	✗	20	488	III	3.9
Atrazine (H)	589 852	598 206	+1	✔*	16-77	33	III	4.5-11
Glyphosate (H)	414 821	647 494	+56	✗	3-174	11 600	III	86
Dichloro-propene (N)	410 512[f]	177 000	-57	✗	2-17	2 000	III	3.9
Dicamba (H)	255 528	205 522	-20	✗	<14	6 500	III	135
Metribuzin (H)	254 276	71 761	-72	✔	-	1050	III	76
2,4-D[g] (H)	222 746	145 720	-35	✔*	<7	311	II	>100
Cyanazine (H)	215 480	49 038	-77	✔*	14	171	II	16[h]
MCPA (H)	161 605[i]	119 700	-26	✗	<7	734	III	232
Mancozeb (F)	155 463	156 269	+1	✔	6-15	6.2	III	2.2
Captan (F)	151 468	101 276	-33	✗	1	3.3	III	0.072[j]
Chlorothalonil (F)	115 613	120 751	+5	✔*	5-36	0.81	III	0.049
EPTC[k] (H)	113 030	46 312	-59	✗	-	375	II	19
Trifluralin (H)	83 945	23 250	-72	✔	57-126	0.184	III	0.01-0.04
Sulphur (F)	72 338	55 670	-23	✗	-	insoluble	III	non-toxic
Azinphos-methyl (I)	71 983	14 120	-80	✗	weeks	28	I[b]	0.02
Metiram (F)	57 230	131 113	+129	✔	-	insoluble	III	1.1
Pendimethalin (H)	51 414	115 687	+125	✔[t]	90-120	0.3	III	0.14
Maneb (F)	49 440	1 873	-96	✔	25	insoluble	III	1.8l
Bromoxynil (H)	45 317	58 854	+30	✔*	10	130	II	0.46[m]
Terbufos (I)	38 282	1 297	-97	✗	9-27	4.5	I[a]	0.01
Endosulfan (I)	25 930	6 909	-73	✔	30-70	0.32	II	0.002[n]
Carbaryl (I)	16 882	15 334	-9	✔	7-28	120	II	1.3
Carbofuran (I)	15 213	2 652	-83	✗	30-60	320-350	I[b]	22-29
Cypermethrin (I)	12 780	6 310	-51	✔	5	0.004	II	0.001

[a] Use estimate based on 1800 farm surveys (1993) [37] and 1200 surveys (1998) [38]. [b] Potential for endocrine-disruption as per the US EPA [27] and the World Wildlife Fund (http://www.wwfcanada.org/hormone-disruptors/); a * signifies that it was only listed by WWF, while a [t] signifies that it was only listed by the US EPA. [c] source: Tomlin [69]; [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-hr 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-hr LC_{50}. [i] the sum of MCPA ([4-chloro-2-methylphenoxy]acetic acid) and MCPA/MCPB. [j] Bluegill sunfish 96-hr LC_{50}. [k] EPTC = dipropylcarbamothioic acid S-ethyl ester = dipropylthiocarbamic acid S-ethyl ester. [l] Carp 96-hr LC_{50}. [m] Goldfish 48-hr LC_{50}. [n] Golden orfe 96-hr LC_{50}. (H)-herbicide, (I)-insecticide, (F)-fungicide, (N)-nematocide, (-)-not available.

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 will likely be present in small quantities in the commercial formulation [1, 63].

Also, 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 [27].

In addition to these factors that complicate assessments of toxicity, there are others that complicate assessments of exposure. Water concentrations are generally a poor surrogate for 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 [64-68]. These chemicals include DDT, mirex, dieldrin, and polychlorinated biphenyls (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 which are most frequently discussed as potential or known endocrine-disrupters [1,27].

1.6 References

1. Foster, W.G. 1995. The reproductive toxicology of Great Lakes contaminants. Environ. Health Perspect. 103 (Suppl. 9):63-69.
2. Fry, D.M. 1995. Reproductive effects in birds exposed to pesticides and industrial chemicals. Environ. Health Perspect. 103 (Suppl. 7):165-171.
3. Hose, J.E. and L.J. Guillette. 1995. Defining the role of pollutants in the disruption of reproduction in wildlife. Environ. Health Perspect. 103 (Suppl. 4):87-91.
4. Gilbertson, M. 1997. Advances in forensic toxicology for establishing causality between Great Lakes epizootics and specific persistent toxic chemicals. Environ. Toxicol. Chem. 16:1771-1778.
5. Vonier, P.M., D.A. Crain, J.A. McLachlan, L.J. Guillette Jr, and S.F. Arnold. 1996. Interactions of environmental chemicals with the estrogen and progesterone receptors from the oviduct of the American alligator. Environ. Health Perspect. 104:1318-1322.
6. Crain, D.A., L.J. Guillette Jr, A.A. Rooney, and D.B. Pickford. 1997. Alterations in steroidogenesis in alligators (*Alligator mississippiensis*) exposed naturally and experimentally to environmental contaminants. Environ. Health Perspect. 105:528-533.
7. Guillette, L.J. Jr. T.S. Gross, G.R. Masson, J.M. Marter, H.F. Percival and A.R. Woodward. 1994. Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. Environ. Health. Perspect. 102:680-688.
8. Cooper, R.L., and R.J. Kavlock. 1997. Endocrine disrupters and reproductive development: a weight-of-evidence overview. J. Endocrinol. 152:159-166.
9. Carlsen, E. A. Giwercman, N. Keiding, N.E. Skakkeback. 1992. Evidence for decreasing quality of semen during past 50 years. Fr. Med. J. 305:609-613.
10. Tyler, C.R., S. Jobling and J. P. Sumpter. 1998. Endocrine disruption in wildlife: a critical review of the evidence. Crit. Rev. Toxicol. 28:319-361.
11. Van Der Kraak, G., R. Munkittrick, M.E. McMaster and D.L. MacLatchy. 1998. A comparison of bleached Kraft mill effluent, 17b-estradiol and B-sitosterol effects on reproductive function in fish. In:

Principles and Processes for Evaluating Endocrine Disruption in Wildlife. Kendall R., R. Dickerson J. Giesy and W. Suk Eds. A technical publication of SETAC Press. Pp. 249-266.
12. Ankley, G., E. Mihaich, R. Stahl, D. Tillitt, et al. 1998. Overview of a workshop on screening methods for detecting potential (anti) estrogenic/androgenic chemicals in wildlife. Environ. Toxicol. Chem. 17:68-87.
13. Bryan, G.W., P.E. Gibbs and G.R. Burt. 1988. A comparison of the effectiveness of tri-n-butyltin chloride and five other organotin compounds in promoting the development of imposex in the dog-whelk, *Nucella lapilus.* J. Mar Biol. Assoc. UK 68:733-744.
14. Poskanzer, D. and A. Herbst. 1997. Epidemiology of vaginal adenosis and adenocarcinoma associated with exposure to stilbestrol *in utero*. Cancer 39:1892-1895.
15. Gill, W.B., F.B. Schumacher, M. Bibbo, F.H. Straus, and H.W. Schoenberg. Association of diethylstilbestrol exposure in utero with cryptorchidism, testicular hypoplasia and semen abnormalities. J. Urol. 122:36-39.
16. Barton, H.A., and M.E. Andersen. 1998. Endocrine active compounds: from biology to dose response assessment. Crit. Rev. Toxicol.. 28:363-423.
17. Chapin, R. E., J.T. Stevens, C.L. Hughes, W.R. Kelce, et al. 1996. Symposium overview: endocrine modulation of reproduction. Fund. Appl. Toxicol. 29:1-17.
18. Golden, R.J., K.L. Noller, L. Titus-Ernstoff, R.H. Kaufman, R. Mittendorf, et al. 1998. Environmental endocrine modulators and human health: an assessment of the biological evidence. Crit. Rev. Toxicol. 28:109-227.
19. Gillesby, B.E. and T.R. Zacharewski. 1998. Exoestrogens: mechanisms of action and strategies for identification and assessment. Environ. Toxicol. Chem. 17:3-14
20. Arcand-Hoy, L.D., and W.H. Benson. 1998. Fish reproduction: an ecologically relevant indicator of endocrine disruption. Environ. Toxicol. Chem. 17:49-57.
21. Safe, S.H. and K. Gaido. 1998. Phytoestrogens and anthropogenic estrogenic compounds. Environ. Toxicol. Chem. 17:119-126
22. Stahlschmidt-Allner, P., B. Allner, J. Rombke and T. Knacker. 1997. Endocrine disrupters in the aquatic environment. Environ. Sci. Pollut. 4:155-162.
23. Campbell, P.M. and T.H. Hutchinson. 1998. Wildlife and endocrine disrupters: requirements for hazard identification. Environ. Toxicol. Chem. 17:127-135.
24 Palmer, B.D., L.K. Huth, D.L. Pieto and K.W. Selcer. 1998. Vitellogenin as a biomarker for xenobiotic estrogens in an amphibian model system. Environ. Toxicol. Chem. 17:30-36.
25. Kime, D.E. 1999. A strategy for assessing the effects of xenobiotics on fish reproduction. Sci. Tot. Environ. 225:3-11.
26. Kendall R., R. Dickerson, J. Giesy, and W. Suk Eds. 1998. Principles and Processes for Evaluating Endocrine Disruption in Wildlife. A Technical Publication of SETAC Press.
27. U.S. EPA. 1997. Special report on environmental endocrine disruption: an effects assessment and analysis. Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. Report No. EPA/630/R-96/012.
28. Cooper, R.L., J.M. Goldman, and T.E. Stoker. 1999. Neuroendocrine and reproductive effects of contemporary-use pesticides. Toxicol. Indust. Health 15:26-36.
29. Porter, W.P., J.W. Jaeger, and I.H. Carlson. 1999. Endocrine, immune, and behavioral effects of aldicarb (carbamate), atrazine (triazine), and nitrate (fertilizer) mixtures at groundwater concentrations. Toxicol. Indust. Health 15:133-150.
30. Short, P. and T. Colborn. 1999. Pesticide use in the US and policy implications: a focus on herbicides. Toxicol. Indust. Health 15:240-275.
31. Ashby, J., E. Houthoff, S.J. Kennedy, J. Stevens, R. Bars, F.W. Jekat, P. Campbell, J. Van Miller, F.M. Carpanini, and G.L.P. Randall. 1997. The Challenge Posed by Endocrine-disrupting Chemicals. Environ. Health Perspect. 105:164-169.
32 Kavlock, R.J., G.P. Daston, C. DeRosa, P. Fenner-Crisp, et al. 1996. Research needs for the assessment of health and environmental effects of endocrine disrupters: a report of the US EPA sponsored

workshop. Environ. Health Perspect. 104:715-740.
33. Ankley, G.T., R.D. Johnson, F.Toth, L.C. Fomar, N.E. Detenbeck, S.P. Bradbury, et al. 1996. Development of a research strategy for assessing the ecological risk of endocrine disrupters. Rev. Environ. Toxicol. 1:231-267.
34. European Community. 1997. European workshop on the impact of endocrine disrupters on human health and wildlife. Workshop Publication EUR 17549. Paris, France.
35. Frank, R., H.E. Braun, and B.D. Ripley. 1989. Monitoring Ontario-grown apples for pest control chemicals used in their production, 1978-86. Food Addit. Contam. 6:227-234.
36. OMAFRA. 1994. 1993 agricultural statistics for Ontario. Ontario Ministry of Agriculture, Food and Rural Affairs, Publication No. 20. Statistical Services Unit, Policy Analysis Branch, OMAFRA, Toronto, ON, Canada.
37. Hunter, C. and B. McGee. 1994. Survey of pesticide use in Ontario, 1993. Estimates of Pesticides Used on Field Crops, Fruit and Vegetable Crops, Provincial Highway Roadsides, and by Licensed Pesticide Applicators. Ontario Ministry of Agriculture, Food and Rural Affairs, Economics Information Report No. 94-01. Policy Analysis Branch, OMAFRA, Toronto, ON, Canada.
38. Hunter, C., and B. McGee. 1999. Survey of pesticide use in Ontario agriculture, 1998. Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), Policy Analysis Branch. Guelph, Ontario, Canada.
39. Fletcher J.S., T.G. Pfleeger, H.C. Ratsch, and R. Hayes. 1996. Potential impact of low levels of chlorosulfuron and other herbicides on growth and yield of nontarget plants. Environ. Toxicol. Chem. 15:1189-1196.
40. Boutin, C., H.B. Lee, T.E. Peart, S.P. Batchelor, and R.J. Maquire. In press. Effect of the sulfonylurea herbicide metsulfuron methyl on growth and reproduction of five wetland and terrestrial species. Environ. Toxicol. Chem.
41. Braun, H.E. and R. Frank. 1980. Organochlorine and organophosphorus insecticides: their use in eleven agricultural watersheds and their loss to stream waters in southern Ontario, Canada, 1975-1977. Sci. Total Environ. 15:169-192.
42. Frank, R., G.J. Sirons, and B.D. Ripley. 1979. Herbicide contamination and decontamination of well waters in Ontario, Canada, 1969-78. Pestic. Monit. J. 13:120-127.
43. Frank, R., H.E. Braun, and M.V.H. Holdrinet. 1981. Residues from past uses of organochlorine insecticides and PCB in waters draining eleven agricultural watersheds in southern Ontario, Canada, 1975-1977. Sci. Tot. Environ. 20:255-276.
44. Frank, R., H.E. Braun, M.V.H. Holdrinet, G.J. Sirons, and B.K. Ripley. 1982. Agriculture and water quality in the Canadian Great Lakes Basin: V. Pesticide use in 11 agricultural watersheds in southern Ontario, Canada, 1975-1977. Sci. Tot. Environ. 20:255-276.
45. Frank, R., B.D. Ripley, H.E. Braun, B.S. Clegg, R. Johnston, and T.J. O'Neill. 1987a. Survey of farm wells for pesticide residues, southern Ontario, Canada, 1981-1982, 1984. Arch. Environ. Contam. Toxicol. 16:1-8.
46. Frank, R., B.S. Clegg, B.D. Ripley, and H.E. Braun. 1987b. Investigations of pesticide contaminations in rural wells, 1979-1984, Ontario, Canada. Arch. Environ. Contam. Toxicol. 16:9-22.
47. Frank, R., and L. Logan. 1988. Pesticide and industrial chemical residues at the mouth of the Grand, Saugeen and Thames Rivers, Ontario, Canada, 1981-85. Arch. Environ. Contam. Toxicol. 17:741-754.
48. Frank, R., H.E. Braun, B.D. Ripley, and B.S. Clegg. 1990. Contamination of rural ponds with pesticide, 1971-85, Ontario, Canada. Bull. Environ. Contam. Toxicol. 44:401-409.
49. Frank., R., L. Logan, and B.S. Clegg. 1991. Pesticide and polychlorinated biphenyl residues in waters at the mouth of the Grand, Saugeen and Thames Rivers, Ontario, Canada, 1986-1990. Arch. Environ. Contam. Toxicol. 21:585-595.
50. Bishop, C.A., N.A. Mahony, J. Struger, P. Ng., and K.E. Petit. 1999. Anuran development, density and diversity in relation to agricultural activity in the Holland River watershed, Ontario, Canada (1990-1992). Environ. Monit. Assess. 57:21-43.
51. Harris, M.L., C.A. Bishop, J. Struger, M.R. van den Heuvel, G.J. Van Der Kraak, D.G. Dixon, B.

Ripley, and J.P. Bogart. 1998. The functional integrity of northern leopard frog (*Rana pipiens*) and green frog (*Rana clamitans*) populations in orchard wetlands. I. Genetics, physiology, and biochemistry of breeding adults and young-of-the year. Environ. Toxicol. Chem. 17:1338-1350.

52. Struger, J., D. Boyter, Z. J. Licsko, and B.D. Johnson. 1994. Environmental concentrations of urban pesticides. In: Current Practices in Modeling the Management of Stormwater Impacts. CRC Press. W. James Ed., pp 85-98.
53. Merriman, J., J. Struger, and R.S. Szawiola. 1991. Distribution of 1,3-dichloroporpene and 1,2-dichloropropane in Big Creek watershed. Bull. Environ. Contam. Toxicol. 47:572-579.
54. Struger, J., B. Ripley, and G. Gris. 1999. Pesticide concentrations in urban aquatic environments. IAGLR Conference. Cleveland, Ohio. May 1999.
55. Struger J. Organophosphorus insecticides and endosulfan in surface waters of the Niagara fruit belt, Ontario, Canada. IAGLR Conference. Cleveland, Ohio. May 1999.
56. Struger J., S. Painter, B. Ripley, B. Thorburn, D. Boyd, and R. Bilyea. Agricultural pesticide concentrations in Canadian Lake Erie tributaries. IAGLR Conference. Cleveland, Ohio. May 1999.
57. Gianessi and Anderson. 1995. National Pesticide Use Database. National Center for Food and Agricultural Policy, Washington D.C.
58. Thompson, H.M. 1996. Interactions between pesticides; a review of reported effects and their implications for wildlife risk assessment. Ecotoxicol. 5:59-81.
59. Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). 1997. 1997-1998 Ontario mushroom pesticide recommendations. OMAFRA Publication No. 367. OMAFRA, Toronto, ON, Canada.
60. Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). 1998. Pest management recommendations for greenhouse crops. OMAFRA Publication No. 365. OMAFRA, Toronto, ON, Canada.
61. Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). 1998. Vegetable production recommendations, 1998-1999. OMAFRA Publication No. 363. OMAFRA, Toronto, ON, Canada.
62. Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). 1998. Fruit production recommendations, 1998-1999. OMAFRA Publication No. 360. OMAFRA, Toronto, ON, Canada.
63. Tobin, P. 1986. Known and potential sources of hexachlorobenzene. In: C.R. Morris and J.R.R. Carbal, eds. Hexachlorobenzene: Proceedings of an International Symposium. Lyon: International Agency for Research on Cancer 77: 3-11.
64. Bishop, C.A., R.J. Brooks, J.H. Carey, P. Ng, R.J. Norstrom, and D.R.S. Lean. 1991. The case for a cause-effect linkage between environmental contamination and development in eggs of the common snapping turtle (*Chelydra s. serpentina*) from Ontario, Canada. J. Toxicol. Environ. Health 33:521-547.
65. Bishop, C.A., D.V. Weseloh, N.M. Burgess, J. Struger, R.J. Norstrom, R. Turle, and K.A. Logan. 1992. An atlas of contaminants in eggs of fish-eating colonial birds of the Great Lakes (1970-1988). Vol. I - Accounts by Chemical. Canadian Wildlife Service Technical Report Series No. 153.
66. Struger, J., J.E. Elliott, C.A. Bishop, M.E. Obbard, R.J. Norstrom, D.V. Weseloh, M. Simon, and P. Ng. 1993. Environmental contaminants in eggs of the common snapping turtle (*Chelydra serpentina serpentina*) from the Great Lakes - St. Lawrence River basin of Ontario, Canada (1981, 1984). J. Great Lakes Res. 19:681-694.
67. Russell, R.W., S.J. Hecnar, and G.D. Haffner. 1995. Organochlorine pesticide residues in southern Ontario spring peepers. Environ. Toxicol. Chem. 14:815-817.
68. Russell, R.W., K.A. Gillan, and G.D. Haffner. 1997. Polychlorinated biphenyls and chlorinated pesticides in southern Ontario, Canada, green frogs. Environ. Toxicol. Chem. 16:2258-2263.
69. Tomlin, C. 1994. The Pesticide Manual: Incorporating the Agrochemicals Handbook (10th ed.). The British Crop Protection Council and the Royal Society of Chemistry. The Bath Press, Bath, England.

2.0 ENDOSULFAN

Use of organochlorine (chlorinated hydrocarbon) insecticides in North American agriculture has been gradually phased out due to their highly persistent and toxic nature. Banned organochlorines which persist in Ontario environments (from past use and atmospheric deposition) include DDT and its derivatives, dieldrin, chlordanes, lindane, and heptachlor epoxide [1-5]. Endosulfan, dicofol and methoxychlor were the only organochlorines used as pesticides in 1993, according to the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) [6]. Dicofol and methoxychlor are DDT-type insecticides with limited use in Ontario fruit- and vegetable-growing industries.

In 1993, the use estimate for the miticide, dicofol was 8,372 kg active ingredient (a.i.), while that for methoxychlor was 3, 791 kg a.i. [6]. Although they are recommended for use on greenhouse (dicofol) and mushroom (methoxychlor) crops [7,8], these applications were not accounted for in the 1993 estimates. Endosulfan is a cyclodiene insecticide with far wider application in Ontario agriculture. On fruit and vegetable crops alone, 25,930 kg endosulfan (a.i.) were applied in 1993 [6]; greenhouse industries also rely heavily on endosulfan [8], but provincial estimates of use are not available.

Organochlorines characteristically exhibit low water solubility, high lipid solubility, and environmental persistence; their chief toxic action is on the nervous system [9]. Endosulfan was chosen for further investigation in this report, because it is the most heavily applied organochlorine in Ontario.

2.1 Description and Use

The cyclodiene, endosulfan was introduced as a broad spectrum insecticide in 1956 [10]. It is used globally as an alternative to more persistent organochlorines, for control of insect pests on a multitude of crops, including fruits, vegetables, tea, coffee, rice, cereals, cotton, sugar cane and tobacco [11,12]. In addition, a good portion of available toxicity data has been compiled due to its use in tropical countries for tsetse fly control [13-18].

Endosulfan ([1,4,5,6,7,7-hexachloro-8,9,10-trinorbon-5-en-2,3-ylenebismethylene]sulfite; CAS No. 115297) is marketed in a variety of commercial- and domestic-use formulations in Ontario, predominantly as wettable powders or emulsifiable concentrates, but also in dusts (at <3% active ingredient). Table 2.1 lists its recommended uses on Ontario fruit, vegetable and greenhouse crops; it is applied principally for control of leafhoppers, aphids, tarnished plant bug, silver-leaf whitefly, and cyclamen mite [8,19,20].

Several domestic and some commercial products include both endosulfan and an ethylenebisdithiocarbamate (EBDC, see chapter 3). The principal commercial products, Thiodan 50WP and Thiodan 4EC, contain 47-50% and 4 lb/gallon endosulfan, respectively. The remaining inert ingredients are unknown, although the EC (emulsifiable concentrate) must contain some form of petroleum distillate as an emulsifier and the WP (wettable powder) some form of wetting agent [21]. One author reported that the EC formulation available in Australia contained 620 g/L xylene as an emulsifier [22]. Technical grade endosulfan is a mixture of two stereoisomers; the alpha and beta isomers are present in approximately a 7:3 ratio, respectively [21]. Without the addition of emulsifiers or wetting agents, solubility in water is low (60-150 mg/L).

Use of endosulfan in Ontario is shown on a county-wide basis in Figure 2.1. Applications were among the highest recorded in the Great Lakes basin. Heaviest use was recorded in southern and western portions of the province (i.e. Lambton, Kent, Elgin, Haldimand-Norfolk, Niagara, Durham, York, and Simcoe counties). This likely reflects its frequent use for apple, strawberry and greenhouse pests.

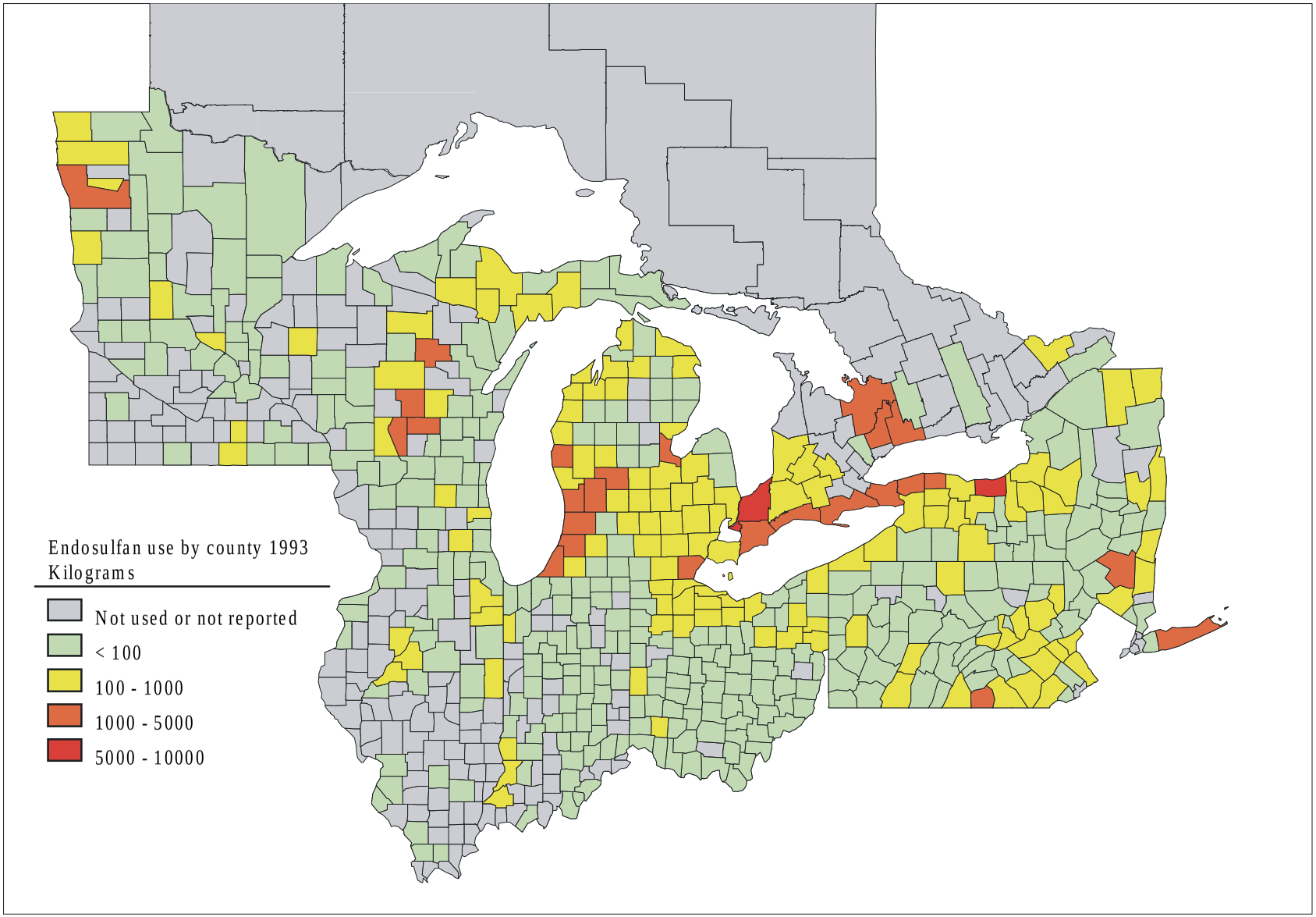

Figure 2.1 Endosulfan use in theGreat Lakes Basin.

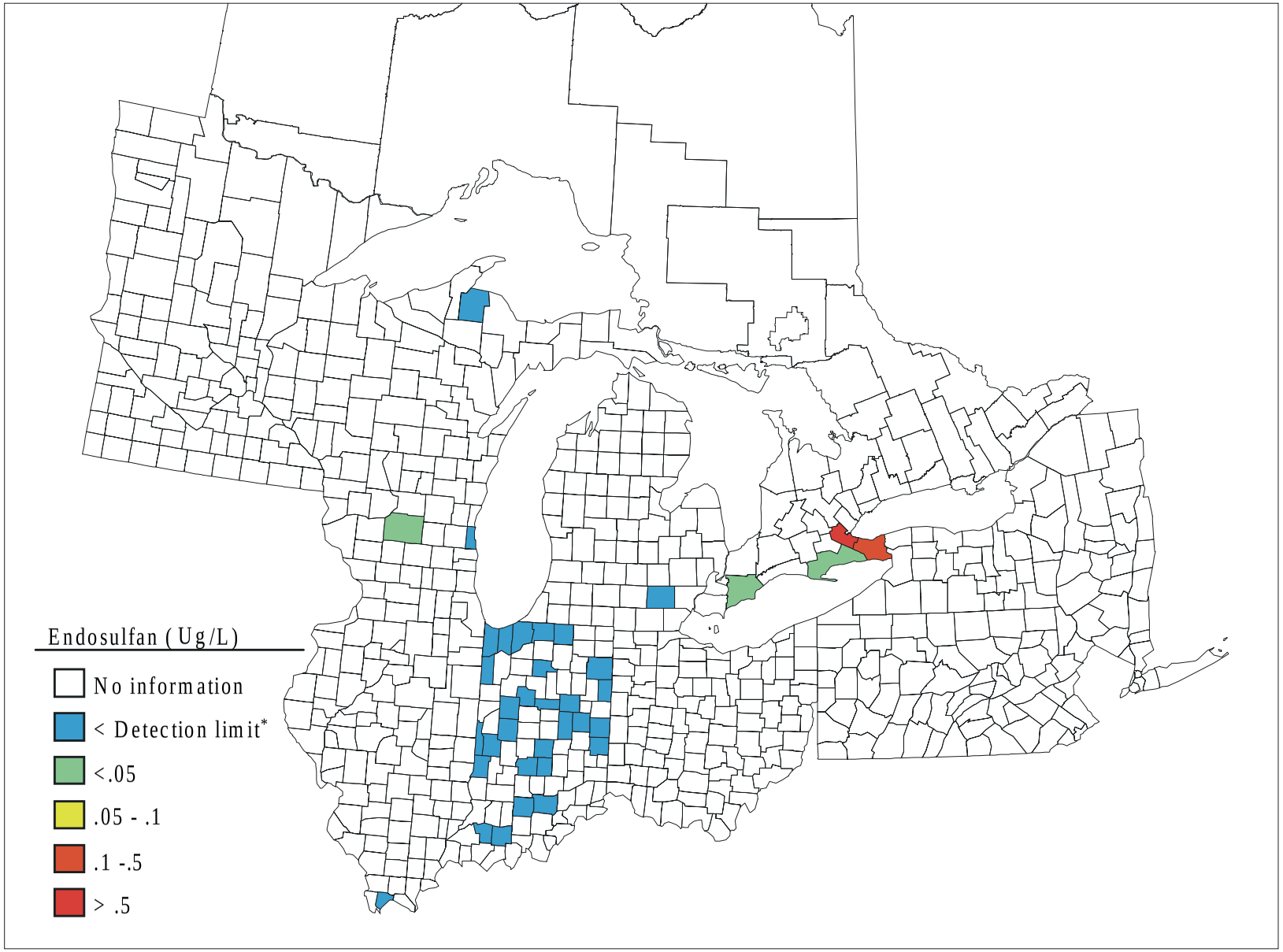

Figure 2.2 Maximum concentration of endosulfan found in surface water by county. *Detection limits range from .01 -.2ug/L.

Table 2.1 Recommended [8,19,20] applications of endosulfan in 1998 Ontario agriculture.

Crop(s) Protected	Insect(s) Controlled	Application Rate[a]
beans (green, wax, lima)	leafhoppers, Mexican bean beetle, green cloverworm	2.1 L/ha
cabbage, cauliflower, broccoli, brussels sprouts	flea beetle, cabbageworm, cabbage looper, diamondback moth caterpillar	2.0 L/ha
kale	cabbageworm, cabbage looper, diamondback moth	1.5-2.0 L/ha
celery	tarnished plant bug, aphids, cabbage looper	1.75 kg/ha
squash	squash vine borer	1.0 kg/ha
cucumber	cucumber beetle	1.5 L/ha
cucumber, muskmelon, watermelon, pumpkin, squash, pepper (sweet, hot, mini, pimento), tomato	aphids	1.5 L/ha
eggplant	flea beetle, aphids, Colorado potato beetle[b]	1.1-2.25 kg/ha
head lettuce	aphids, cabbage looper, tarnished plant bug	2.1 L/ha
pepper (sweet, hot, mini, pimento)	pepper maggot	1.1 kg/ha
rutabaga	flea beetle, aphids, cabbageworm, cabbage looper, zebra caterpillar	2.0 L/ha
spinach	aphids, cabbage looper	2.0 L/ha
tomato	hornworm	1.1 kg/ha
apple	green apple aphid, rosy apple aphid, white apple leafhopper	3.25 kg/ha
apple	potato leafhopper	2.6 kg/ha
apple	woolly apple aphid	4.25 kg/ha
cherry (sour), plum	aphids	3.25 kg/ha
cherry (sour), peach, nectarine, plum	peach tree borer	1.5 kg/1000L
cherry (sweet)	black cherry aphid	4.5 kg/ha
grape	grape phylloxera	2.25 kg/ha
peach	tarnished plant bug	4.5 kg/ha
pear	pear psylla, plum curculio	5-6.75 kg/ha
pear	plant bugs, blister mite	3.25-4.5 kg/ha

Table 2.1 Continued.

Crop(s) Protected	Insect(s) Controlled	Application Rate[a]
greenhouse flower crops[c]	aphids, cyclamen mite, whitefly, two-spotted spider mite	1.25 L/1000L
greenhouse tomato, cucumber, lettuce	aphids, whitefly	1.25-1.5 L/1000L
greenhouse pepper	lygus bug, tarnished plant bug	1.24-1.5 L/1000L

[a] - Two or more forms of endosulfan were usually listed in OMAFRA publications [8,19,20] (primarily Thiodan 50WP, 4EC, Endosulfan 50WP, 400EC), but only one was chosen as an example for this table. Note that recommendations for vegetables listed an applied mass (kg) or volume (L) only [19]; it was assumed that these values were to be applied on a 'per hectare' basis. Fruit tree 'per hectare' recommendations were based on 4.5-5.5 m high trees.
[b] - although endosulfan is still listed as a potential control agent for Colorado potato beetle on potato crops, the resistance of this beetle to many historically effective insecticides has virtually eliminated endosulfan use for its control in Ontario.
[c] - includes African violet, azalea, begonia, tulip (bulb), calceolaria, chrysanthemum, cineraria, cyclamen, geranium, gloxinia, hydrangea, easter lily, rose, snapdragon.

2.2 Environmental Concentrations

Endosulfan is typically applied to crops using air-blast or ground boom sprayers, thus allowing for chance of drift and longer-range atmospheric transport [23]. Surface water concentrations detected in Ontario over the past 2 decades ranged from <0.01 mg/L to 0.54 mg/L (Fig. 2.2). Samples collected from the open waters of Lake Erie in 1993 showed less than 1.0 ng/L endosulfan (J. Struger, Environment Canada, pers. comm.).

However, water monitoring was sporadic and limited. One study of residues in sediment and green frog fat bodies collected from ponds in apple orchards suggests that environmental exposure to endosulfan is more widespread than estimated by random surface water collections [24]. Sediment concentrations of b-endosulfan ranged from <2.9 ng/g to 64.5 ng/g (n=8 samples), while frog fat body concentrations of endosulfan sulfate ranged from 0.7 mg/g to 10.0 mg/g (n=6 samples). Sediment and frogs were collected from Ontario apple orchard ponds in the spring and fall, thus endosulfan was persisting over a year in some environments. Tree swallow eggs collected from the same orchards contained very little endosulfan (maximum residue of 0.057 mg/g wet weight), suggesting there was limited food chain transfer of contamination to a terrestrial insectivore [25]. The isomers a- and b-endosulfan (as well as methoxychlor) were detected in precipitation at several locations along the northern edges of Lakes Ontario, Erie and Superior [2,5] during comprehensive scans for persistent chlorinated contaminants. This suggests that some atmospheric transport of endosulfan occurs within and perhaps into Ontario.

In soils and sediments, a- and b-endosulfan degrades primarily via microbial biotransformation to endosulfan sulphate (the major metabolite); other minor products include the diol, ether, a-hydroxy ether, and lactone forms. a-Endosulfan disappears quickly in the environment, within 60 days in soil and within 7 days in river water [26,27]; b-endosulfan and endosulfan sulphate may not disappear for over two years [21]. During the winter, degradation is effectively halted, and it may also be considerably slowed under conditions of low pH (below pH 7) or dissolved oxygen, or in highly organic soils or sediments [21]. For example, Greve [28] found that, under anaerobic conditions and neutral pH, the half-life of endosulfan in water was five weeks rather than one week; under anaerobic, acidic (pH 5.5) conditions, the half-life was extended to five months. In Ontario, this suggests that the environments most likely to act as sinks for endosulfan are marshy wetland adjacent to sprayed croplands, or muck crop areas. Stagnant water in such wetlands are subject to eutrophication, resulting in highly anaerobic sediments and seasonal lows in dissolved oxygen concentrations. Where peaty bottoms exist (an infrequent phenomenon given the predominance of limestone deposits in Ontario soils), there will be additional acidity.

2.3 Bioconcentration and Metabolism of Endosulfan

Bioconcentration data for endosulfan is available for several species of freshwater fish and invertebrates (Table 2.2). Estimates of a bioconcentration factor vary by almost four orders of magnitude ranging from 1.97 to 11583, likely due to the lipid content and the metabolic ability of the organisms tested. The endosulfan sulfate metabolite is generally included in the calculation of the bioconcentration factor (BCF), since it is just as toxic as the parent compound. The only indigenous species for which data was available was the white sucker, and endosulfan showed only a moderately low BCF ranging between 65 in muscle to 550 in liver (gill, kidney, gut and skin accumulation was also measured).

Although endosulfan sulfate is typically the only metabolite included in bioconcentration calculations, investigators have detected many other metabolites; some have suggested that the sulfate is just an intermediate metabolic form in vertebrates [35] and endosulfan ether is the main product of detoxification [37]. Rao et al. [37] report finding ether, alcohol, a-hydroxy ether and lactone forms of endosulfan as well as two unidentified metabolites in tissues of the fish *Macrognathus aculeatum*.

2.4 Toxic Mechanisms of Action

Unlike the DDT-type organochlorines (methoxychlor, dicofol) which act primarily on the peripheral nervous system, cyclodienes like endosulfan are thought to act on the central nervous system by binding at the picrotoxinin site in the g-aminobutyric acid (GABA) chloride ionophore complex [9,38,39]. GABA is an inhibitory neurotransmitter that operates through membrane hyperpolarization as mediated by increased chloride flux into nerve cells. By impairing the inhibitory actions of this complex, and, thus, chloride influx into the nerve, hyper excitation results which, when prolonged, may lead to respiratory failure. External symptoms include depressed activity a few hours after exposure followed by hyper excitability, tremors and convulsions [9]. Convulsions can lead to death by interfering with pulmonary gas exchange and by generating severe metabolic acidosis [9].

An alternate hypothesis of cyclodiene toxicity is based on the observation that a-endosulfan inhibits $Ca^{+}Mg$-ATPase and Ca-ATPase [9,40]. This effect may be specific to the stereo chemistry of the a- isomer, since b-endosulfan did not elicit the same response in rat brain during *in vitro* studies [40]. An energetic mode of action is further supported by the observation that endosulfan impairs respiration in rat liver mitochondria, *in vitro* [41], and in rainbow trout and catfish (*Clarias batrachus*) liver mitochondria, *in vitro* [42] and *in vivo* [42,43].

2.5 Acute Toxicity

Field data on acute toxicity is limited and the bulk of observations were collected after aerial spray applications of endosulfan, which are not routinely conducted in Ontario. Several studies were completed in the Okavango Delta in Botswana, where endosulfan was tested as an alternative tsetse fly control to previously applied DDT and dieldrin. With a single application of 200 g active ingredient/ hectare, all fish in rivers within the spray area were killed [17]. With six applications of 6-12 g/ha, fish kills were reduced to 0-2350 fish/ha, but mortalities still represented up to an estimated 60% of the population of some species [15]. The distribution of mortality was extremely patchy and possibly related to amount of submerged vegetation and silt. During the second study, a piscivorus bird species, the pied kingfisher (*Ceryle rudis*), was also assessed for effects higher in the food chain. Although kingfisher were observed feeding on poisoned fish, they suffered no acute ill effects [14]. Endosulfan residues in the brains of three kingfishers were 0.2 mg/g wet weight; similar concentrations were measured in fish species, suggesting that bioaccumulation was not occurring. In Canada, the aerial application of endosulfan to potato fields in Prince Edward Island resulted in 90% mortality of threespine stickleback (*Gasterosteus aculeatus*) 200 m downwind where water concentrations never exceeded 4 mg/L [53].

Table 2.2 Bioconcentration factors in invertebrate and fish species for a- and b-isomers of endosulfan as well as for the principal metabolite, endosulfan sulfate.

Species	α	β	α + β + Sulfate	R
Louisiana crayfish (*Procambarus clarkii*)	-	1.97	-	29
Mussel (*Mytilus edulis*)	-	-	600	30
Scallop (*Chlamys opercularis*)	-	-	26	31
Zebra fish (*Brachydanio rerio*)	2006	1398	2650	32
Yellow tetra (*Hyphessobrycon bifasciatus*)	10994	9908	11583	33
Mosquitofish (*Gambusia affinis*)	71.5	15.5	86.5	34
White sucker (*Catostomus commersoni*)	-	-	65-550	35
Goldldfish (*Carassius auratus*)	-		350	36

Within Ontario, fish kills have been observed occasionally as a result of accidental spills of endosulfan into waterways. In 1969, 300-400 dace (*Chrosomus* sp.), white sucker, rock bass (*Ambloplites rupestris*) and other species were killed in the North Thames River upon exposure to 0.096 - 0.26 mg/L endosulfan originating from off-target spraying of potato fields [21].

Acute toxicity of endosulfan to non-target organisms has been documented in the field and the laboratory. The sensitivity of aquatic invertebrates ranges over several orders of magnitude (Table 2.3); not surprisingly, the arthropods, specifically crustaceans and insects, were the most sensitive invertebrates. Physiologically, these species are most like the target terrestrial insect pests that organochlorines are meant to kill. Nonetheless, some fish species, notably young rainbow trout, mosquitofish, and several Australian species (Table 2.4), were more sensitive than aquatic insects to endosulfan. The 96-hr LC50s for rainbow trout fry, mosquitofish, bony bream, golden perch, European carp and Harlequin fish were 0.1 - 0.5 mg/L, while that for naiads of the stonefly, *Pteronarcys californicus*, was 2.3 mg/L.

Amphibians, the only other aquatic group to be tested in the laboratory, exhibited a wide range of toxicities (Table 2.5), but were all less sensitive than the afore-mentioned fish. Two indigenous species, white sucker (*Catostomus commersoni*) and northern pike (*Esox lucius*), appeared slightly less sensitive than fish species already mentioned, with LC50s in the range of 3 - 6.6 mg/L [21].

In general, bird and mammal studies used a small number of species to test the toxicity of endosulfan (Table 2.5), which was almost always administered orally (a few dermal and intra peritoneal applications were discussed in WHO [23]). Mallards, starlings and rats all exhibited LD50s in the range of 30 - 70 mg/kg body weight. This form of exposure may best estimate toxicity associated with food chain transfer of endosulfan.

In 1972, rainbow trout in a pond near Simcoe died from a similar application of endosulfan to tobacco fields [21]. Frank et al. [78] also recorded three more recent (1971-1985) fish kills in rural ponds contaminated with 0.11-2.0 mg/l endosulfan. In addition, fall applications of endosulfan to cole crops are suspected as the cause of several recent fish kills in southern Ontario (Paul McCubben, Ontario Ministry of the Environment, pers. comm.). Fall sprays pose a unique risk to temperate environments because of the reduced rates of degradation in colder months [21].

As with most chemicals, there were several factors noted within the original laboratory data that mitigated or exacerbated toxicity. Several authors described an increase in toxicity (i.e. lower LC_{50} with increasing water temperature in aqueous fish exposures [55,61,67]. Since endosulfan is sprayed mostly during the summer months in Ontario, elevated surface water temperatures in shallow or stagnant water bodies may have important consequences for toxic effects in wild fish.

Sunderam et al. [55] also noted that toxicity in Australian fish species was greater during flow-through tests compared to static tests, a finding with considerable ramifications, since many LC50s are derived under static conditions. The effects of age, life stage and body size on toxicity were observed in both invertebrates and fish. Early larval stages of tiger prawn were more sensitive than juveniles [50], while among adult catfish, smaller individuals were more sensitive than larger ones [61]. Among bird species, the egg-laying condition of hens was proposed as an explanation for inter-species differences in sensitivity [72]. Delayed mortality in egg-laying pheasant hens exposed to endosulfan was thought to be partly related to the presence of eggs as an additional route of toxicant metabolism. Since endosulfan is lipophilic, it is probably eliminated from the adult by deposition into the egg yolk.

One relative constant across test groups was the description of symptoms accompanying acute toxicity. All behavioural anomalies in invertebrates, fish, amphibians, birds and mammals suggested the initiation of nervous and respiratory dysfunction. In worms [48], stoneflies [52], mosquitofish [56], and green frog and Indian toad tadpoles [69,71] the sequence of behavioural changes was excitation or irritability (including erratic swimming in fish and frogs) followed by loss of equilibrium, tremors or twitching, then either convulsions or immobility just prior to death. In addition to these signs, yellow tetra, zebrafish [58], snakehead fish [59,60], and eel [68] displayed multiple signs of respiratory distress including erratic opercular movements.

The latter two species also reacted with mucous secretions and a general loss of colour. In birds dosed orally with endosulfan, symptoms of toxicity included ataxia, jerkiness, high carriage, tremors, wing shivers and falling [72]. In an accidental poisoning of calves (they were dusted with endosulfan for lice control and all five animals soon died), toxic reactions were tremors, ear twitching, eyelid snapping, body jerks severe enough to result in falls, and various signs of disorientation [79].

The symptoms were also fairly uniform across chemical variations of endosulfan. The tests with formulations (wettable powders and emulsifiable concentrates) and a- and b-isomers produced the same behavioural anomalies as tests with technical grade endosulfan. This suggests that endosulfan, rather than the inert ingredients, is responsible for acute effects. However, where different forms were compared in concurrent tests with fish, an EC formulation was 1 to 2 times more toxic [59,60,63], and the isomer a-endosulfan was 3 to 30 times more toxic [59,63] than the technical (96% endosulfan) form. This does not conform with mammalian studies, which have shown equivalent toxicity of both stereoisomers, endosulfan sulphate and the technical form [23]. Formulations have not been intentionally tested on higher vertebrates.

There is a limited data set on critical body residues of endosulfan. Residues in the accidentally exposed calves mentioned above ranged from 0.73 mg/kg in brain to 3.78 mg/kg in liver [79]. In another cattle poisoning, the a- and b-isomers, and sulphate metabolite of endosulfan were measured and reported as 0.083, 0.065, and 4.23 mg/kg respectively. Lethal dose in carp (total endosulfan) was 3.5 mg/kg in liver 4.9 mg/kg in gill [80].

Although the volume of information summarized here implies that the acute toxicity of endosulfan to wildlife has been thoroughly evaluated, closer examination reveals that there is little data for species indigenous to Ontario. In particular, only seven fish species and one frog species from Ontario have been subject to acute toxicity tests. Information on reptiles and wild mammals (e.g. mink, otter, voles, etc.) is lacking in the open literature. This gap increases uncertainty when attempting to establish the risk endosulfan use in Ontario agriculture poses to surrounding environments.

Table 2.3 Acute toxicity of endosulfan to aquatic invertebrates. Species indigenous to Ontario are identified by an (I) after the species name. Sample size (N) = no. animals/replicate x no. replicates. R = reference/source.

Group	Species	Common Name	Age	N	Chemical Form	Dose Method	Test Type	LC50 (mg/L)[a]	R
Monogonta (Rotifera)	*Brachionus calyciflorus (I)*	rotifer	neonates	10 x 9	96% endosulfan[b]	static	24-hr	5150 (n)	44[c]
Gastropoda (Mollusca)	*Aplexa hypnorum*	snail	adults	10 x 6	Thiodan-2 (23.5%)[b]	static renewal	96-hr	>1890 (m)	47
Polychaeta (Annelida)	*Nereis virens*	polychaete worm	adults	5 x -(1)	-	static renewal (salt-water)	12-d	100 (m)	48
Crustacea (Arthropoda)	*Daphnia magna (I)*	water flea	neonates	10 x -(2+)	96% endosulfanb	static	24-, 48-hr	620, 220 (n)	45
Crustacea (Arthropoda)	*Daphnia magna (I)*	water flea	< 24 hr	5 x 4	technical gradeb	static	48-hr	271-343 (m)[d]	49
Crustacea (Arthropoda)	*Penaeus monodon*	Tiger prawn	mysids	10 x 2	35 EC[b]	static renewal	24-hr	2.41	50
Crustacea (Arthropoda)	*Penaeus monodon*	Tiger prawn	post-larvae, juveniles	10 x 2	35 EC[b]	static renewal	24-, 48-hr	7.53, 4.65 17.6, 12.2	50
Crustacea (Arthropoda)	*Gammarus fasciatus (I)*	amphipod	adults	-	96% endosulfan	static	24-, 96-hr	10, 6	51
Crustacea (Arthropoda)	*Gammarus lacustris (I)*	amphipod	adults	-	96% endosulfan	static	24-, 96-hr	9.2, 5.8	51
Insecta (Arthropoda)	*Pteronarcys californica (I)*	stonefly	naiads	10 x -(2+)	technical grade[b]	static	24-, 48-, 96-hr	24, 5.6, 2.3	52
Insecta (Arthropoda)	*Sigara alternata (I)*	water boatman	-	-	Thiodan 50WP (47%)	static	24-, 48-hr	>50, 12.3 (n)	53

[a] - always relates to the amount of endosulfan, regardless of the chemical form of exposure, and, where available, values are defined as nominal (n) or measured (m).
[b] - exposure was conducted using a solvent carrier; rotifer, prawn, water flea = acetone, snail = dimethyl formamide (DMF), water flea [49] = triethylene glycol, stonefly = ethanol
[c] - same results presented in Ferrando et al. [45], and Fernandez-Casalderry et al. [46].
[d] - values represent EC50s.

Table 2.4 Acute toxicity (mg/l) of endosulfan to freshwater fish. Species indigenous to Ontario are identified by an (I) after the

Species	Common Name	Age	N	Chemical Form	Dose Method	Test Type	LC50 (mg/L)[a]	R
Gasterosteus aculeatus (I)	Threespine stickleback	adults	-	Thiodan 50WP (47%)	aerated	24-, 48-hr	7.75, 6 (n)	53
Pimephales promelas (I)	Fathead minnow	juveniles[b]	10 x 2	technical grade[c]	static	96-hr	0.8-1.3 (m)	49
Pimephales promelas (I)	Fathead minnow	juveniles[b]	10 x 2	technical grade[c]	flowthrough	96-hr	1-1.7 (m)	49
Pimephales promelas (I)	Fathead minnow	adults[b]	-	technical (96%)	static	24-, 96-hr	2.4, 1.5	51
Oncorhynchus mykiss (I)	Rainbow trout	fry[b]	10 x 2	technical grade[c]	static	96-hr	1.6-1.7	49
Oncorhynchus mykiss (I)	Rainbow trout	fry[b]	10 x 2	technical grade[c]	flowthrough	96-hr	0.3-0.4	49
Oncorhynchus mykiss (I)	Rainbow trout	fry[b]	-	technical (96%)	static	24-, 96-hr	2.3-13, 1.1-2.9	51
Oncorhynchus mykiss (I)	Rainbow trout	juveniles[b]	10 x 2	technical[c] (96.2%)	static	96-hr	0.7-1.6 (m)	55
Lepomis macrochirus (I)	Bluegill sunfish	adults[b]	-	technical (96%)	static	24-, 96-hr	3.3, 1.2	51
Ictalurus punctatus (I)	Channel catfish	juveniles[b]	-	technical (96%)	static	24-, 96-hr	1.8, 1.5	51
Catostomus commersoni (I)	White sucker	-	-	-	-	24-, 48-hr	6.6, 4.3	21
Gambusia affinis	Mosquitofish	adults	10 x 6	Thiodan 3EC	static	96-hr	0.467d	56
Gambusia affinis	Mosquitofish	adults[b]	10 x 2	technical[c] (96.2%)	static	96-hr	2.3 (n)	55
Melanotaenia duboulayi	Eastern rainbow fish	-	10 x 2	technical[c] (96.2%)	static	96-hr	5 (m)	55
Melanotaenia duboulayi	Eastern rainbow fish	-	10 x 2	technical[c] (96.2%)	static renewal	96-hr	2.4-2.5 (m)	55
Melanotaenia duboulayi	Eastern rainbow fish	-	10 x 2	technical[c] (96.2%)	flowthrough	96-hr	0.5 (m)	55
Bidyanus bidyanus	Silver perch	-	10 x 2	technical[c] (96.2%)	static renewal	96-hr	2.3-2.4 (m)	55
Macquaria ambigua	Golden perch	-	10 x 2	technical[c] (96.2%)	static renewal	96-hr	0.3-0.5 (m)	55
Nematolosa erebi	Bony bream	-	10 x 2	technical[c] (96.2%)	static renewal	96-hr	0.2 (m)	55

Table 2.4 Continued.

Species	Common Name	Age	N	Chemical Form	Dose Method	Test Type	LC50 (mg/L)[a]	R
Cyprinus carpio	European carp	-	10 x 2	technica[c] (96.2%)	static renewal	96-hr	0.1 (m)	55
Rasbora daniconius	Carp minnow	adult	-	35% EC	static renewal	96-hr	5	57
Hyphessobrycon bifasciatus	Yellow tetra	adult	16 x 5	technical[c] (97%)	static renewal	24-hr	2.6 (m)	58
Brachydanio rerio	Zebra fish	adult	16 x 5	technical[c] (97%)	static renewal	24-hr	1.6 (m)	58
Channa punctata(us)	Snake head fish	-	10 x 2	technical[c] (96%)	flowthrough	96-hr	4.8 (m)	59
Channa punctata(us)	Snake head fish	-	10 x 2	35% Ec[c]	flowthrough	96-hr	2.5	59
Channa punctata(us)	Snake head fish	-	10 x 2	4% dust[c]	flowthrough	96-hr	16	59
Channa punctata(us)	Snake head fish	-	10 x 2	a-endosulfan[c]	flowthrough	96-hr	0.16 (m)	59
Channa punctata(us)	Snake head fish	-	10 x 2	b-endosulfan[c]	flowthrough	96-hr	6.6 (m)	59
Channa punctata(us)	Snake head fish	adult	10 x -	technical grade[c]	static renewal	24-, 48-, 96-hr	11.4, 9.8, 6.1	60
Channa punctata(us)	Snake head fish	adult	10 x -	Thiodan 35Ec[c]	static renewal	24-, 48-, 96-hr	7.9, 6.3, 3.3	60
Heteropneustes fossilis	catfish	-	5 x -	Thiodan 35EC[c]	static	96-hr	1.8-6.5d	61
Heteropneustes fossilis	catfish	-	10 x 3	technical grade[c]	flowthrough	96-hr	1.1	62
Mystus cavasius	catfish	-	10 x 3	technical grade[c]	flowthrough	96-hr	1.9	62
Mystus vittatus	catfish	-	10 x 3	technical grade[c]	flowthrough	96-hr	2.2	62
Labeo rohita	carp/ rohu	juveniles	10 x 3	technical[c] (96%)	flowthrough	96-hr	1.1	63
Labeo rohita	carp/ rohu	juveniles	10 x 3	35% Ec[c]	flowthrough	96-hr	1	63
Labeo rohita	carp/ rohu	juveniles	10 x 3	4% dust[c]	flowthrough	96-hr	1.25	63
Labeo rohita	carp/ rohu	juveniles	10 x 3	a-endosulfan[c]	flowthrough	96-hr	0.33	63
Labeo rohita	carp/ rohu	juveniles	10 x 3	b-endosulfan[c]	flowthrough	96-hr	7.1	63
Tilapia sparrmanii	tilapia	-	10 x 1	35% EC	static renewal	24-hr	7.35	15

Table 2.4 Continued.

Species	Common Name	Age	N	Chemical Form	Dose Method	Test Type	LC50 (mg/L)[a]	R
Sarotherodon mossambicus	tilapia	3-mo adult	10 x 1	35% EC	static renewal	24-, 48-, 96-hr	10.4, 6.7, 4.3	64
Barbus spp.	barbs	-	10 x 1	35% EC	static renewal	24-hr	1.22	15
Aplocheilichthys johnstonii	Top-minnow	-	10 x 1	35% EC	static renewal	24-hr	2.57	15
Macrognathus aculeatum	-	-	10 x 3	technical (96%)	flowthrough	96-hr	3.5	37
Anabus scandens	perch	-	- x 3	technical[c] (96%)	-	96-hr	10	65
Anguilla anguilla	European eel	-	10 x 3	-	aerated	24-, 48-, 96-hr	23-40, 22-42, 20-42	66
Esox lucius (I)	Northern pike	-	-	-	-	24-hr	5.0f	21

species name. Sample size (N) = no. animals/replicate x no. replicates. R = reference/source.

[a] - always relates to the amount of endosulfan, regardless of the chemical form of exposure, and, where available, values are defined as nominal (n) or measured (m).
[b] - age was not given, but inferred from body size (using data from Scott and Crossman [54]).
[c] - exposure was conducted using a solvent carrier; Nebeker et al. [49] = triethylene glycol, all other listed = acetone.
[d] - estimated based on LC50 values given for chemical formulations; mosquitofish, catfish - 3 lbs endosulfan/US gallon, assume a density of 1.
[e] - similar results were described in Ferrando and Andreu-Moliner [67] and Ferrando et al. [68].
[f] - value represents LC100 at 24 hr.

Table 2.5 Acute toxicity of endosulfan to amphibians, birds and mammals. Species indigenous to Ontario are identified by an (I) after the species name. Sample size (N) = the no. animals/replicate x no. replicates or no. animals (=replicates) for most birds. R = reference/source.

Species	Common Name	Age	N	Chemical Form	Dose Method	Test Type	Concentration[a]	R
Rana clamitans (I)	Green frog	tadpoles	10 x 2	Thiodan 50WP	Aqueous, static renewal	13-, 16-d LC50	15	69
Rana tigrina	Indian bullfrog	tadpoles	-	-	Aqueous	24-, 48-, 96-hr LC50	2.1, 2.0, 1.8	70
Bufo melanostictus	Common Indian toad	tadpoles	-	technical grade[b]	Aqueous, static renewal	24-, 48-, 96-hr LC50	142, 134, 123	71
Anas platyrhynchos(I)	Mallard	3 mo	12	technical (96%)	oral, gavage	14-d LD50	33	72
Anas platyrhynchos(I)	Mallard	1 yr	20 x 2	technical (96%)	oral, gavage	14-d LD50	31.2-45.0	72
Anas platyrhynchos(I)	Mallard	juveniles	-	Thiodan (35%)	diet	<10-d LC50	1000	73
Phasianus colchicus (I)	Ring-necked pheasant	3-4 mo	7, 12	technical (96%)	oral, gavage	14-d LD50	80-190	72
Phasianus colchicus (I)	Ring-necked pheasant	1 yr	16	technical (96%)	oral, gavage	14-d LD50	> 320	72
Sturnus vulgaris (I)	European starling	adults	-	technicalb	gavage	LD50	35	74
Colinus virginianus (I)	Northern bobwhite	juveniles	-	Thiodan (35%)	diet	<10-d LC50	300	73
Rattus norvegicus (I)	Rat	adults	-	technical[b]	oral	LD50	64	75
Rattus norvegicus (I)	Rat	adults	-	technical[b]	oral	LD50	40-50	76
Rattus norvegicus (I)	Rat	adults	-	technical[b]	oral	LD50	18	77
Mesocricetus sp.	Hamster	adults	-	technical[b]	oral	LD50	118	75

[a] - in mg/L for amphibians, mg/kg body weight or diet for birds and mammals; always relates to the amount of endosulfan, regardless of the chemical form of exposure, and, where available, values are defined as nominal (n) or measured (m).
[b] - exposure was conducted using a solvent carrier; Indian toad = acetone, European starling = propylene glycol, [75] = olive oil, [76] = 95% alcohol, [77] = peanut oil.

2.6 Chronic Effects

The mechanisms of action described for endosulfan, and organochlorines in general, relate to central nervous system reactions (see section 2.4). Hence, it is likely that other effects documented in reproductive and detoxification (hepatic) systems represent secondary (physiological) responses to the initial effects on the neurological network.

2.6.1 Chronic effects in mammals

Studies of endosulfan chronic toxicity in mammals, *in vivo*, are limited to only a few studies. One set of evaluations focused on the issue of mutagenicity/carcinogenicity. Endosulfan inhibited chemical communication between cells across gap junctions in several *in vitro* experiments [81-84]. Inhibition of gap junction communication is thought to be one mode of action by which tumour promoters operate, thus implicating carcinogenicity as a chronic effect of endosulfan exposure. However, endosulfan was not mutagenic in the Ames test [85], and a comprehensive rat *in vivo* carcinogenicity study by Hack et al. [86] indicated no tumour formation at endosulfan doses ranging from 3 to 75 mg/kg. Thus, the studies of gap junction communication inhibition may have little environmental (i.e. *in vivo*) relevance.

Only two studies directly examined mammalian reproductive toxicity. Gupta et al. [87] showed an increased rate of litter resorption in rat dosed orally with 5 or 10 mg/kg technical endosulfan. This was accompanied by a twofold increase in abnormal fetuses at the same doses. Experiments by Pandey et al. [88] showed mutation in the sperm of male rats, but at essentially irrelevant doses that approached the LD50 level. Also, sperm mutations did not result in any effects on fertility when these male rats were mated.

Behaviour of rats was examined by Lakshmana and Raju [89] upon exposure to sublethal levels of 6 mg/kg endosulfan. Deficits in memory acquisition and retention were observed and were associated with fluctuations in levels of brain neurotransmitters. In an independent study, increases in foot shock-induced aggressive behaviour upon exposure to 1 mg/kg endosulfan was related to increased binding of serotonin to serotonergic receptors in the rat pup brains [90].

2.6.2 Chronic effects in fish

Chronic effects in fish were linked to primary responses of the nervous and respiratory systems, and secondary responses of the respiratory, digestive (hepatic) and reproductive systems.

Behavioural manifestations of nervous disorders described by several investigators [55,56 58-60,91-93] were further evaluated via histological examination of the brain and pituitary gland. After a 20-day exposure to 1 mg/L Thiodan 35EC (sublethal level), tilapia (*Tilapia rendalli*) pituitary cells became structurally compromised, the axons of the neurohypophysis deteriorated, and neurosecretory material in the pars intermedia was reduced compared to that in control fish [94]. Wild tilapia and catfish exposed to aerial sprays of endosulfan in Botswana also showed severe damage in brain cells. Effects on the brain included edema, meningitis and encephalitis, which the authors linked causally to hyperactivity and uncoordinated swimming observed in surviving fish in sampled rivers [95]. Continued monitoring showed that most histological changes in the brain disappeared within a couple of weeks of spray termination; however, glial scarring in brain tissue persisted for 20 months post-spray.

Respiratory responses observed in several species might be partly due to primary as well as secondary toxic reactions. Studies with both rats [41] and fish [42,96] suggest that endosulfan acts as an oxidative phosphorylation uncoupler, inhibiting respiration by impairing membranes and respiratory interfaces. When the short-term respiratory response of rainbow trout exposed to an acutely lethal concentration of endosulfan was evaluated, the predominant effects were an increase in cough frequency and oxygen consumption with a decrease in arterial oxygen and carbon dioxide [96].

Rao et al. [63] examined oxygen consumption in an Indian carp species (*Labeo rohita*) over a concentration gradient, and also found that consumption increased during sublethal exposures up to the 96-hr LC50

concentration; individuals exposed for short periods to greater endosulfan levels showed decreased consumption. This pattern of initial stimulation then inhibition of oxygen consumption along a chemical concentration gradient is characteristic of oxidative uncouplers [97]. Histological studies also showed severe structural damage of gills with sublethal endosulfan concentrations [58,98]; however, these effects may be partly the result of a different toxic action, namely that of a surficial irritant. Johal and Dua [99] found that endosulfan disrupted the calcareous construction of snakehead fish (*Channa punctatus*) scales, thereby reducing their adhesive capacity and disrupting the principal protective interface of the fish body.

In vivo and *in vitro* studies of liver mitochondria in catfish (*Clarias batrachus*) and rainbow trout also suggest inhibition of respiratory function by endosulfan. Technical grade material decreased the respiratory capacity of liver mitochondria to oxidize isocitrate and succinate entering the electron transport pathway in catfish; it also increased ATPase activity [42]. The authors suggest that endosulfan prevents ATP formation, thereby blocking the utilization of oxygen. Arnold et al. [43] propose that loss of ultra structural integrity seen in rainbow trout liver mitochondria with endosulfan exposure represented the morphological counterpart to respiration disturbances.

Several studies described reproductive and endocrine response patterns in exposed fish that likely originated with primary reactions in the hypothalamo-pituitary-gonadal axis. In the well-studied tsetse fly spray region of Botswana, the nest-building behaviour of tilapia (*Tilapia rendalli*) during spawning season was reduced by 75% in sprayed river reaches, and juvenile recruitment declined by 25% [100]. Supporting laboratory studies with a closely related species (*Sarotherodon mossambicus*) found that exposure to sublethal levels of endosulfan (0.5 mg/L) delayed male breeding behaviour, and, in turn, female egg-laying by 15-20 days [64]. This cichlid does not build nests, but broods young in the mouth; hence, with delays in male sexual activity, several clutches were aborted by females by discontinuing brooding behaviour before clutches were fertilized.

Although no changes in steroid hormone concentrations were observed with these behavioural changes, other *in vitro* and *in vivo* assays suggest that steroid pathways are influenced by endosulfan. When endosulfan was introduced to leutinizing-hormone (LH)-induced carp (*Cyprinus carpio*) oocytes *in vitro*, the process of germinal vesicle breakdown was significantly inhibited [101].

This process is a critical step in oocyte maturation, thought to be controlled by the action of 17a- and 20b-dihydroxyprogesterone on the germinal vesicle. The progesterones are produced by 3b-hydroxysteroid dehydrogenase (3b-HSD), which the authors believed to be the point of inhibition by endosulfan. Their hypothesis is supported by histochemical studies with snakehead fish (*Channa punctatus*) ovaries (*in vivo*) that detected inhibition of 3b-HSD and glucose-6-phosphate dehydrogenase (G-6-PD), along with the complete disappearance of mature oocytes upon exposure to sublethal endosulfan (0.24-0.72 mg/L) for 120 days [102].

Shorter exposure times (20-75 days) with other fish species (*Rasbora daniconius*, *Sarotherodon mossambicus*, and *Colisa fasciatus*) identified comparable disruptions in ovarian tissues [57,92,94]. In general, the frequency of immature and atretic (dead) oocytes increased, while numbers of maturing and mature oocytes declined disproportionately. Cellular damage in all stages of oocytes was also detected, including increased lumen size, fibrous matting, and reabsorption and clumping of yolk in mature stages (a precursor to cell death).

Other changes in reproductive and endocrine function were observed in the vitellogenic catfish. Plasma vitellogenin levels were decreased upon endosulfan exposure, but effects could be partially reversed by supplementing concentrations of 17b-estradiol, triiodothyronine (T_3), and luteinizing hormone-releasing hormone (LHRH). The authors suggested that endosulfan was acting by decreasing estrogen production or by impairing the estradiol trigger for vitellogenin production in the liver [103]. However, given the primary neurological mode of action of organochlorines, it seems more likely that vitellogenin production was reduced because of dysfunction at multiple points along the hypothalamo-pituitary-gonadal axis. Related studies by the same research group found that the extrathyroidal conversion of thyroxine (T_4) to triiodothyronine (T_3) was impaired in catfish [104]. This supports our suggestion that endosulfan produces a general endocrine dysfunction.

Several investigators described endosulfan-induced changes in hepatic tissues, some of which were

likely linked to detoxification processes (Jensen et al. [105] reported that endosulfan induced both P450-1A and 2B in rainbow trout liver), but others of which were purported signs of liver degeneration [22,43,58,95, 106]. During morphological studies of rainbow trout liver, Arnold et al. [43] distinguished alterations which were likely adaptive from those which were degenerative effects. Adaptive changes were not specific to endosulfan, and were reported to disappear in wild fish livers within 40 days of spray cessation in Botswana [95].

Degenerative changes were also generally not specific to endosulfan, except perhaps for observed increases in cellular heterogeneity and loss of mitochondrial integrity, which were indicative of the already described inhibition of respiration. Nowak [22] measured endosulfan residues in catfish (*Tandanus tandanus*) liver coincident with histological examinations and found a correlation between a degenerative effect, namely percentage of pyknotic nuclei, and residues of b-endosulfan. The same author suggested that the b-isomer also produced a disproportionate amount of the damage seen in gill tissue [98].

This implication has important ramifications for wild fish populations, because the b-isomer persists in the environment for far greater periods. Acute toxicity comparisons in fish had suggested that the a-isomer was more toxic [59,63], but it may simply produce a more immediate response.

The remainder of chronic effects studies with endosulfan in fish investigated changes induced in carbohydrate and protein metabolism [37,65,91,93,107-117]. These authors report symptoms that indicate the mobilization of a general physiological stress response [118]. The response patterns were non-specific and typical of those described for many pollutants as well as abiotic factors (e.g. fluctuating water temperatures, low dissolved oxygen, eutrophied conditions). They, therefore, have less relevance in specifically describing the toxic action of endosulfan.

2.6.3 Chronic effects in invertebrates

Chronic studies of endosulfan in invertebrates are restricted mostly to cladoceran species. Two Australian cladocerans *Ceriodaphnia dubia* and *Moinodaphnia macleayi* had NOECs for reproductive impairment of 10 and 20 mg/L respectively [119]. Levels of 320 mg/L were observed to reduce *Daphnia carinata* brood size in laboratory experiments [120], and 160 mg/L endosulfan are sufficient to reduce population density of *Daphnia carinata* in microcosms [121]. In *Moina micrura*, levels representing 25% of the LC50 (4 mg/L) were sufficient to induce decreases in egg production and growth [122].

A crayfish species, *Procambarus clarkii*, showed no reductions in reproduction or growth after a 20 week exposure to 400 mg/L endosulfan [123]. These limited data for invertebrates indicate that all chronic effects occur at concentrations higher than the lethal levels for most fish species.

2.7 Potential for Endosulfan to Act as an Endocrine Disrupter

Our working definition of an endocrine disrupting compound:

> *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.*

Recent projections that endosulfan is an endocrine disrupter [124-126] have been almost exclusively based on observations that endosulfan was weakly estrogenic when applied to the *in vitro* estrogenicity bioassay or 'E-screen' [127,128]. E-screens are extremely sensitive and can detect estrogen receptor-binding chemicals over ten orders of magnitude concentration range [126]. Endosulfan is six orders of magnitude less potent than estradiol in the E-screen bioassay.

There are no *in vivo* studies to support an estrogenic mode of action for endosulfan. To the contrary,

studies in fish demonstrate that endosulfan inhibits vitellogenesis - making endosulfan an antiestrogen [103,129,130]. The endosulfan example clearly illustrates the danger of classifying a compound based on *in vitro* bioassays of little or no known relevance.

There is, nonetheless, sufficient evidence that endosulfan meets the criteria of our definition of an endocrine disrupter. It is clear that endosulfan is an extremely potent neurotoxin, and, therefore, it is likely that neurological tissue would be the primary site of endosulfan action. Chronic neural damage can clearly mediate other effects in an organism through endocrine pathways. One such example is the behavioural changes observed in rats due to alterations in neurotransmitter concentrations and binding. We also would hypothesize that reproductive effects observed in fish [57,64,92,94,101-103] originate at the hypothalamo-pituitary-gonadal axis. Neurological damage in this area would result in problems with hormonal release, synthesis and feedback regulation, the ultimate result of which would be impaired gonadal development.

Histological data on brains of fish exposed to endosulfan would tend to support this hypothesis. Although little data are available from field studies, we feel that there is sufficient evidence from chronic studies to classify endosulfan as an endocrine disrupter. We would further suggest that any neurologically-active pesticide (e.g. other organochlorines) is likely to act as an endocrine disrupter in a related fashion.

2.8 Risk to Ontario Environments and Recommendations

Based on very limited information on environmental concentrations of endosulfan in Ontario, some biota inhabiting aquatic ecosystems may be exposed to potentially acutely lethal as well as more frequently chronic levels of contamination. Surface water concentrations of <0.01 - 0.54 mg/L lie within the range for acute and chronic aquatic toxicity. The following issues should be priorities for further evaluation of the extent and degree of risk posed by endosulfan:

- a more comprehensive survey of surface water, sediment and tissue concentrations must be completed to identify levels of contamination
- more precise use information would help narrow the geographic focus for monitoring in high-risk counties;
- high-risk environments to be targeted for residue monitoring include shallow wetlands, slow-moving small streams and dugout ponds within or adjacent to sprayed crops;
- terrestrial environments may be at risk, but there is little relevant toxicity data on wild birds and small mammals - an information gap that should be addressed with well-designed studies of critical body residues and related effects;
- aquatic environments are most likely at risk, but aquatic-based food chains should also receive contaminant evaluation - for example, endosulfan residues in the food chain sediment aquatic invertebrate/frog/benthic fish muskrat/tree swallow/great blue heron/green-backed heron should characterize the maximum trophic transfer of chemical and its metabolites from aquatic to terrestrial systems;
- the range of acute toxicity among fish species is extremely narrow so indigenous species' sensitivity probably falls within the range outlined; however, given that surface water concentrations lie just within the acute range of the most sensitive fish species, acute toxicity tests with some benthic (e.g. suckers) and small stream (e.g. dace, minnows) fish species would be advisable;
- the lack of acute toxicity data for indigenous frog, toad and snake species should be rectified;
- the potential endocrine-disruption effects produced in wild fish and amphibians should be evaluated with indigenous species by *in vivo* tests of nervous and reproductive indicators in lab-exposed and field-exposed animals - histological examinations could be paired with biochemical assays such as analysis of plasma vitellogenin concentrations;
- residues of related organochlorines (DDE, dicofol, dieldrin, methoxychlor) should be assessed and combined effects on reproduction and nervous function evaluated;
- no information is readily available on possible spray mixtures which include endosulfan, although it is likely that it is sprayed with ethylenebisdithiocarbamates - characterization of mixtures should be completed.

2.9 References

1. Frank, R. and L. Logan. 1988. Pesticide and industrial chemical residues at the mouth of the Grand, Saugeen and Thames Rivers, Ontario, Canada, 1981-85. Arch. Environ. Contam. Toxicol. 17:741-754.
2. Johnson, M.G., J.R.M. Kelso, and S.E. George. 1988. Loadings of organochlorine contaminants and trace elements to two Ontario lake systems and their concentrations in fish. Can. J. Fish. Aquat. Sci. 45:170-178.
3. Bishop, C.A., D.V. Weseloh, N.M. Burgess, J. Struger, R.J. Norstrom, R. Turle, and K.A. Logan. 1992. An atlas of contaminants in eggs of fish-eating colonial birds of the Great Lakes (1970-1988). Vol. II - accounts by chemical. Canadian Wildlife Service Technical Report Series No. 153.
4. Struger, J., J.E. Elliott, C.A. Bishop, M.E. Obbard, R.J. Norstrom, D.V. Weseloh, M. Simon, and P. Ng. 1993. Environmental contaminants in eggs of the common snapping turtle (*Chelydra serpentina serpentina*) from the Great Lakes - St. Lawrence River basin of Ontario, Canada (1981, 1984). J. Great Lakes Res. 19:681-694.
5. Chan, C.H., G. Bruce, and B. Harrison. 1994. Wet deposition of organochlorine pesticides and polychlorinated biphenyls to the Great Lakes. J. Great Lakes Res. 20:546-560.
6. Hunter, C. and B. McGee. 1994. Survey of pesticide use in Ontario, 1993. Estimates of pesticides used on field crops, fruit and vegetable crops, provincial highway roadsides, and by licensed pesticide applicators. Ontario Ministry of Agriculture, Food and Rural Affairs, Economics Information Report No. 94-01. Policy Analysis Branch, OMAFRA, Toronto, ON, Canada.
7. Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). 1997. 1997-1998 Ontario mushroom pesticide recommendations. OMAFRA Publication No. 367. OMAFRA, Toronto, ON, Canada.
8. Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). 1998. Pest management recommendations for greenhouse crops. OMAFRA Publication No. 365. OMAFRA, Toronto, ON, Canada.
9. Coats, J.R. 1990. Mechanisms of toxic action and structure-activity relationships for organochlorine and synthetic pyrethroid insecticides. Environ. Health Perspect. 87:255-262.
10. Maier-Bode, H. 1968. Properties, effect, residues and analytics of the insecticide endosulfan. Resid. Rev. 22:1-44.
11. Tomlin, C. 1994. The Pesticide Manual: Incorporating the Agrochemicals Handbook (10th ed.). The British Crop Protection Council and the Royal Society of Chemistry. The Bath Press, Bath, England.
12. Goebel, H., S. Gorbach, W. Knauf, R.H. Rimpau, and H. Hhttenbach. 1982. Properties, effects, residues, and analytics of the insecticide endosulfan. Resid. Rev. 83:1-165.
13. Matthiessen, P. 1981. Haematological changes in fish following aerial spraying with endosulfan insecticide for tsetse fly control in Botswana. J. Fish Biol. 18:461-469.
14. Douthwaite, R.J. 1982. Changes in pied kingfisher (*Ceryle rudis*) feeding related to endosulfan pollution from tsetse fly control operations in the Okavango Delta, Botswana. J. Appl. Ecol. 19:133-141.
15. Fox, P.J. and P. Matthiessen. 1982. Acute toxicity to fish of low-dose aerosol applications of endosulfan to control tsetse fly in the Okavango Delta, Botswana. Environ. Pollut. 27A:129-142.
16. Matthiessen, P. and R.J. Roberts. 1982. Histopathological changes in the liver and brain of fish exposed to endosulfan insecticide during tsetse fly control operations in Botswana. J. Fish Dis. 5:153-159.
17. Everts, J.W. 1983. Animal indicators for side-effects of chemical vector control. Environ. Monit. Assess. 3:229-236.
18. Knauer, J., J.R. Duncan, and G.S. Merron. 1993. Sublethal effects of an organochloride/ synthetic pyrethroid insecticide cocktail on *Tilapia rendalli rendalli* (Pisces: Cichlidae). S. Afric. J. Sci. 89:249-251.
19. Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). 1998. Vegetable production recommendations, 1998-1999. OMAFRA Publication No. 363. OMAFRA, Toronto, ON, Canada.
20. Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). 1998. Fruit production recom-

mendations, 1998-1999. OMAFRA Publication No. 360. OMAFRA, Toronto, ON, Canada.
21. National Research Council of Canada (NRCC). 1975. Endosulfan: its effects on environmental quality. National Research Council (NRC) Associate Committee on Scientific Criteria for Environmental Quality Report No. 11./ NRCC-14098. NRCC Publications, Ottawa, ON, Canada.
22. Nowak, B. 1996. Relationship between endosulfan residue level and ultra structural changes in the liver of catfish, *Tandanus tandanus*. Arch. Environ. Contam. Toxicol. 30:195-202.
23. World Health Organization (WHO). 1984. Endosulfan. International Programme on Chemical Safety (IPCS) Environmental Health Criteria No. 40. WHO, Geneva, Switzerland.
24. Harris, M.L., C.A. Bishop, J. Struger, M.R. van den Heuvel, G.J. Van Der Kraak, D.G. Dixon, B. Ripley, and J.P. Bogart. 1998. The functional integrity of northern leopard frog (*Rana pipiens*) and green frog (*Rana clamitans*) populations in orchard wetlands. I. Genetics, physiology and biochemistry of breeding adults and young-of-the-year. Environ. Toxicol. Chem.17:1338-1350.
25. Bishop, C.A., H.J. Boermans, P. Ng, G.D. Campbell, and J. Struger. 1998. Health of tree swallows (*Tachycineta bicolor*) nesting in pesticide-sprayed apple orchards in Ontario, Canada. I. Immunological parameters. J. Toxicol. Environ. Health. 55:531-559.
26. Eichelberger, J.W. and J.J. Lichtenberg. 1971. Persistence of pesticides in river water. Environ. Sci. Technol. 5:541-544.
27. Steward, D.K.R. and K.G. Cairns. 1974. Endosulfan persistence in soil and uptake by potato tubers. J. Agric. Food Chem. 22:984-986.
28. Greve, P.A. 1971. The persistence of endosulfan in surface water. Meded. Fac. Landbouwwet. Rijksuniv. Gent. 36:439-447.
29. Naqvi, S.M. and D.J. Newton. 1990. Bioaccumulation of endosulfan (Thiodan[7] insecticide) in the tissues of Louisiana crayfish, *Procambarus clarkii*. J. Environ. Sci. Health 25B:511-526.
30. Roberts, D. 1975. Differential uptake of endosulfan by the tissues of *Mytilus edulis*. Bull. Environ. Contam. Toxicol. 13:170.
31. Ernst, W. 1977. Determination of the bioconcentration potential of marine organisms - a steady state approach. I. Bioconcentration data for seven chlorinated pesticides in mussels (*Mytilus edulis*) and their relation to solubility data. Chemosphere 6:731-740.
32. Toledo, M.C.F. and C.M. Jonsson. 1992. Bioaccumulation and elimination of endosulfan in zebra fish (*Brachydanio rerio*). Pestic. Sci. 36:207-211.
33. Jonsson, C.M. and M.C.F. Toledo. 1993. Bioaccumulation and elimination of endosulfan in the fish yellow tetra (*Hyphessobrycon bifasciatus*). Bull. Environ. Contam. Toxicol. 50:572-577.
34. Novak, B. and N. Ahmad. 1989. Residues in fish exposed to sublethal doses of endosulfan and fish collected from cotton growing area. J. Environ. Sci. Health 24B:97-109.
35. Schoettger, R.A. 1970. Toxicology of Thiodan in several fish and aquatic invertebrates. Invest. Fish Contr. No. 35. United States Department of the Interior (USDI), Bureau of Sport Fisheries and Wildlife, Washington, DC, U.S.A.
36. Oeser, H., S.G. Gorbach, and W. Knauf. 1971. Endosulfan and the environment. Presented at the Workshop on Phytopathology (>Giornate Fitopathologie), Udine, Italy, May 1971.
37. Rao, D.M.R., A.P. Devi, and A.S. Murty. 1981. Toxicity and metabolism of endosulfan and its effect on oxygen consumption and total nitrogen excretion of the fish *Macrognathus aculeatum*. Pestic. Biochem. Physiol. 15:282-287.
38. Abalis, I.M., M.E. Eldefrawi, and A.T. Eldefrawi. 1986. Effects of insecticides on GABA-induced chloride influx into rat brain microsacs. J. Toxicol. Environ. Health 18:13-23.
39. Gant, D.B., M.E. Eldefrawi, and A.T. Eldefrawi. 1987. Cyclodiene insecticides inhibit $GABA_A$ receptor-regulated chloride transport. Toxicol. Appl. Pharmacol. 88:313-321.
40. Srikanth, N.S., P.K. Seth, and D. Desaiah. 1989. Inhibition of calmodulin-activated Ca^{2-}-ATPase by endosulfan in rat brain. J. Toxicol. Environ. Health 28:473-481.
41. Dubey, R.K., M.U. Beg, and J. Singh. 1984. Effects of endosulfan and its metabolites on rat liver mitochondrial respiration and enzyme activities *in vitro*. Biochem. Pharmacol. 33:3405-3410.
42. Mishra, R. and S.P. Shukla. 1994. Effects of endosulfan on bioenergetic properties of liver mitochondria from the freshwater catfish *Clarias batrachus*. Pestic. Biochem. Physiol. 50:240-246.

43. Arnold, H., H-J. Pluta, and T. Braunbeck. 1996. Cytological alterations in the liver of rainbow trout *Oncorhynchus mykiss* after prolonged exposure to low concentrations of waterborne endosulfan. Dis. Aquat. Org. 25:39-52.
44. Fernandez-Casalderrey, A., M.D. Ferrando, M. Gamin, and E. Andreau-Moliner. 1991. Acute toxicity and bioaccumulation of endosulfan in rotifer (*Brachionus calyciflorus*). Comp. Biochem. Physiol. 100C:61-63.
45. Ferrando, M.D., E. Andreu-Moliner, and A. Fernandez-Casalderrey. 1992. Relative senisitivity of *Daphnia magna* and *Brachionus calyciflorus* to five pesticides. J. Environ. Sci. Health B27:511-522.
46. Fernandez-Casalderrey, A., M.D. Ferrando, and E. Andreau-Moliner. 1992. Acute toxicity of several pesticides to rotifer (*Brachionus calyciflorus*). Bull. Environ. Contam. Toxicol. 48:14-17.
47. Holcombe, G.W., G.L. Phipps, and J.T. Fiandt. 1983. Toxicity of selected priority pollutants to various aquatic organisms. Ecotoxicol. Environ. Saf. 7:400-409.
48. McLeese, D.W., L.E. Burridge, and J. Van Dinter. 1982. Toxicities of five organochlorine compounds in water and sediment to *Nereis virens*. Bull. Environ. Contam. Toxicol. 28:216-220.
49. Nebeker, A.V., J.K. McCrady, R. Mshar, and C.K. McAuliffe. 1983. Relative sensitivity of *Daphnia magna*, rainbow trout and fathead minnows to endosulfan. Environ. Toxicol. Chem. 1:69-72.
50. Joshi, H.C. and M.K. Mukhopadhyay. 1990. Toxicity of quinalphos and endosulfan to different life-stages of Tiger prawn (*Penaeus monodon*). Environ. Conserv. 17:266-267.
51. Mayer, F.L. and M.R. Ellersieck. 1986. Manual of acute toxicity: interpretation and data base for 410 chemicals and 66 species of freshwater animals. United States Department of the Interior (USDI), Fish and Wildlife Service Resource Publication No. 160. USDI, Washington, D.C., U.S.A.
52. Sanders, H.O. and O.B. Cope. 1968. The relative toxicities of several pesticides to naiads of three species of stoneflies. Limnol. Oceanogr. 13:112-117.
53. Ernst, W.R., P. Jonah, K. Doe, G. Julien, and P. Hennigar. 1991. Toxicity to aquatic organisms of off-target deposition of endosulfan applied by aircraft. Environ. Toxicol. Chem. 10:103-114.
54. Scott, W.B. and E.J. Crossman. 1973. Freshwater Fishes of Canada. Fisheries Research Board of Canada Bulletin No. 184. Department of Fisheries and Oceans, Ottawa, ON, Canada
55. Sunderam, R.I.M., D.M.H. Cheng, and G.B. Thompson. 1992. Toxicity of endosulfan to native and introduced fish in Australia. Environ. Toxicol. Chem. 11:1469-1476.
56. Naqvi, S.M. and R. Hawkins. 1988. Toxicity of selected insecticides (Thiodan, Security, Spartan, and Sevin) to mosquitofish, *Gambusia affinis*. Bull. Environ. Contam. Toxicol. 40:779-784.
57. Rastogi, A. and S.K. Kulshrestha. 1990. Effect of sublethal doses of three pesticides on the ovary of a carp minnow *Rasbora daniconius*. Bull. Environ. Contam. Toxicol. 45:742-747.
58. Jonsson, C.M. and M.C.F. Toledo. 1993. Acute toxicity of endosulfan to the fish *Hyphessobrycon bifasciatus* and *Brachydanio rerio*. Arch. Environ. Contam. Toxicol. 24:151-155.
59. Devi, A.P., D.M.R. Rato, K.S. Tilak, and A.S. Murty. 1981. Relative toxicity of the technical grade material, isomers, and formulations of endosulfan to the fish *Channa punctata*. Bull. Environ. Contam. Toxicol. 27:239-243.
60. Haider, S. and R.M. Inbaraj. 1986. Relative toxicity of technical material and commerical formulation of malathion and endosulfan to a freshwater fish, *Channa punctatus* (Bloch). Ecotoxicol. Environ. Saf. 11:347-351.
61. Singh, B.B. and A.S. Narain. 1982. Acute toxicity of thiodan to catfish (*Heteropneustes fossilis*). Bull. Environ. Contam. Toxicol. 28:122-127.
62. Rao, D.M.R. and A.S. Murty. 1982. Toxicity and metabolism of endosulfan in three freshwater catfishes. Environ. Pollut. 27A:223-231.
63. Rao, D.M.R., A.P. Devi, and A.S. Murty. 1980. Relative toxicity of endosulfan, its isomers, and formulated products to the freshwater fish, *Labeo rohita*. J. Toxicol. Environ. Health 6:825-834.
64. Matthiessen, P. and J.W.M. Logan. 1984. Low concentration effects of endosulfan insecticide on reproductive behaviour in the tropical cichlid fish *Sarotherodon mossambicus*. Bull. Environ. Contam. Toxicol. 33:575-583.
65. Yasmeen, R., S.J. Tulasi, and J.V.R. Rao. 1991. Metabolic changes in the air-breathing fish *Anabas scandens* on long-term exposure to endosulfan. Pestic. Biochem. Physiol. 40:205-211.

66. Ferrando, M.D., E. Andreu-Moliner, M.M. Almar, C. Cebrian, and A. Nu ez. 1987. Acute toxicity of organochlorined pesticides to the European eel, *Anguilla anguilla*: the dependency on exposure time and temperature. Bull. Environ. Contam. Toxicol. 39:365-369.
67. Ferrando, M.D. and E. Andreu-Moliner. 1989. Effects of temperature, exposure time and other water parameters on the acute toxicity of endosulfan to European eel, *Anguilla anguilla*. J. Environ. Sci. Health 24B:219-224.
68. Ferrando, M.D., E. Sancho, and E. Andreu-Moliner. 1991. Comparative acute toxicities of selected pesticides to *Anguilla anguilla*. J. Environ. Sci. Health 26B:491-498.
69. Harris, M.L., C.A. Bishop, J. Struger, B. Ripley, and J.P. Bogart. 1998. The functional integrity of northern leopard frog (*Rana pipiens*) and green frog (*Rana clamitans*) populations in orchard wetlands. II. Effects of pesticides and eutrophic conditions on early life stage development. Environ. Toxicol. Chem. 17:1351-1363.
70. Gopal, K., R.N. Khanna, M. Anand, and G.S.D. Gupta. 1981. The acute toxicity of endosulfan to freshwater organisms. Toxicol. Lett. 7:453-456.
71. Vardia, H.K., P.S. Rao, and V.S. Durve. 1984. Sensitivity of toad larvae to 2,4-D and endosulfan pesticides. Arch. Hydrobiol. 100:395-400.
72. Hudson, R.H., R.K. Tucker, and M.A. Haegele. 1984. Handbook of Toxicity of Pesticides to Wildlife. United States Department of the Interior (USDI), Fish and Wildlife Service Resource Publication No. 153. USDI, Washington, D.C., U.S.A.
73. Dewitt, J.B., W.H. Stickel, and P.F. Springer. 1963. Wildlife studies, Patuxent Wildlife Research Centre. In: Pesticide-wildlife Studies: A Review of Fish and Wildlife Service Investigations During 1961 and 1962. U.S. Department of the Interior (USDI), Fish and Wildlife Service Circular No. 167. USDI, Gulf Breeze, FL, U.S.A.
74. Schafer, E.W.Jr., W.A. Bowles Jr., and J. Hurlbut. 1983. The acute oral toxicity, repellency, and hazard potential of 998 chemicals to one or more species of wild and domestic birds. Arch. Environ. Contam. Toxicol. 12:355-382.
75. Truhaut, R., J.C. Gak, and C. Graillot. 1974. Study of the modalities and action mechanisms of organochlorine insecticides. I. Comparative study of the acute toxicity in hamster and rat. J. Eur. Toxicol. 7:159-166.
76. FAO/WHO. 1968. Endosulfan. In: FAO. 1967 Evaluations of Some Pesticide Residues in Food. FAO, Rome.
77. Gaines, T.B. 1969. Acute toxicity of pesticides. Toxicol. Appl. Pharmacol. 14:515-534.
78. Frank, R., H.E. Braun, B.D. Ripley, and B.S. Clegg. 1990. Contamination of rural ponds with pesticide, 1971-85, Ontario, Canada. Bull. Environ. Contam. Toxicol. 44:401-409.
79. Mount, M.E. and F.W. Oehme. 1981. Insecticide levels in tissues associated with toxicity: a literature review. Vet. Hum. Toxicol. 23:34-42.
80. Nowak, B., A. Goodsell, and M. Julli. 1995. Residues of endosulfan in carp as an indicator of exposure conditions. Ecotoxicol. 4:363-371.
81. Kang, K-S., M.R. Wilson, T. Hayashi, C-C. Chang, and J.E. Trosko. 1996. Inhibition of gap junctional intercellular communication in normal human breast epithelial cells after treatment with pesticides, PCBs, and PBBs, alone or in mixtures. Environ. Health Perspect. 104:192-200.
82. Ruch, R.J., R. Fransson, S. Flodstrom, L. Warngard, and J.E. Klaunig. 1990. Inhibition of hepatocyte gap junctional intercellular communication by endosulfan, chlordane and heptachlor. Carcinogenesis 11:1097-1101.
83. Kenne, K. R. Fransson-Steen, S. Honkasalo, and L. Warngdrd. 1994. Two inhibitors of gap junctional intercellular communication, TPA and endosulfan: different effects on phosphorylation of connexin 43 in the rat liver epithelial cell line, IAR 20. Carcinogenesis 15:1161-1165.
84. Fransson-Steen, R. and L. Warngdrd. 1992. Inhibitory effects of endosulfan on gap junctional intercellular communication in WB-F344 rat liver cells and primary rat hepatocytes. Carcinogenesis 13:657-662.
85. Pednekar, M.D., S.R. Gandhi, and M.S. Netrawali. 1987. Evaluation of mutagenic activities of endosulfan, phosalone, malathion, and permithrin, before and after metabolic activation, in the Ames

Salmonella test. Bull. Environ. Contam. Toxicol. 38:925-933.
86. Hack, R., E. Ebert, and K-H. Leist. 1995. Chronic toxicity and carcinogenicity studies with the insecticide endosulfan in rats and mice. Fd. Chem. Toxic. 33:941-950.
87. Gupta, P.K., S.V. Chandra, and D.K. Saxena. 1978. Teratogenic and embryotoxic effects of endosulfan in rats. Acta Pharmacol. Toxicol. 42:150-152.
88. Pandey, N., F. Gundevia, A.S. Prem, and P.K. Ray. 1990. Studies on the genotoxicity of endosulfan, an organochlorine insecticide, in mammalian germ cells. Mutat. Res. 242:1-7.
89. Lakshmana, M.K. and T.R. Raju. 1994. Endosulfan induces small but significant changes in the levels of noradrenaline, dopamine and serotonin in the developing rat brain and deficits in the operant learning performance. Toxicology 91:139-150.
90. Zaidi, N.F., A.K. Agrawal, M. Anand, and P.K. Seth. 1985. Neonatal endosulfan neurotoxicity: behavioural and biochemical changes in rat pups. Neurobehav. Toxicol. Teratol. 7:439-442.
91. Matthiessen, P. 1981. Haematological changes in fish following aerial spraying with endosulfan insecticide for tsetse fly control in Botswana. J. Fish Biol. 18:461-469.
92. Pandey, A.C. 1988. Impact of endosulfan (Thiodan) EC 35 on behaviour and dynamics of oocyte development in the teleostean fish, *Colisa* (Trichogaster) *fasciatus*. Ecotoxicol. Environ. Saf. 15:221-225.
93. Singh, N.N. and A.K. Srivastava. 1981. Effects of endosulfan on fish carbohydrate metabolism. Ecotoxicol. Environ. Saf. 5:412-417.
94. Shukla, L. and A.K. Pandey. 1986. Effects of endosulphan on the hypothalamo- hypophysial complex and fish reproductive physiology. Bull. Environ. Contam. Toxicol. 36:122-131.
95. Matthiessen, P. and R.J. Roberts. 1982. Histopathological changes in the liver and brain of fish exposed to endosulfan insecticide during tsetse fly control operations in Botswana. J. Fish Dis. 5:153-159.
96. Bradbury, S.P., R.W. Carlson, G.J. Niemi, and T.R. Henry. 1991. Use of respiratory-cardiovascular responses of rainbow trout (*Oncorhynchus mykiss*) in identifying acute toxicity syndromes in fish: Part 4. central nervous system seizure agents. Environ. Toxicol. Chem. 10:115-131.
97. Corbett, J.R. 1974. The Biochemical Mode of Action of Pesticides. Academic Publishers, New York, NY, U.S.A.
98. Nowak, B. 1992. Histological changes in gills induced by residues of endosulfan. Aquat. Toxicol. 23:65-84.
99. Johal, M.S. and A. Dua. 1994. SEM study of the scales of freshwater snakehead, *Channa punctatus* (Bloch) upon exposure to endosulfan. Bull. Environ. Contam. Toxicol. 52:718-721.
100. Douthwaite, R.J., P.J. Fox, P. Matthiessen, and A. Russell-Smith. 1981. The environmental impact of endosulfan applied for tsetse fly control in the Okavango Delta, Botswana. Overseas Development Administration, London, England.
101. Haider, S. and R.M. Inbaraj. 1988. *In vitro* effect of malathion and endosulfan on the LH-induced oocyte maturation in the common carp, *Cyprinus carpio* (L.). Water Air Soil Pollut. 39:27-31.
102. Inbaraj, R.M. and S. Haider. 1988. Effect of malathion and endosulfan on brain acetylcholinesterase and ovarian steroidogenesis of *Channa punctatus* (Bloch). Ecotoxicol. Environ. Saf. 16:123-128.
103. Chakravorty, S., B. Lal, and T.P. Singh. 1992. Effect of endosulfan (thiodan) on vitellogenesis and its modulation by different hormones in the vitellogenic catfish *Clarias batrachus*. Toxicology 75:191-198.
104. Sinha, N., B. Lal, and T.P. Singh. 1991. Effect of endosulfan on thyroid physiology in the freshwater catfish, *Clarias batrachus*. Toxicology 67:187-197.
105. Jensen, E.G., J.U. Skaare, E. Egaas, and A. Goksryr. 1991. Response of xenobiotic metabolizing enzymes in rainbow trout (*Oncorhynchus mykiss*) to endosulfan, detected by enzyme activities and immunochemical methods. Aquat. Toxicol. 21:81-92.
106. Gill, T.S., J. Pande, and H. Tewari. 1990. Hepatopathotoxicity of three pesticides in a freshwater fish, *Puntius conchonius* Ham. J. Environ. Sci. Health 25A(6):653-663.
107. Sharma, R.M. 1988. Effect of endosulfan on adenosine triphosphatase (ATPase) activity in liver, kidney, and muscles of *Channa gachua*. Bull. Environ. Contam. Toxicol. 41:317-323.
108. Gimeno, L., M.D. Ferrando, S. Sanchez, L.O. Gimeno, and E. Andreu. 1995. Pesticide effects on eel metabolism. Ecotoxicol. Environ. Saf. 31:153-157.
109. DangJ, A.D. 1986. Changes in carbohydrate metabolism in tilapia, *Oreochromis* (*Sarotherodon*)

mossambicus, during short-term exposure to different types of pollutants. Environ. Pollut. 41A:165-177.
110. Sastry, K.V. and A.A. Siddiqui. 1983. Metabolic changes in the snake head fish *Channa punctatus* chronically exposed to endosulfan. Water Air Soil Pollut. 19:133-141.
111. Murty, A.S. and A.P. Devi. 1982. The effect of endosulfan and its isomers on tissue protein, glycogen, and lipids in the fish *Channa punctata*. Pestic. Biochem. Physiol. 17:280-286.
112. Gill, T.S., J. Pande, and H. Tewari. 1991. Effects of endosulfan and phosphamidon poisoning on the peripheral blood of fish (*Barbus conchonius* Hamilton). J. Environ. Sci. Health 26A(2):249-255.
113. Gill, T.S., J. Pande, and H. Tewari. 1991. Effects of endosulfan on the blood and organ chemistry of freshwater fish, *Barbus conchonius* Hamilton. Ecotoxicol. Environ. Saf. 21:80-91.
114. Gill, T.S., J. Pande, and H. Tewari. 1990. Enzyme modulation by sublethal concentrations of aldicarb, phosphamidon, and endosulfan in fish tissues. Pestic. Biochem. Physiol. 38:231-244.
115. Arora, N. and S.K. Kulshrestha. 1985. Effects of chronic exposure to sublethal doses of two pesticides on alkaline and acid phosphatase activities in the intestine of a fresh water teleost, *Channa striatus* Bl. (Channidae). Acta Hydrochim. Hydrobiol. 13:619-624.
116. Sharma, R.M. 1990. Effect of endosulfan on acid and alkaline phosphatase activity in liver, kidney, and muscles of *Channa gachua*. Bull. Environ. Contam. Toxicol. 44:443-448.
117. Pandey, A.C. 1986. Response of corticosteroidogenic, catecholamine-secreting cells, corpuscles of Stannius, and Dahlgren cells of snake headed murrel *Ophiocephalus punctatus* (Bloch) to Thiodan treatment - a karyometric investigation. Ecotoxicol. Environ. Saf. 12:101-104.
118. Mayer, F.L., D.J. Versteeg, M.J. McKee, L.C. Folmar, R.L. Graney, D.C. McCume, and B.A. Rattner. 1992. Physiological and nonspecific biomarkers. In: R.J. Huggett, R.A. Kimerle, R.M. Mehrle Jr., and H.L. Bergman, eds. Biomarkers: Biochemical, Physiological, and Histological Markers of Anthropogenic Stress. Lewis Publishers, Boca Raton, FL, U.S.A.
119. Sunderam, R.I.M., G.B. Thompson, J.C. Chapman, and D.M.H. Cheng. 1994. Acute and chronic toxicity of endosulfan to two Australian cladocerans and their applicability in deriving water quality criteria. Arch. Environ. Contam. Toxicol. 27:541-545.
120. Barry, M.J., D.C. Logan, J.T. Ahokas, and D.A. Holdway. 1995. Effect of algal food concentration on toxicity of two agricultural pesticides to *Daphnia carinata*. Ecotoxicol. Environ. Saf. 32:273-279.
121. Barry, M.J. 1996. Effects of an organochlorine pesticide on different levels of biological organization in *Daphnia*. Ecotoxicol. Environ. Saf. 34:239-251.
122. Krishnan, M. and S. Chockalingam. 1989. Toxic and sublethal effects of endosulfan and carbaryl on growth and egg production of *Moina micrura* Kurz (Cladocera:Moinidae). Environ. Pollut. 56:319-326.
123. Naqvi, S.M. and D.J. Newton. 1991. Chronic toxicity of Thiodan (endosulfan) insecticide to Louisiana crayfish, *Procambarus clarkii*. J. Environ. Sci. Health 26B:437-447.
124. Colborn, T., F.S. vom Saal, and A.M. Soto. 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ. Health Perspect. 101:378-384.
125. US EPA. 1997. Special report on environmental endocrine disruption: an effects assessment and analysis. Risk Assessment Forum, US Environmental Protection Agency, Washington, D.C. Report No. EPA/630/R-96/012.
126. Katzenellenbogen, J.A. 1995. The structural pervasiveness of estrogenic activity. Environ. Health Perspect. 103(Suppl.7):99-101.
127. Soto, A.M., K.L. Chung, and C. Sonnenschein. 1994. The pesticides endosulfan, toxaphene, and dieldrin have estrogenic effects on human estrogen-sensitive cells. Environ. Health Perspect. 102:380-383.
128. Soto, A.M., C. Sonnenschein, K.L. Chung, M.F. Fernandez, N. Olea, and F.O. Serrano. 1995. The E-SCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. Environ. Health Perspect. 103(Suppl.7):113-122.
129. Palmer, B.D., L.K. Huth, D.L. Pieto, and K.W. Selcer. 1998. Vitellogenin as a biomarker for xenobiotic estrogens in an amphibian model system. Environ. Toxicol. Chem. 17:30-36.
130. Sumpter, J.P. and S. Jobling. 1995. Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. Environ. Health Perspect. 103:173-178.

3.0 ETHYLENE BISDITHIOCARBAMATE (EBDC) FUNGICIDES

Dithiocarbamates are a class of compounds widely applied in industry and agriculture for their biocidal properties as well as for their metal chelating abilities [1,2]. They may be subdivided by chemical structure into two distinct groups: 1) the N,N'-ethylene bisdithiocarbamates (EBDCs) which are derived from primary amines, and 2) the N-substituted mono- and di-alkyl dithiocarbamates which are derived from secondary amines.

Most chemicals in both groups contain metals and share many structural elements; however, there are some fundamental differences in how they degrade and react within environmental - and specifically biological - systems. In general, the EBDCs induce less acute and chronic responses than the alkyl dithiocarbamates [1-3]. Since the EBDCs are the predominant class of dithiocarbamates used in Ontario agriculture [4], this chapter will discuss their presence in Ontario environments, with only a peripheral mention of other dithiocarbamates where relevant.

3.1 Description and Use

The ethylene bisdithiocarbamates (EBDCs) were developed as broad spectrum contact fungicides during and shortly after World War II [1]. In Ontario, 1993 agriculture surveys listed three EBDCs in use on fruit and vegetable crops. Mancozeb (155 463 kg), metiram (57 230 kg), and maneb (49 440 kg) were applied as fungicides to control rusts, blights, scabs and mildews [4]. Table 3.1 elaborates on EBDC use as recommended by the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) [5-9].

Maneb (CAS No. 12427-38-2) was developed in 1950 [10] by adding manganese sulphate or manganese chloride to nabam (carbon disulfide and ethylene diamene combined in the presence of sodium hydroxide); if zinc salts are substituted for manganese, the EBDC zineb (CAS No. 12122-67-7) is formed [3].

Mancozeb (CAS No. 8018-01-7) was developed later, in 1961 [10] by forming a complex of maneb with zinc salts. In addition to the applications listed in Table 3.1, mancozeb is also used in several combinations: with copper to control bacterial diseases; with metalaxyl (Ridomil MZ 72WP) to control downy mildew and root rot; with dimethomorph (Acrobat MZ) to control potato blights; and with benomyl to control gummy stem blight and powdery mildew [5,7,9]. Metiram (CAS No. 9006-42-2) is a polymer complex of zineb and ethylenebisthiuram disulfide. Metiram or mancozeb may be combined with myclobutanil or Superior oil to control scabs, and with benomyl to control powdery mildew [8]. Zineb was recommended for application to several crops in 1997/98 spray guidelines (Table 3.1), but was not evident in 1993 provincial use estimates [4]. The EBDC compounds are all very closely related, and their use is interchangeable on many crops [5-9].

Commercial formulations of maneb, mancozeb, metiram and zineb registered in Canada [11] include wettable powders (containing 75-80% active ingredient), wettable or soluble granules (75% active ingredient), solutions (37% active ingredient), and dusts (7-50% active ingredient, for seed treatments only). Domestic use products are not widely available which likely indicates Canadian support of a United States Environmental Protection Agency (EPA) recommendation.

Upon special review of EBDCs, the EPA cancelled several home-and-garden uses, as they assumed non-registered users would not wear the protective clothing essential during application and would, thus, expose themselves to the potential teratogenic, carcinogenic and goitrogenic properties of this class of dithiocarbamates [12,13]. The limited presence of inert ingredients in most formulations reduces the potential for non-active ingredient toxic effects, but does not eliminate it.

A group of Italian researchers reported that the commercial formulation, Maneb 80, contained 20% sodium lignin sulfonate and n-butyl-naphthalene sulfonate as dispersal and wetting agents, respectively [14-16]; their inert ingredient control did not produce teratogenicity in test subjects. The latter compound is a substituted polycyclic aromatic hydrocarbon (PAH).

The presence of PAHs in wettable powders and solutions available in Ontario is likely but not confirmed. The mancozeb dispersable granular product available in Ontario, Dithane DG, contains sodium lignosulfonate, sodium sulfate, and manganese sulfate, with a further 1-2% in unknown "related reaction

products" [17] which are probably ethylenethiourea and derivatives thereof.

Use of mancozeb and maneb in Ontario are shown on a countywide basis in Figures 3.1 and 3.2. Mancozeb applications are widespread throughout the southern portion of the province, and even further north into Georgian Bay and Ottawa regions. Increased use in Middlesex, Niagara, Grey, Dufferin and Simcoe counties reflects applications to fruit and cole crops. Metiram and maneb were not relied upon as heavily for fungicidal uses, with a combined total still several thousand kilograms less than mancozeb. Reported use of maneb was limited to Elgin, Simcoe and York counties. It should be noted that these use maps do not include ginseng, greenhouse, or mushroom treatments; thus, they likely underestimate the magnitude and distribution of EBDC use in Ontario.

3.2 Environmental Concentrations

EBDCs may be applied to Ontario crops by ground boom or airblast sprayers and infrequently, aircraft. These application methods in some conditions increase the risk of off-target spraying and drift. Despite this increased probability and the substantial use of EBDCs in Ontario agriculture, routine chemical analyses have not detected residues in surface waters [18]. However, the lack of detections may be the result of several factors other than the lack of EBDC introductions to surface waters, and, more importantly, may not be indicative of the lack of associated toxicity in Ontario wildlife.

Analytical methods developed for EBDCs in several media were essentially created in an effort to quantify human exposure. They were intended to assess the presence of hazardous residues on food, and consequently exhibit relatively high detection limits around 10-50 ppb - i.e. mg/L, mg/kg [18-20]. If this limit lies above that associated with toxicity, then lack of detections does not imply lack of toxic concentrations in various environmental media. More sensitive analytical methods have since been developed, but are still not widely applied [20].

The second and perhaps more critical factor in terms of toxicity concerns the degradation behaviour of EBDCs in soil and water. EBDCs are highly unstable in the presence of moisture and oxygen. Rhodes [21] determined that maneb had a half-life of 4-8 weeks in a Keyport silt loam soil. Maneb also adsorbs strongly to soil particles, decreasing the likelihood of groundwater contamination [22]. Van Leeuwen et al. [23] provide half-conversion times for hydrolysis of several dithiocarbamates, including maneb and zineb. They conclude that their stability in water is highly dependent on pH and the metal present; maneb half-conversion times varied from 0.08 h at pH 3.8 to 265 h at pH 8.0 (data taken from Klisenko and Vekshtein 1971, cited in [23]). Mancozeb has a reported half-life of less than one day in sterile water, pH 5-9 [1].

The principle breakdown products of all EBDCs are ethylene thiourea (ETU), ethylene urea (EU), ethylene diamine (EDA), ethylene bisisothiocyanate sulfide (EBIS), carbon disulfide, and inorganic metallic salts [3,24]. Some of these products, especially ETU, produce high chronic toxicity in laboratory animals [20]. Despite the greater likelihood of the presence of breakdown products in environmental compartments and their sometimes high relative toxicity, routine analytical methods do not detect ETU, EU or EBIS. Sensitive analytical methods exist for some of these compounds, but have not been applied to water, plant or soil/sediment samples collected in Ontario. For example, Hogendoorn et al. [25] describe a reverse-phase liquid chromatography procedure with a reported detection limit for ETU in ground-water of 0.05 ppb.

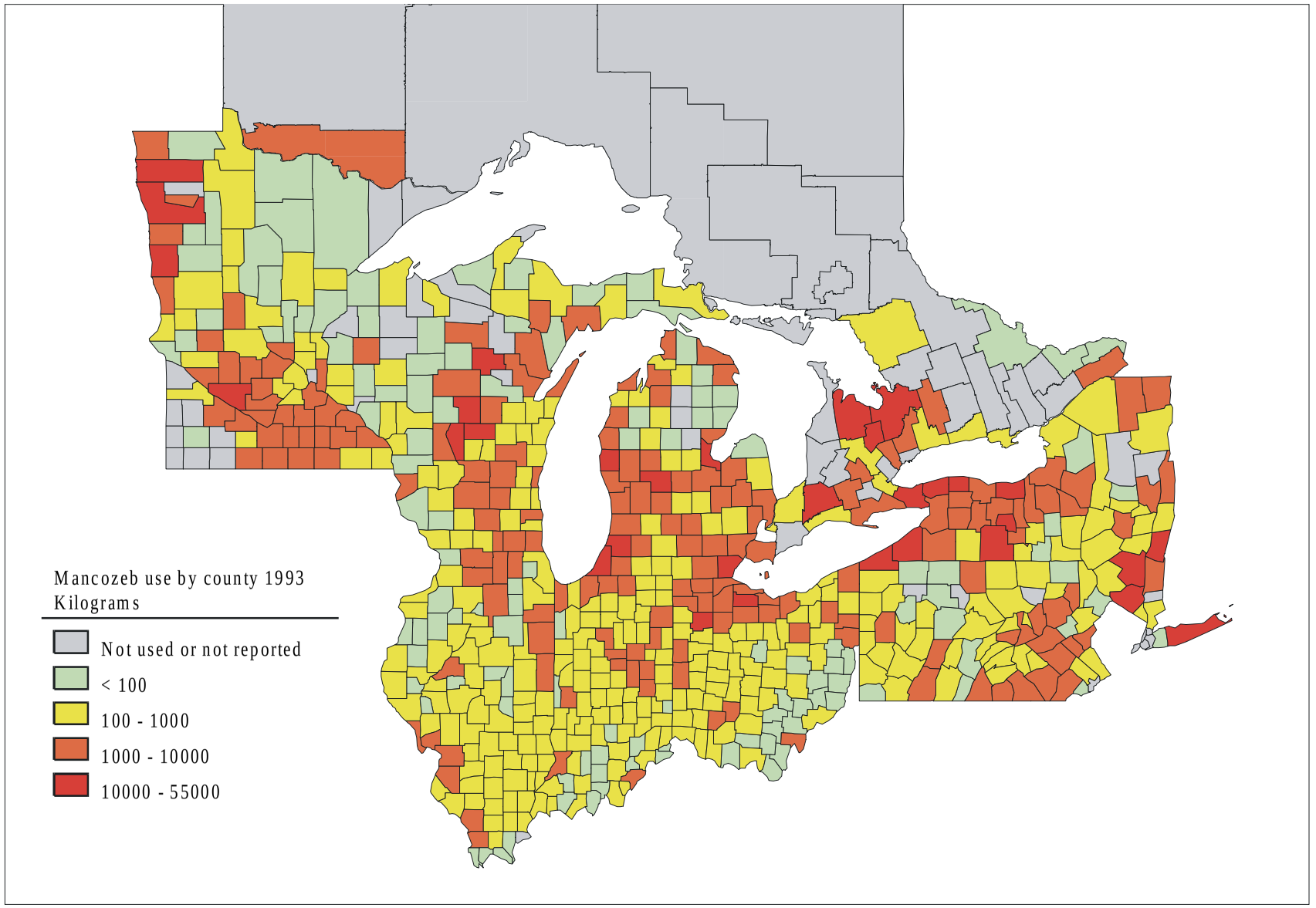

Figure 3.1 Mancozeb use in theGreat Lakes Basin.

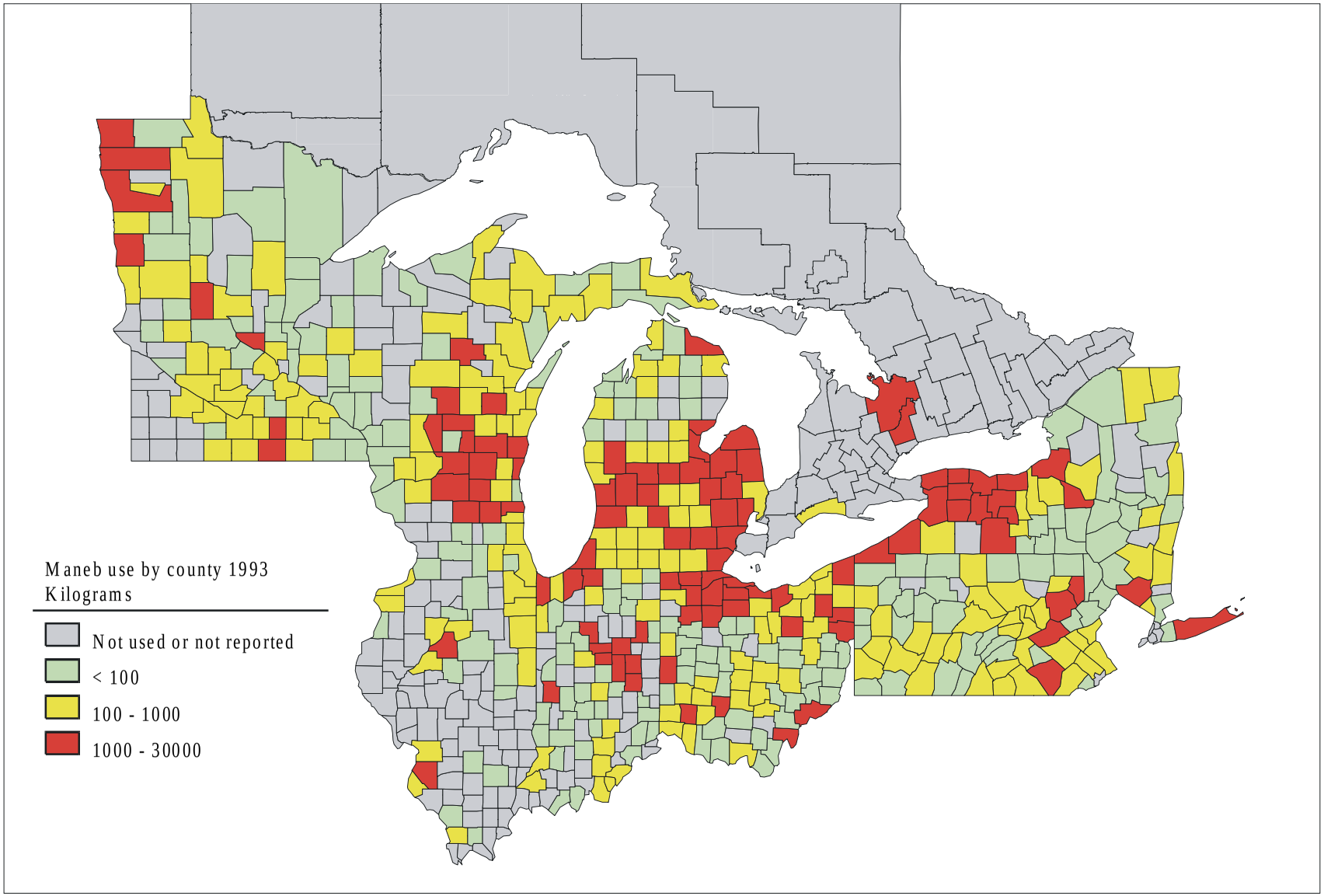

Figure 3.2 Maneb use in theGreat Lakes Basin.

Table 3.1 Recommended [5-9] applications of EBDCs in Ontario agriculture, 1998.

Fungicide	Crop(s) Protected	Pest(s) Controlled	Application Rate[a]
amancozeb	ginseng	leaf blight, stem canker, head blight	4.4 kg/ha in 2000L water
	carrot	leaf spots, blight	2.25 kg/ha
	celery	early, late & bacterial blights	2.25 kg/ha
	cucumber, melon, pumpkin, squash	cucumber scab, anthracnose, leaf spot	1.1-3.25 kg/ha
	greenhouse tomato	early & late blight, Septoria leaf spot	2 kg/ha
	apple	scabs, cedar apple rust, quince rust	6.0 kg/ha
	grape	downy mildew	3.25-5.5 kg/a
maneb	cabbage	black leaf spot	2.25-3.25 kg/ha
	celery, potato	early, & late blights	2.25 kg/ha
	lettuce, endive	downy mildew	2.25-3.25 kg/ha
	onion	downy mildew, leaf blight, purple blotch	3.25 kg/ha
	tomato	early blight, Septoria leaf spot, anthracnose	3.25 kg/ha
metiram	asparagus	rust, gray mold	2.25-3.25 kg/ha
	celery	early, late & bacterial blights	2.25 kg/ha seed
	potato	seed-piece decay	225-325 g/50 kg
	grape	downy mildew, black rot	3.25-5.5 kg/ha
zineb	mushroom	dry & wet bubble, green & cobweb molds	60-125 g/100 m^2
	cabbage, cauliflower, broccoli, brussels sprouts	downy mildew, black leaf spot	2.25-3.25 kg/ha
	greenhouse lettuce	downy mildew	1.75-2.25 kg/ha

[a] - Two or more formulations (often with differing EBDC active ingredients) were usually listed in OMAFRA publications, but only one was chosen as an example for this table. Note that recommendations for vegetables listed an applied mass (kg) or volume (L) only [7,9]; it was assumed that these values were to be applied on a 'per hectare' basis. Fruit tree 'per hectare' recommendations were based on 4.5 - 5.5 m high trees.

3.3 Bioaccumulation and Metabolism of EBDCs

Several studies in mammals and fish have shown that EBDCs do not bioaccumulate or bioconcentrate, and are rapidly excreted or metabolized [1,20,26]. However, metabolism pathways are distinct in several test species [20,27,28], suggesting that species differences in metabolite production and subsequent toxicity may be great.

When Brocker and Schlatter [29] examined the metabolism of maneb in female rats, they found that over 95% of the radio-labelled compound was excreted in faeces, urine and respired air within 72 hours of oral exposure. When treatment was supplemented with metal ions, excretion rates were essentially unchanged, although less was actually absorbed by the intestine since the proportion excreted via the faeces increased considerably. Similar excretion rates have been reported in mice administered maneb and zineb [30]. Although Van Leeuwen et al. [26] could not distinguish between parent and metabolite compounds in a retention study in rainbow trout, retention times appeared considerably longer in this fish species with a 96-h aqueous exposure to radio-labelled zineb. Substantial activity was still discernable in some tissues (e.g. melanophores and thyroid follicles) after 16 days.

The metabolism of EBDCs is complex and involves decomposition into carbon disulfide, EDA, EBIS, ETU, 5,6-dihydro-3H-imidazo (2,1-c)-1,2,4-dithiazole-3-thion (DIDT), polymeric ethylenethiuram disulfide (PETD), 2-imidazoline, and hydrogen sulfide [1,2]. Subtle differences in metabolism pathways have been suggested as possible explanations for toxicity differences amongst species. For instance, Iverson et al. [28] found that ETU was rapidly metabolized to S-methyl ETU in cats, but not in rats; they suggested that this explained why teratogenicity was associated with ETU exposure only in rats.

Additionally, researchers have observed either inhibition or induction of the cytochrome P450 detoxification system in different species exposed to EBDCs, suggesting that this basic mechanism for metabolism may be functional in only some species with respect to this set of compounds (discussed further in [20]). These findings have important ramifications for assessing risk to wildlife, because one cannot predict how a particular EBDC fungicide will be metabolized in untested wild species based on available information from laboratory animals.

3.4 Toxic Mechanisms of Action

The rapid and complex degradation and metabolism pathways characteristics of EBDCs hinder investigations of modes of action. Few published studies that apply EBDCs to biological systems attempt to differentiate between effects produced by specific parent compounds and their metabolites. A general *in vitro* study of acute toxicity of dithiocarbamates suggested that there was a common cellular/cytotoxic mechanism of action [31]. Aside from the reasonably well-documented effects of ETU on the pituitary-thyroid axis [1,32-34], most effects may be best explained by a general cytotoxic mechanism, with multiple pathways tied to the redistribution and action of metals on enzyme biochemistry.

3.5 Acute Toxicity

Concerns of human health effects from exposure to EBDCs have been expressed, with respect to the suspected carcinogenic and teratogenic properties of ETU. ETU residues were detected in food and were found to be enhanced by some cooking processes [1,20]. Consequently, the vast majority of toxicity studies have assessed responses of laboratory animals (rats, mice, chickens) with little effort made to extract environmental relevance from the derived data.

Acute toxicity tests are summarized in Tables 3.2 and 3.3. The majority of analyses were completed on laboratory mammals, but some limited information was also available for fish, amphibians and birds. Although differences in exposure and test conditions make it difficult to compare, the aquatic species appear to be more acutely sensitive to EBDCs than mammals or birds by several orders of magnitude. In addition, the one invertebrate (*Daphnia magna*) and one amphibian (green frog) tested, along with the most sensitive fish species (rainbow trout) tested, showed approximately equal sensitivity to a common EBDC, mancozeb.

This suggests that the acute lethality response in water occurs within a relatively narrow range of concentrations for all aquatic groups. A comprehensive study by Van Leeuwen provided most of the aquatic data listed, and showed considerably greater toxicity associated with some metabolites compared to parent compounds. While ETU and EU exhibited very low acute toxicity, less studied metabolites like PETD and DIDT exhibited toxicity about an order of magnitude greater than the parent compounds mancozeb and maneb.

Aquatic LC50 values for maneb, mancozeb and metiram could still be considered high compared to other pesticides. For example, endosulfan produced 96-hr LC50s for most sensitive fish species in the range 0.0002 - 0.001 mmol/L (0.0001 - 0.0005 mg/L), whereas these EBDCs produced 96-hr LC50s in the range 1.3 - 13.9 mmol/L (0.34 - 6.4 mg/L). The EBDC LC50s also lie above the current detection limits (0.01 - 0.05 mg/L); however, even though the three major aquatic groups tested appear to have similar sensitivity to parent compounds, the actual number of species tested is extremely low - one invertebrate, one amphibian and three fish. The possibility that other indigenous aquatic species might be more sensitive than those assessed needs to be investigated, given the relatively narrow margin of safety provided by currently used analytical detection limits. In addition, the more toxic metabolites, which have not been assayed for in Ontario surface waters and which are more likely to be present in higher concentrations need further evaluation.

Among tests with birds and mammals, LD50s for most species could not be accurately derived because they lay above the range of test concentrations. Since bioaccumulation and bioconcentration of EBDCs is low, the most likely scenario for substantial intake of EBDCs by these animal groups might be by incidental ingestion while foraging in treated fields. Even then, it seems unlikely that wildlife could ingest enough to attain acutely lethal body burdens.

Persistence of organophosphorus insecticides in granular form on treated fields in B.C. has been identified as a major source of waterfowl and raptor poisonings [37], but EBDCs are less likely to persist as granules, given that the only granular formulations are soluble or dispersable slurries more amenable to environmental breakdown. Accessibility of granules to waterfowl, gulls, small birds or mammals in agricultural fields has not been evaluated and cannot be dismissed, but it does seem unlikely to be of a magnitude capable of producing acute effects.

Many studies with rats, mice and birds described a common set of neurological symptoms associated with exposure to lethal concentrations of EBDCs. Animals exposed to EBDCs for extended periods in their diet exhibited ataxia and, in mammals, paralysis of hind limbs [1,24,40,41,44]. Unresponsiveness, loss of muscular tone, and alopecia were also observed. When investigators searched for nervous lesions to explain these symptoms they could find none [24,41], and they concluded that the behaviour was a manifestation of biochemical processes, rather than a specific neurological mode of action.

This summary of acute information and available monitoring data for water and sediment suggests that the EBDCs are likely not present in Ontario environments at acutely toxic concentrations. However, this conclusion is based on a very small number of tests on indigenous wildlife species and a slightly larger number of tests on laboratory species. Data for invertebrates, fish, amphibians and birds is sparse, while that for reptiles and wild mammals is nonexistent. The apparent low risk posed by EBDCs and some of the common metabolites to wildlife is still connected with a high degree of uncertainty.

Table 3.2 Aquatic toxicity of EBDCs and their metabolites. Species indigenous to Ontario are identified by an (I) after the species name. Sample size (N) indicates the no. animals/replicate x no. replicates.

Group	Species	Common Name	Age	N	Chemical Form	Dose Method	Test Type	LC50 (mg/L)	R
Crustacea	*Daphnia magna (I)*	Water flea	-	-	maneb (90%)	static renewal	48-hr	1.0	2
Crustacea	*Daphnia magna (I)*	Water flea	-	-	mancozeb (64%)	static renewal	48-hr	1.3	2
Crustacea	*Daphnia magna (I)*	Water flea	-	-	mancozeb	-	48-hr	1.0	17
Crustacea	*Daphnia magna (I)*	Water flea	-	-	metiram (61.5%)	static renewal	48-hr	2.2	2
Crustacea	*Daphnia magna (I)*	Water flea	-	-	PETD (97%)	static renewal	48-hr	0.28	2
Crustacea	*Daphnia magna (I)*	Water flea	-	-	DIDT (98%)	static renewal	48-hr	0.21	2
Crustacea	*Daphnia magna (I)*	Water flea	-	-	ETU (99%)	static renewal	48-hr	26.4	2
Crustacea	*Daphnia magna (I)*	Water flea	-	-	EU (97%)	static renewal	48-hr	5600	2
Cyprinodontiformes	*Poecilia reticulata*[a]	Guppy	-	-	maneb (90%)	static renewal	96-hr	3.7	2
Cyprinodontiformes	*Poecilia reticulata*[a]	Guppy	-	-	mancozeb (64%)	static renewal	96-hr	2.6	2
Cyprinodontiformes	*Poecilia reticulata*[a]	Guppy	-	-	metiram (61.5%)	static renewal	96-hr	6.4	2
Cyprinodontiformes	*Poecilia reticulata*[a]	Guppy	-	-	PETD (97%)	static renewal	96-hr	0.88	2
Cyprinodontiformes	*Poecilia reticulata*[a]	Guppy	-	-	DIDT (98%)	static renewal	96-hr	0.49	2
Cyprinodontiformes	*Poecilia reticulata*[a]	Guppy	-	-	ETU (99%)	static renewal	96-hr	7500	2
Cyprinodontiformes	*Poecilia reticulata*[a]	Guppy	-	-	EU (97%)	static renewal	96-hr	13000	2

Table 3.2 Continued.

Group	Species	Common Name	Age	N	Chemical Form	Dose Method	Test Type	LC50 (mg/L)	R
Salmonidae	*Oncorhynchus mykiss (I)*	Rainbow trout	embryo-larval	100 x 2	maneb (90%)	static renewal	60-d	0.165	2
Salmonidae	*Oncorhynchus mykiss (I)*	Rainbow trout	embryo-larval	100 x 2	zineb (95%)	static renewal	60-d	0.211	2
Salmonidae	*Oncorhynchus mykiss (I)*	Rainbow trout	embryo-larval	100 x 2	ETU (99%)	static renewal	60-d	1800	2
Salmonidae	*Oncorhynchus mykiss (I)*	Rainbow trout	embryo-larval	100 x 2	EU (97%)	static renewal	60-d	10000	2
Salmonidae	*Oncorhynchus mykiss (I)*	Rainbow trout	early fry	10 x 2	maneb (90%)	static renewal	96-hr	0.34	35
Salmonidae	*Oncorhynchus mykiss (I)*	Rainbow trout	-	-	mancozeb	-	96-hr	0.46	17
Centrarchidae	*Lepomis macrochirus (I)*	Bluegill sunfish	-	-	mancozeb	-	96-hr	1.35	17
Amphibia	*Rana clamitans (I)*	Green frog	embryo-larval	10 x 2	Dithane DG	static renewal	96-hr	0.96-2.21	36
Amphibia	*Rana clamitans (I)*	Green frog	embryo-larval	10 x 2	Dithane DG	static renewal	2(96-hr)[b]	0.2	36
Amphibia	*Rana clamitans (I)*	Green frog	embryo-larval	10 x 2	Dithane DG	static renewal	13-d	0.023	36

[a] - not indigenous, but introduced to Cave and Basin Hotspring, Banff National Park.
[b] - test consisted of two 96-hr exposures, separated by 7.5 days in which animals were transferred to clean reference water.

Table 3.3 Mammalian and avian toxicity of EBDCs and their metabolites. Species indigenous to Ontario are identified by a (I) after the species name. Sample size (N) = no. animals (replicates).

Species	Common Name	Age	N	Chemical Form	Dose Method	Test Type	Concentration[a]	R
Agelaius phoeniceus (I)	Red-winged blackbird	adults	-	maneb (technical)	oral, gavage	LD50	>100	38
Agelaius phoeniceus (I)	Red-winged blackbird	adults	-	zineb (technical)	oral, gavage	LD50	>100	38
Sturnus vulgaris (I)	European starling	adults	-	zineb (technical)	oral, gavage	LD50	>100	38
Coturnix japonica	Japanese quail	14-d	10	manzate (maneb)	diet	5-d LC50	>5000	39
Coturnix japonica	Japanese quail	14-d	10	zineb	diet	5-d LC50	>5000	39
Coturnix sp.	Quail	-	-	mancozeb	oral	LD50	6400	17
Anas platyrhynchos (I)	Mallard	1-yr	6	zineb (95%)	oral, gavage	14-d LD50	>2000	40
Anas platyrhynchos (I)	Mallard	-	-	mancozeb	oral	LD50	>6400	17
Phasianus colchicus (I)	Ring-necked pheasant	3-4 mo.	3	zineb (95%)	oral, gavage	14-d LD50	>2000	40
Rattus norvegicus (I)	Rat	juveniles	8-16	maneb	single oral	28-d LD50	>8000	41
Rattus norvegicus (I)	Rat	juveniles	8-16	zineb	single oral	28-d LD50	8200-8900	41
Rattus norvegicus (I)	Rat	-	-	mancozeb	oral	LD50	>8000	42
Rattus norvegicus (I)	Rat	-	-	metiram	oral	LD50	>10000	42
Mus muscaris (I)	Mouse	juveniles	8-16	maneb	single oral	28-d LD50	>8000	41
Mus muscaris (I)	Mouse	-	-	maneb	oral	LD50	4100	1
Mus muscaris (I)	Mouse	juveniles	8-16	zineb	single oral	28-d LD50	7600-7700	41
Mus muscaris (I)	Mouse	-	-	metiram	oral	LD50	>5400	43-
	Guinea pig	-	-	metiram	oral	LD50	2400-4800	43

[a] - active ingredient in mg/kg body weight

3.6 Chronic Effects

The complexity associated with investigating chronic, sublethal effects in organisms exposed to a highly metabolized set of compounds is likely responsible for what often seems to be contradictory interpretations of effects in the published literature. The predominant effects described with some consensus involve the pituitary-thyroid axis and are ramified in the thyroid and in the early development process.

3.6.1 Chronic effects in mammals

The bulk of experiments with laboratory rats and mice have focussed on the mutagenic and carcinogenic properties of EBDCs and their metabolites. Although most investigators found that ETU was a carcinogen and not a mutagen, there was little consensus regarding the parent compounds, maneb, mancozeb, metiram and zineb. Reviews of the early literature on mutagenicity of maneb and zineb concluded that the burden of evidence was not indicative of mutagenic effects [1,45]. Another assessment of over 90 genotoxicity studies also confirmed that ETU is not mutagenic [46].

However, more recent investigations, all with mancozeb, detected a low level of mutagenicity associated with prolonged exposure to high doses [47-53]. All of these recent tests based their positive conclusions on increased frequency of sperm head abnormalities or bone marrow cell structural chromosomal aberrations, which became significant at concentrations approaching LD50 or maximum tolerable dose values. In another mancozeb experiment with high doses but much shorter exposure times, no evidence of mutagenicity was found [54].

Given the negative results of the Ames test, combined with the extreme nature of exposure conditions in other tests, one could argue that observed aberrations represented a general cytotoxic response triggered by the toxic stress, and were not indicative of mutagenicity per se. At the very least, the experimental design provides little context for environmentally relevant exposure and suggests that there would be little risk of mutagenicity in natural environments.

Similar contradictory information exists for the carcinogenic potential of EBDCs. Again, reviews of the early literature suggest that maneb and zineb are not carcinogenic in mice, but may be in rats, and ETU is carcinogenic in both species [1,45,55]. Pulmonary and thyroid tumours were most frequently encountered in positive tests with ETU and zineb in rats. More recent studies, again all with mancozeb, found that it was a tumour-promoter and co-carcinogen when applied to mouse skin [56-58]. The mouse studies all found that only benign tumours resulted from exposure, while another similar experiment with rats found carcinomas when mancozeb was used as a promoter with nitrosomethylurea [59]. As in the mutagenicity studies, however, exposure conditions were characterized by high doses, often incurring substantial mortality, over prolonged periods. The relevance for environmental exposure is questionable.

The teratogenicity of EBDCs in rats has been fairly well-documented (reviewed in [1,55]), but there is some question of how widespread this property is amongst other exposed species. When Larsson et al. [60] dosed pregnant female mice and rats once by gavage with maneb or mancozeb on the 9th - 13th day of gestation, they found significantly greater frequencies of skeletal and internal deformities in rat foetuses but not in mouse foetuses. They noted that additions of zinc reduced effects and also that the zinc-complexed mancozeb was less potent than maneb; these observations supported their theory that rats were particularly susceptible to embryonic zinc deficiency caused by the chelating properties of EBDCs, which in turn made the species particularly susceptible to teratogenic effects.

The teratogenic properties of ETU also appear to be species-specific; exposure to ETU during pregnancy produced teratogenicity in rats [61,62] and (at very high doses) in mice, but not in guinea pigs or hamsters (reviewed in [1]). Rats given a single oral dose of ETU (30 mg/kg) on the 15th day of gestation had foetuses with brain deformities ranging from hydrocephalus to a condition resembling hydrancephaly, defined as the complete absence of cerebral hemispheres [61]. They could not determine if the anomalies were the result of negative effects on the maternal thyroid gland or developing thyroid gland (which matures on the 18th day of gestation) or the result of some other toxic mechanism. Another study found that

teratogenic abnormalities produced by ETU in rats could be prevented by simultaneous exposure to sodium nitrite [63].

Further investigation found that addition of the nitrite resulted in the transformation of ETU to the apparently non-teratogenic form, N-nitrosoethylenethiourea. Even though rats show the most convincing evidence for teratogenicity of EBDCs, the route of exposure also appeared to influence the teratogenic potential; researchers exposing females to mancozeb during the gestational period via inhalation rather than oral gavage did not observe any effects on the foetuses [44].

The suggested primary mechanism of action for EBDCs and/or their metabolites is linked to the pituitary-thyroid axis. Colborn et al. [64] used a thyroid study by Laisi et al. [65] as justification for including maneb and zineb in their list of potential endocrine disrupters. Laisi and collaborators found that an acute intra peritoneal exposure to either compound decreased cold-stimulated thyroid-stimulating hormone (TSH) secretion in rats. From their findings, they hypothesized that the EBDCs were acting on endogenous thyrotropin-releasing hormone (TRH) at the hypothalamic or pituitary level, possibly by inhibiting dopamine b-hydroxylase.

Inhibition of this enzyme leads to accumulation of dopamine and depletion of noradrenaline in the brain. Some support for their theory may come from a related study with the dithiocarbamate thiram in which exposure resulted in an increase in adrenal dopamine via a decrease in the b-hydroxylase assisted conversion of dopamine to noradrenaline [66]. The authors suggested that plasma levels of the enzyme were undergoing chelation with copper released during the metabolism of thiram.

Other qualitative studies with maneb, mancozeb and zineb found that thyroids were enlarged in treated rats [67,68], but no mechanistic pathway could be determined. Other peripheral evidence of thyroid dysfunction involves the consistent observation of body weight loss upon exposure to EBDCs [24,32,33,41,68,69] and the teratogenic properties discussed above. Thyroid hormones play key roles in processes of growth and development [34].

Laisi et al. [65] also tested the effects of the metabolite ETU on rat TSH secretion and found no effect. Earlier studies with ETU found it to be goitrogenic, causing severe thyroid hyperplasia (reviewed in [1]). Rats developed both benign and malignant thyroid tumours after prolonged (12-mo.) dietary exposure to elevated (500 mg/kg) ETU concentrations [32].

More recent enzyme studies found that ETU competitively inhibited thyroid peroxidase activity [70] and decreased plasma thyroxine (T_4) concentrations [33]. In keeping with the high degree of inter-species variability in responses, Nebbia et al. [69] found that zineb exposure produced no effect on serum TSH concentrations (contrary to Laisi's findings with rats), but decreased serum triiodothyronine (T_3) and T_4 concentrations in rabbits. These later studies suggest that more than one metabolic route may be affected in thyroid tissue of EBDC-exposed animals. Arnold et al. [33] also suggested that ETU-induced thyroid lesions may be partly reversible when a recovery period is allowed.

3.6.2 Chronic effects in birds

Most studies involving birds and EBDC exposure concerned teratogenic effects and, as with mammalian experiments, the results were often contradictory. Embryo-toxicity tests with chickens were conducted by dipping eggs in a maneb solution for 30 seconds and then incubating treated eggs for a set period, around 19 days. One set of investigators found that concentrations as low as 1/5 the recommended application rate increased the frequency of unilateral limb deformities in developing embryos [71]. A second set of investigators repeated the study with minor modifications and found no teratogenicity [72]; however, closer examination of their data showed that the authors dismissed a potentially important increase in the frequency of 'dead-in-shell' deformities.

Both sets of authors showed some bias in design and interpretation that detracted from the credibility of their findings. The latter study by Munk and Schulz [72] suggested that design elements such as the dipping procedure and experimental termination before chick hatching might have influenced the results obtained in the first study by Maci and Arias [71]. Nonetheless, Munk and Schulz (employees of the manufacturer of maneb) seemed to be more concerned with negating the results of the earlier study and

actually interpreted some potentially important findings in their own study (mentioned above) rather peripherally. An earlier study in which chicken eggs were injected with the metabolite ETU, Korhonen et al. [73] concluded that, with an ED50 of 4.5 mmol/egg, ETU was a relatively weak teratogen.

De Snoo [3] reviewed the reproductive toxicity of dithiocarbamates in birds, reporting on studies with maneb and zineb as well as with the alkyldithiocarbamates (the other division of dithiocarbamates besides EBDCs). While the alkyldithiocarbamates produced dramatic effects on ovaries and testes, the EBDCs had no effect on egg production or ovary weight in laying hens. It was hypothesized that the reproductive effects produced by some of the non-EBDCs were related to inhibition of dopamine b-hydroxylase, a primary toxic mechanism discussed previously in the mammalian section. The lack of inhibition by EBDCs was attributed to steric restrictions that prevented their access to the active site [74].

3.6.3 Chronic effects in amphibians

The bulk of data available on amphibians was conducted by one set of investigators and concerned the teratogenic potential of maneb in newts. Zavanella and collaborators used the regenerating limb of the newt as an experimental model to evaluate teratogenicity of aquatic pollutants [15,75,76]. Adult limbs were bilaterally amputated under anaesthesia, followed by a brief recovery period before percutaneous discontinuous exposure to maneb solutions.

This protocol produced delayed growth and numerous skeletal deformities at a concentration of 5 mg/L [15,76]. Secondary observations of increased skeletal bulk, decreased muscular development, and vascular hyperplasia supported their theory that deformities were related to vascular disruptions; they suggested that observed large hemorrhagic regions might have acted as mechanical or biochemical obstacles to normal morphogenesis of cartilaginous skeletal elements [15]. No effects on thyroid or nervous tissues were seen during histological examinations [15,16].

Other embryo-larval studies also detected teratogenic effects in frogs. Notochord deformities were observed in African clawed frog (*Xenopus laevis*) larvae exposed to 2.5 mg/L maneb [77], and in green frog (*Rana clamitans*) larvae exposed to 0.08 mg/L mancozeb [37]. Tests with the transparent larvae of the clawed frog showed internal abnormalities of the gut and the otoliths (an organ of balance) as well. The latter were suggested as the cause for observed behavioural anomalies where tadpoles were unable to orient themselves to swim. Both Bancroft and Prahlad [77] and Zavanella et al. [15] also observed delayed pigmentation in developing tissues. The latter authors suggested that EBDCs may inhibit tyrosinase activity, thus interfering with normal melanogenesis.

Maneb and mancozeb delayed growth in regenerating limbs of newts [15] and in developing tadpoles of clawed frogs [77] and green frogs [37]. Green frog tadpoles exposed to mancozeb appeared to be most sensitive, showing significant growth inhibition at 0.08 mg/L [37].

3.6.4 Chronic effects in fish

A compilation of studies completed in the Netherlands by Van Leeuwen is the only examination of EBDC chronic effects in fish [2]. In 60-day early life stage tests, rainbow trout were exposed to several EBDCs and related metabolites from fertilized egg to juvenile stages. Growth was reduced at 18 mg/L maneb and 32 mg/L zineb. An increased frequency of deformities was also observed and most were notochordal in nature; they included lateral (scoliosis), ventral (lordosis), and dorsal (kyphosis) curvature/flexure, and dwarfing of the trunk section.

Histological examination showed that deformities were the result of ectopic osteogenesis of the bone and to irregularities in surrounding muscle tissue. Teratogenicity became apparent at 32 mg/L maneb, but was more prevalent upon exposure to some of the metabolites, such as PETD (effects at 10 mg/L) and DIDT (effects at 3.2 mg/L). Van Leeuwen suggested that teratogenic effects might be induced by chelation of metal ions. By chelating copper, EBDCs could reduce the amount available for the enzyme lysyl oxidase which is necessary for reactions strengthening the structural integrity of collagen in the developing notochord.

Some similarities were seen between effects in rainbow trout and early life stage tests with other animals. Van Leeuwen [2] found that the 21-day LC50 values for *Daphnia magna* correlated strongly (r=0.93-0.96) with the rainbow trout 60-d EC50 values. Also, impaired balance and other swimming behavioural anomalies observed were identical to those described by Bancroft and Prahlad [77] in tadpoles of clawed frogs.

Vascular disruptions reported were similar to those described by Zavanella et al. [15] in regenerating newt limbs. Retarded rates of pigmentation noted in rainbow trout juveniles were also seen in amphibians [15,77]. Further analysis of pigmentation effects in rainbow trout showed that EBDCs or their metabolites were accumulating in melanophores [23]; the authors suggested that EBDCs inhibit phenoloxidase (an enzyme involved in melanin synthesis) via chelation with copper. Similar accumulation of EBDCs in thyroid follicles was tentatively related to other chelating properties or the affinity for sulfhydryl groups of thyroglobulin [23].

3.6.5 Chronic effects in invertebrates

Van Leeuwen et al. [78] also provided the only information on invertebrate chronic effects. During *Daphnia magna* life cycle tests, reduced population growth with EBDC exposure was due to both increased mortality and fewer clutches per female. Since effects were typically not observed until the LC50 concentration was approached, Van Leeuwen suggested that all life cycle effects could be explained by general cytotoxicity mechanisms.

3.7 Potential for EBDCs to Act as Endocrine Disruptors

Our working definition of an endocrine disrupting compound:

> *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 rationale for including EBDCs such as maneb, mancozeb and metiram in lists of potential endocrine disruptors is founded on *in vivo* observations of effects originating along the pituitary-thyroid axis [1,32-34,64,65]. Although several effects studies found similar symptomology and enzyme studies supported theories of a point of action somewhere in the hypothalamic-pituitary region, there has never been clear evidence that the parent compounds elicit the toxic mechanisms. The major metabolite, ETU, has been implicated as the causative agent in many cases, but it is also not clear that all thyroid-related effects resulted from this metabolite alone [65]. This ambiguity does not negate the endocrine disruption capabilities of the class of compounds; however; it does imply that these capabilities may be of a species-specific nature (given observed differences in metabolism pathways). There is no evidence from any organism class that EBDCs act via the endocrine disruption pathway of most concern - that is, via effects on reproductive hormones.

To meet the criteria of our definition of an endocrine disrupter, a compound must interfere with endogenous chemical messengers at non-lethal doses that are relevant to environmental or tissue concentrations (see chapter 1). The discussions above imply that experimental mammals only show thyroid interference at concentrations approaching lethal doses which are most definitely not environmentally relevant; effects in these species would therefore, not meet our criteria for endocrine disruption. However, teratogenicity observed in amphibians and fish did occur at environmentally relevant concentrations (at or below analytical detection limits) and may qualify EBDCs or their metabolites as endocrine disruptors in these animal groups.

Although some investigators postulated a pituitary-thyroid foundation for teratogenic effects in rats [62], others suggested that they were the result of a general cytotoxic mechanism [2]. Further mechanistic investigations with aquatic species must be completed to clarify the endocrine disrupting potential of EBDCs in lower vertebrates.

3.8 Risk to Ontario Environments and Recommendations

One of the primary limitations for evaluations of risk associated with EBDCs is the high analytical detection limits characteristic of surveys for the environmental presence of residues in surface waters, soil and tissues. Although limited assessments of toxicity in wildlife suggest that parent compounds are not present at levels capable of producing acutely lethal effects, there is insufficient information to also dismiss acute lethality induced by some of the more toxic breakdown products (PETD, DIDT). In addition, there are fish and amphibian studies using indigenous species that describe chronic effects manifested at aqueous concentrations at or below currently applied analytical detection limits. The following issues should be priorities for further evaluation of the extent and degree of risk posed by EBDCs and their metabolites and degradation products:

- analytical chemistry methods used in environmental monitoring must be modified to improve detection limits for EBDCs, and also must be expanded to include some of the most important/toxic metabolites, such as ETU, DIDT and PETD;
- high-use regions should be identified so that they may be the focus of a multi-year survey (which uses improved chemical methods) to quantify the worst-case scenario for environmental concentrations of EBDCs and metabolites in Ontario;
- surveys of environmental concentrations should include evaluations of tissue concentrations of ETU in wild mammals, birds, reptiles, amphibians and fish. This metabolite shows the most convincing evidence of thyroid goitrogenic effects and pathological studies suggest that Great Lakes fish and bird populations are particularly susceptible to goiter induction [79,80]. Surveys should include seed-eating birds foraging in fields in the spring where potato seeds or other treated seeds have been recently sown;
- chronic effects studies with indigenous fish and amphibians should be expanded to identify relevant variations in metabolic pathways, and to explore mechanisms of action, specifically as they relate to endocrine disrupter potential.

Further studies may build on these suggested priorities, but currently the emphasis should be placed on improved field quantification of exposure and laboratory expansion of data on chronic effects in lower vertebrates.

3.9 References

1. World Health Organization (WHO). 1988. Dithiocarbamate Pesticides, Ethylenethiourea, and Propylenethiourea: a General Introduction. International Programme on Chemical Safety (IPCS) Environmental Health Criteria No. 78. WHO, Geneva, Switzerland.
2. Van Leeuwen, C.J. 1986. Ecotoxicological Aspects of Dithiocarbamates. PhD. Thesis, University of Utrecht, Utrecht, Netherlands.
3. de Snoo, G. 1986. Effects of Dithiocarbamates on Birds. Report of the Ecotoxicology Work Group/ Information Centre for Biology, Free University of Amsterdam, Amsterdam, Netherlands.
4. Hunter, C. and B. McGee. 1994. Survey of pesticide use in Ontario, 1993. Estimates of pesticides used on field crops, fruit and vegetable crops, provincial highway roadsides, and by licensed pesticide applicators. Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), Economics Information Report No. 94-01. Policy Analysis Branch, OMAFRA, Toronto, ON, Canada.
5. Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). 1997. 1997-1998 Ginseng pest control recommendations. OMAFRA Publication No. 610. OMAFRA, Toronto, ON, Canada.
6. Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). 1997. 1997-1998 Ontario mushroom pesticide recommendations. OMAFRA Publication No. 367. OMAFRA, Toronto, ON, Canada.
7. Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). 1998. Vegetable production

recommendations 1998-1999. OMAFRA Publication No. 363. OMAFRA, Toronto, ON, Canada.
8. Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). 1998. Fruit production recommendations 1998-1999. OMAFRA Publication No. 360. OMAFRA, Toronto, ON, Canada.
9. Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). 1998. Pest management recommendations for greenhouse crops. OMAFRA Publication No. 365. OMAFRA, Toronto, ON, Canada.
10. Martin, H. and C.R. Worthing.1976. Insecticide and Fungicide Handbook For Crop Protection (5th ed.). Blackwell Scientific Publications, Oxford, England.
11. North American Compendiums. 1996. Pest Control Canada - A Reference Manual (11th ed.). North American Compendiums Ltd., Hensall, ON, Canada.
12. US Environmental Protection Agency (EPA). 1988. Guidance for the Registration of Pesticide Products Containing Maneb as the Active Ingredient. Office of Pesticides and Toxic Substances, US EPA, Washington, D.C.
13. US Environmental Protection Agency (EPA). 1988. Guidance for the Reregistration of Pesticide Products Containing Metiram as the Active Ingredient. Office of Pesticides and Toxic Substances, US EPA, Washington, D.C.
14. Zaffaroni, N.P., T. Zavanella, and E. Arias. 1979. Peripheral blood cells in the crested newt after long-term exposure to the fungicide manganese ethylenebisdithiocarbamate (maneb). Bull. Environ. Contam. Toxicol. 23:587-591.
15. Zavanella, T., N.P. Zaffaroni, and E. Arias. 1984. Abnormal limb regeneration in adult newts exposed to the fungicide maneb 80. A histological study. J. Toxicol. Environ. Health 13:735-745.
16. Arias, E., N.P. Zaffaroni, and T. Zavanella. 1982. A study on the genesis of maneb-induced malformations of the regenerating limb of the adult crested newt. In: C. Vago and G. Matz, eds. Proceedings of the First International Colloquium on Pathology of Reptiles and Amphibians, Angers, France. pp. 223-226.
17. Rohm and Haas Canada. 1993. Material Safety Data Sheet for Dithane[7] DG Fungicide. Rohm and Haas Canada Inc., West Hill, ON, Canada.
18. Harris, M.L., C.A. Bishop, J. Struger, M.R. van den Heuvel, G.J. Van Der Kraak, D.G. Dixon, B. Ripley, and J.P. Bogart. 1998. The functional integrity of northern leopard frog (*Rana pipiens*) and green frog (*Rana clamitans*) populations in orchard wetlands. I. Genetics, physiology and biochemistry of breeding adults and young-of-the-year. Environ. Toxicol. Chem. 17:1338-1350.
19. Ripley, B.D. 1979. Residues of ethylenebisdithiocarbamates on field-treated fruits and vegetables. Bull. Environ. Contam. Toxicol. 22:182-189.
20. Houeto, P., G. Bindoula, and J.R. Hoffman. 1995. Ethylenebisdithiocarbamates and ethylenethiourea: possible human health hazards. Environ. Health Perspect. 103:568-573.
21. Rhodes, R.C. 1977. Studies with manganese [^{14}C]ethylenebis(dithiocarbamate) ([^{14}C]maneb) fungicide and [^{14}C]ethylenethiourea ([^{14}C]ETU) in plants, soil, and water. J. Agric. Food Chem. 25:528-533.
22. Howard, P.H. (ed). 1989. Handbook of Environmental Fate and Exposure Data for Organic Chemicals. V. III - Pesticides. Lewis Publishers, Chelsea, MI, USA.
23. Van Leeuwen, C.J., J.L. Maas-Diepeveen, G. Niebeek, W.H.A. Vergouw, P.S. Griffioen, and M.W. Luyken. 1985. Aquatic toxicological aspects of dithiocarbamates and related compounds. Short-term tests. Aquat. Toxicol. 7:145-164.
24. Freudenthal, R.I., G.A. Kerchner, R.L. Persing, I. Baumel, and R.L. Baron. 1977. Subacute toxicity of ethylenebisisothiocyanate sulfide in the laboratory rat. J. Toxicol. Environ. Health 2:1067-1078.
25. Hogendoorn, E.A., P. van Zoonen, and U.A.T. Brinkman. 1991. Column-switching RPLC for the trace-level determination of ethylenethiourea in aqueous samples. Chromatographia 31:285-292.
26. Van Leeuwen, C.J., P. Van Hameren, M. Bogers, and P.S. Griffioen. 1986. Uptake, distribution and retention of zineb and ziram in rainbow trout (*Salmo gairdneri*). Toxicology 42:33-46.
27. Newsome, W.H. 1974. The excretion of ethylenethiourea by rat and guinea pig. Bull. Environ. Contam. Toxicol. 11:174-176.
28. Iverson, F., K.S. Khera, and S.L. Hierlihy. 1980. *In vivo* and *in vitro* metabolism of ethylenethiourea in the rat and the cat. Toxicol. Appl. Pharmacol. 52:16-21.
29. Brocker, E.R. and C. Schlatter. 1979. Influence of some cations on the intestinal absorption of maneb.

J. Agric. Food Chem. 27:303-306.
30. Jordan, L.W. and R.A. Neal. 1979. Examination of the *in vivo* metabolism of maneb and zineb to ethylenethiourea (ETU) in mice. Bull. Environ. Contam. Toxicol. 22:271-277.
31. Hodgson, J.R. and C-C. Lee. 1977. Cytotoxicity studies on dithiocarbamate fungicides. Toxicol. Appl. Pharmacol. 40:19-22.
32. Graham, S.L., W.H. Hansen, K.J. Davis, and C.H. Perry. 1973. Effects of one-year administration of ethylenethiourea upon the thyroid of the rat. J. Agric. Food Chem. 21:324-329.
33. Arnold, D.L., D.R. Krewski, D.B. Junkins, P.F. McGuire, C.A. Moodie, and I.C. Munro. 1983. Reversibility of ethylenethiourea-induced thyroid lesions. Toxicol. Appl. Pharmacol. 67:264-273.
34. US Environmental Protection Agency (EPA). 1997. Special Report on Environmental Endocrine Disruption: An Effects Assessment and Analysis. Risk Assessment Forum, US EPA, Washington, D.C. Report No. EPA/630/R-96/012.
35. Van Leeuwen, C.J., P.S. Griffioen, W.H.A.Vergouw, and J.L. Maas-Diepeveen. 1985. Differences in susceptibility of early life stages of rainbow trout (*Salmo gairdneri*) to environmental pollutants. Aquat. Toxicol. 7:59-78.
36. Harris, M.L., C.A. Bishop, J. Struger, B. Ripley, and J.P. Bogart. 1998. The functional integrity of northern leopard frog (*Rana pipiens*) and green frog (*Rana clamitans*) populations in orchard wetlands. II. Effects of pesticides and eutrophic conditions on early life stage development. Environ. Toxicol. Chem. 17:1351-1363..
37. Elliott, J.E., K.M. Langelier, P. Mineau, and L.K. Wilson. 1996. Poisoning of bald eagles and red-tailed hawks by carbofuran and fensulfothion in the Fraser Delta of British Columbia, Canada. J. Wildl. Dis. 32:486-491.
38. Schafer, E.W.Jr., W.A. Bowles Jr., and J. Hurlbut. 1983. The acute oral toxicity, repellency, and hazard potential of 998 chemicals to one or more species of wild and domestic birds. Arch. Environ. Contam. Toxicol. 12:355-382.
39. Hill, E.F. and M.B. Camardese. 1986. Lethal Dietary Toxicities of Environmental Contaminants and Pesticides to Coturnix. US Department of the Interior (USDI), Fish and Wildlife Service Technical Report No. 2. USDI, Washington, D.C., USA.
40. Hudson, R.H., R.K. Tucker, and M.A. Haegele. 1984. Handbook of Toxicity of Pesticides to Wildlife (2nd ed.). US Department of the Interior (USDI), Fish and Wildlife Service Resource Publication No. 153. USDI, Washington, D.C., USA.
41. Lee, C-C., J.Q. Russell, and J.L. Minor. 1978. Oral toxicity of ferric dimethyl-dithiocarbamate (ferbam) and tetramethylthiuram disulfide (thiram) in rodents. J. Toxicol. Environ. Health 4:93-106.
42. Smith, G.J. 1987. Pesticide Use and Toxicology in Relation to Wildlife: Organophosphorus and Carbamate Compounds. US Department of the Interior (USDI), Fish and Wildlife Service Resource Publication No. 170. USDI, Washington, D.C., USA.
43. Tomlin, C. 1994. The Pesticide Manual: Incorporating the Agrochemicals Handbook (10th ed.). The British Crop Protection Council and the Royal Society of Chemistry. The Bath Press, Bath, England.
44. Lu, M-H. and G.L. Kennedy Jr. 1986. Teratogenic evaluation of mancozeb in the rat following inhalation exposure. Toxicol. Appl. Pharmacol. 84:355-368.
45. Woo, Y-T. 1983. Carcinogenicity, mutagenicity and teratogenicity of carbamates, thiocarbamates and related compounds: an overview of structure-activity relationships and environmental concerns. J. Environ. Sci. Health C1(1):97-133.
46. Elia, M.C., G. Arce, S.S. Hurt, P.J. O'Neill, and H.E. Scribner. 1995. The genetic toxicology of ethylenethiourea: a case study concerning the evaluation of a chemical's genotoxic potential. Mutat. Res. 341:141-149.
47. Georgian, L., I. Moraru, T. Draghicescu, I. Dinu, and G. Ghizelea. 1983. Cytogenetic effects of alachlor and mancozeb. Mutat. Res. 116:341-348.
48. Gautam, D.C. and L. Kapoor. 1991. Genotoxic effects of dithane —45 on the bone marrow cells of mice *in vivo*. Experientia 47:280-282.
49. Hemavathi, E. and M.A. Rahiman. 1993. Toxicological effects of ziram, thiram, and dithane —45

assessed by sperm shape abnormalities in mice. J. Toxicol. Environ. Health 38:393-398.
50. Hemavathi, E. and M.A. Rahiman. 1996. Effect of ziram, thiram, and dithane —45 on bone marrow cells of mice - assessed by micronucleus test. Bull. Environ. Contam. Toxicol. 56:190-196.
51. Khan, P.K. and S.P. Sinha. 1993. Antimutagenic efficacy of higher doses of vitamin C. Mutat. Res. 298:157-161.
52. Khan, P.K. and S.P. Sinha. 1994. Vitamin C mediated amelioration of pesticide genotoxicity in murine spermatocytes. Cytobios 80:199-204.
53. Khan, P.K. and S.P. Sinha. 1996. Ameliorating effect of vitamin C on murine sperm toxicity induced by three pesticides (endosulfan, phosphamidon and mancozeb). Mutagenesis 11:33-36.
54. Vasudev, V. and N.B. Krishnamurthy. 1994. *In vivo* cytogenetic analyses of the carbamate pesticides dithane —45 and baygon in mice. Mutat. Res. 323:133-135.
55. Fishbein, L. 1976. Environmental health aspects of fungicides. I. Dithiocarbamates. J. Toxicol. Environ. Health 1:713-735.
56. Shukla, Y., M. Antony, S. Kumar, and N.K. Mehrotra. 1988. Tumour-promoting ability of mancozeb, a carbamate fungicide, on mouse skin. Carcinogenesis 9:1511-1512.
57. Shukla, Y., M. Antony, S. Kumar, and N.K. Mehrotra. 1990. Carcinogenic activity of a carbamate fungicide, mancozeb on mouse skin. Cancer Lett. 53:191-195.
58. Mehrotra, N.K., S. Kumar, and Y. Shukla. 1990. Enhancement of tumor-initiating activity of DMBA by the carbamate fungicide mancozeb. Bull. Environ. Contam. Toxicol. 44:39-45.
59. Monis, B. and M.A. Valentich. 1993. Promoting effects of mancozeb on pancreas of nitrosomethylurea-treated rats. Carcinogenesis 14:929-933.
60. Larsson, K.S., C. Arnander, E. Cekanova, and M. Kjellberg. 1976. Studies of teratogenic effects of the dithiocarbamates maneb, mancozeb, and propineb. Teratology 14:171-184.
61. Khera, K.S. and L. Tryphonas. 1977. Ethylenethiourea-induced hydrocephalus: pre- and postnatal pathogenesis in offspring from rats given a single oral dose during pregnancy. Toxicol. Appl. Pharmacol. 42:85-97.
62. Khera, K.S. and B.G. Shah. 1979. Failure of zinc acetate to reduce ethylenethiourea-induced fetal anomalies in rats. Toxicol. Appl. Pharmacol. 48:229-235.
63. Khera, K.S. 1982. Reduction of teratogenic effects of ethylenethiourea in rats by interaction with sodium nitrite *in vivo*. Fd. Chem. Toxic. 20:273-278.
64. Colborn, T., F.S. vom Saal, and A.M. Soto. 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ. Health Perspect. 101:378-384.
65. Laisi, A., R. Tuominen, P. M@nnist‘, K. Savolainen, and J. Mattila. 1985. The effect of maneb, zineb, and ethylenethiourea on the humoral activity of the pituitary-thyroid axis in rat. Arch. Toxicol. (Suppl.8):253-258.
66. Caroldi, S. and P. de Paris. 1995. Comparative effects of two dithiocarbamates disulfiram and thiram, on adrenal catecholamine content and on plasma dopamine-b-hydroxylase activity. Arch. Toxicol. 69:690-693.
67. Ivanova-Chemishanska, L., D.V. Markov, and G. Dashev. 1971. Light and electron microscopic observations on rat thyroid after administration of some dithiocarbamates. Environ. Res. 4:201-212.
68. SzJpv‘lgyi, J., K. Nagy, K. Sajg\nJ Vuk<n, A. Reg‘ly-MJrei, K. So\s, K. T\th, A. PintJr, and M. Antal. 1989. Subacute toxicological examination of Dithane —45. Fd. Chem. Toxicol. 27:531-538.
69. Nebbia, C., M. Dacasto, F. Valenza, E. Burdino, G. Ugazio, and J. Fink-Gremmels. 1995. Effects of the subchronic administration of zinc ethylene-bis-dithiocarbamate (zineb) to rabbits. Vet. Human Toxicol. 37:137-142.
70. Doerge, D.R. and R.S. Takazawa. 1990. Mechanism of thyroid peroxidase inhibition by ethylenethiourea. Chem. Res. Toxicol. 3:98-101.
71. Maci, R. and E. Arias. 1987. Teratogenic effects of the fungicide maneb on chick embryos. Ecotoxicol. Environ. Saf. 13:169-173.
72. Munk, R. and V. Schulz. 1989. Study of possible teratogenic effects of the fungicide maneb on chick embryos. Ecotoxicol. Environ. Saf. 17:112-118.

73. Korhonen, A., K. Hemminki, and H. Vainio. 1982. Embryotoxicity of industrial chemicals on the chicken embryo: thiourea derivatives. Acta Pharmacol. Toxicol. 51:38-44.
74. Serio, R., R.A. Long, J.E. Taylor, R.L. Tolman, R.M. Weppelman, and G. Olson. 1984. The antifertility and antiadrenergenic actions of thiocarbamate fungicides in laying hens. Toxicol. Appl. Pharmacol. 72:333-342.
75. Zaffaroni, N.P., E. Arias, G. Capodanno, and T. Zavanella. 1978. The toxicity of manganese ethylenebisdithiocarbamate to the adult newt, *Triturus cristatus*. Bull. Environ. Contam. Toxicol. 20:261-267.
76. Arias, E. and T. Zavanella. 1979. Teratogenic effects of manganese ethylenebisdithiocarbamate (maneb) on forelimb regeneration in the adult newt, *Triturus cristatus carnifex*. Bull. Environ. Contam. Toxicol. 22:297-304.
77. Bancroft, R. and K.V. Prahlad. 1973. Effect of ethylenebis[dithiocarbamic acid] disodium salt (nabam) and ethylenebis[dithiocarbamato]manganese (maneb) on *Xenopus laevis* development. Teratology 7:143-150.
78. Van Leeuwen, C.J., F. Moberts, and G. Niebeek. 1985. Aquatic toxicological aspects of dithiocarbamates and related compounds. II. Effects on survival, reproduction and growth of *Daphnia magna*. Aquat. Toxicol. 7:165-175.
79. Moccia, R.D., J.F. Leatherland, and R.A. Sonstegard. 1981. Quantitative interlake comparison of thyroid pathology in Great Lakes coho (*Oncorhynchus kisutch*) and chinook (*Oncorhynchus tschawytscha*) salmon. Cancer Res. 41:2200-2210.
80. Moccia, R.D., G.A. Fox, and A. Britton. 1986. A quantitative assessment of thyroid histopathology of herring gulls (*Larus argentatus*) from the Great Lakes and a hypothesis on the causal role of environmental contaminants. J. Wildl. Dis. 22:60-70.

4.0 THE DINITROANILINE HERBICIDES, TRIFLURALIN AND PENDIMETHALIN

The 2,6-dinitroanilines were first marketed as selective weed control agents in the early 1960s [1-2]. There are three herbicides from this class currently used in Ontario agriculture - trifluralin, pendimethalin and ethalfluralin [3]; only the first two (most commonly applied) will be discussed in this chapter.

4.1 Description and Use

Manufacture and herbicidal use of trifluralin (2,6-dinitro-N, N-dipropyl-4-[trifluoromethyl] =benzenamine, CAS No. 1582-09-8) and pendimethalin (N-[1-ethylpropyl]-3,4-dimethyl-2,6-dinitrobenzenamine, CAS No. 40487-42-1) began in 1960 and 1974, respectively [2]. They may both be used to control a number of annual grass and broadleaf weed species (Table 4.1); 1993 agricultural surveys suggest that trifluralin use (83 945 kg) was slightly greater than pendimethalin use (51 414 kg) in the province [3]. Although they have similar chemical structures and modes of action in target plants [1-2], they are applied in very different ways to Ontario crops. Pendimethalin is applied to fields as a pre- or post-emergence spray, while trifluralin is generally applied by pre-plant injection into crop soils [4].

These two distinct application methods have some obvious ramifications for environmental fate of the two herbicides, particularly since dinitroanilines can adhere very strongly to soil particles [5-6].

Pendimethalin, available in two emulsifiable concentrate (EC) forms with the trade-name PROWL, is strictly limited to agricultural land applications in Ontario. Trifluralin may have broader applications within the commercial (i.e. licenced/permitted) sector; although OMAFRA [4] does not indicate that it is available for domestic use, a 1996 pesticide registration guide indicated that domestic products were available in Canada [7]. Trifluralin is produced for agricultural purposes in emulsifiable concentrate (Bonanza 400, Rival, Treflan) and dry flowable (Rival DF) forms. The EC formulations must contain solvents, but their identity is unknown. Two formulation mixtures are also used in Ontario agriculture, where these dinitroanilines are paired, during production, with an imidazolinone and a triazolopyrimidine sulfonanilide herbicide for application to soybean fields [4]. Tank mixes of trifluralin with either EPTC (a thiocarbamate), imazethapyr (an imidazolinone), metolachlor (an acetanilide) or metribuzin (a triazine) are recommended for the pre-plant control of weeds in bean, tomato, and several fruit crops [4]. Pendimethalin tank mixes with either atrazine (a triazine), cyanazine (another triazine), dicamba (a benzoic acid), imazethapyr or rimsulfuron (a sulfonylurea) are recommended for pre- or post-emergence control of weeds in field corn crops [4]. The latter dinitroaniline is also often mixed and sprayed with fertilizers.

Use estimates of trifluralin and pendimethalin in Ontario are mapped on a county-wide basis in Figures 4.1 and 4.2. Trifluralin applications in Ontario are more limited than in some US states within the Great Lakes basin. Perth county showed the highest provincial use in 1993 (Fig. 4.1), a likely reflection of its incorporation into bean fields. Pendimethalin was used heavily in Middlesex, Bruce and Wellington counties (Fig. 4.2), reflecting a greater density of onion and field corn crops in these regions. As with trifluralin, pendimethalin appears to be applied at much higher rates in some US states bordering the Great Lakes compared to Ontario.

4.2 Environmental Concentrations

As mentioned, the differences in application methods for trifluralin and pendimethalin have repercussions for the availability of the compounds in soil and water. Pendimethalin, which is applied via spray to the surface of the soil, may be more susceptible to runoff during precipitation events than trifluralin, which is injected 8-10 cm deep in the soil [4]. However, trifluralin is considerably more volatile than pendimethalin (vapour pressures of 13.7 and 4.0 mPa, respectively), and high rates of volatilization to the atmosphere have previously been recorded (reviewed in [8]). Hoff et al. [9] recorded a maximum concentration of 3400 pg/m^3 and an annual mean of 270 pg/m^3 trifluralin in southern Ontario air collected during 1988-89.

Table 4.1 Recommended [4] applications of Trifluralin and Pendimethalin in Ontario agriculture.

Herbicide	Crop Protected	Weed(s) Controlled	Application Rate
Trifluralin	beans (white, kidney, lima and snap), soybeans, sunflowers, peppers, rutabaga	barnyard, crab & old witch grasses, fall panicum, foxtail, lambs-quarters, pigweed	0.6 - 1.155 kg/ha
	canola, forage legumes	barnyard, crab & old witch grasses, fall panicum, foxtail, wild oats, lamb's-quarters, pigweed	0.6 - 1.147 kg/ha
	winter wheat, fall rye	silky bentgrass	0.383 - 0.546 kg/ha
	asparagus	barnyard, crab & old witch grasses, fall panicum, foxtail, lamb's-quarters, pigweed	1.0 - 2.0 kg/ha
	cabbage, carrots, cauliflower, broccoli, brussels sprouts, tomatoes	barnyard, crab & old witch grasses, fall panicum, foxtail, lamb's-quarters, pigweed	0.6 - 1.1 kg/ha
	peas	barnyard, crab & old witch grasses, fall panicum, foxtail, lamb's-quarters, pigweed	0.6 - 0.8 kg/ha
Pendimethalin	apples, apricots, cherries, plums, peaches, pears, strawberries, herbaceous ornamentals	barnyard, crab, witch & brome grasses, fall panicum, chickweed, lamb's-quarters, pigweed	0.6 - 1.155 kg/ha
	field corn	barnyard grass, fall panicum, foxtail, lamb's-quarters, pigweed	1.68 kg/ha
	onions	barnyard grass, fall panicum, foxtail, lamb's-quarters, pigweed	1.2 - 1.8, 3.005* kg/ha

* rates for mineral and muck soils, respectively

Environmental persistence of these compounds is related to climate and soil composition [6], particularly moisture content and microbial make-up [8]. Both compounds can bind strongly, almost irreversibly to soil particles [6]. In general, soil persistence increases with decreasing temperature and moisture [8].

Therefore, trifluralin and pendimethalin in Ontario environments might be expected to degrade more slowly than they would in more southern US states where many of the environmental fate studies have been conducted. In southwestern Ontario, Gaynor [10] calculated a soil half-life for trifluralin of 63 to 77 days, while half-lives ranging from 140 to 164 days were observed in more northern (also more sandy) maritime soils [11]. Soil half-lives for pendimethalin ranged from 33 to 67 days, depending on the soil moisture and microbial activity [12].

In the atmosphere, trifluralin is rapidly degraded via vapor phase photochemical pathways into 2,6-dinitro-a,a,a-trifluoro-*p*-toluidine and 2-ethyl-7-nitro-5-trifluoromethyl benzimidazole [8]. A peak trifluralin concentration detected in Ontario air in June coincided closely with its application season; concentrations detected in subsequent months dropped sharply [9]. Similar degradation pathways ultimately produce the same compounds in water. Isensee et al. [13] identified 8 non-polar metabolites as well as some unknown polar compounds in water from treated artificial ecosystems. The product 2-ethyl-7-nitro-5-trifluoromethyl benzimidazole predominated over time. Degradation products for pendimethalin are also numerous, and include N-(1-ethylpropyl)-5,6-dimethyl-7-nitrobenzimidazole [12].

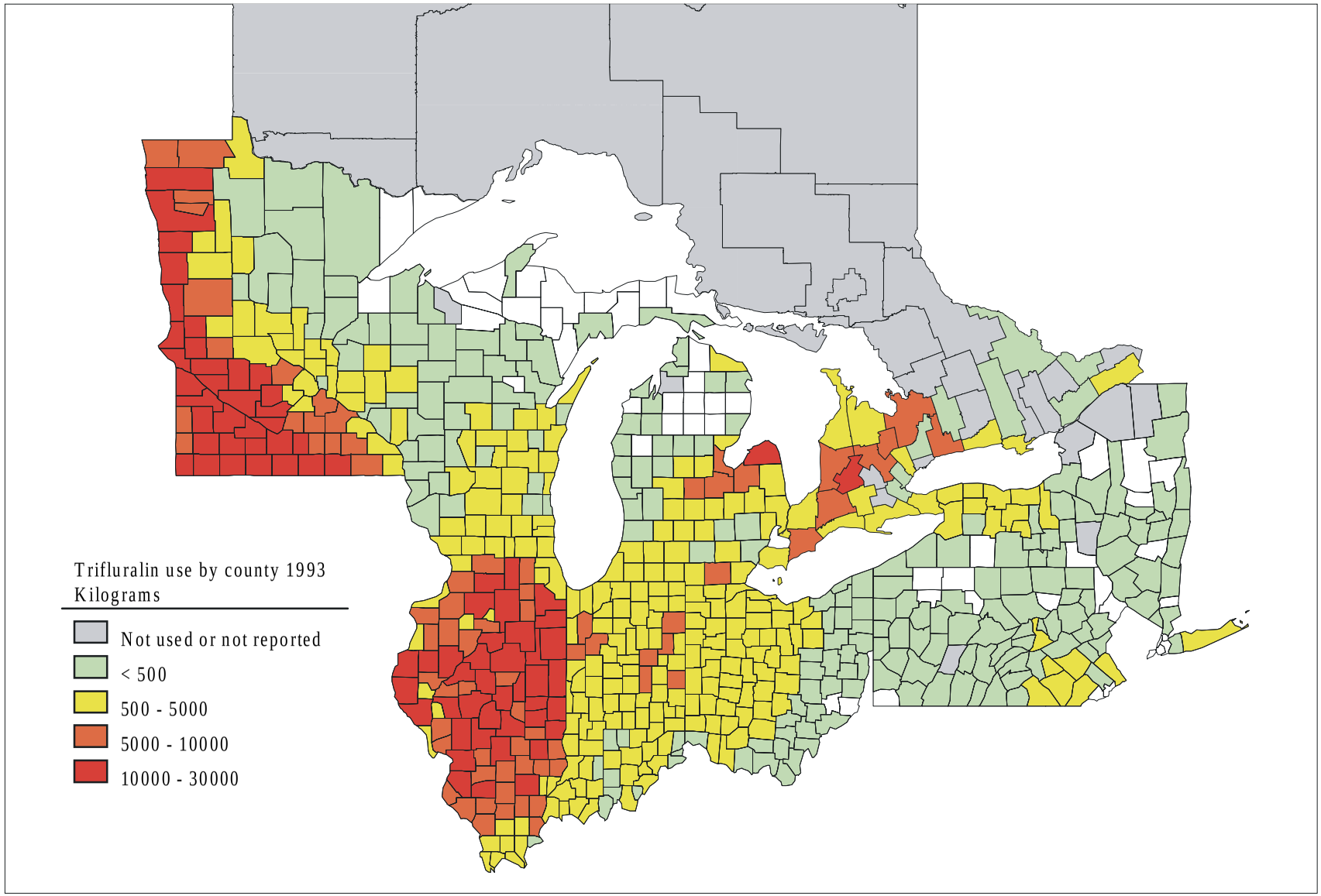

Figure 4.1 Trifluralin use in theGreat Lakes Basin.

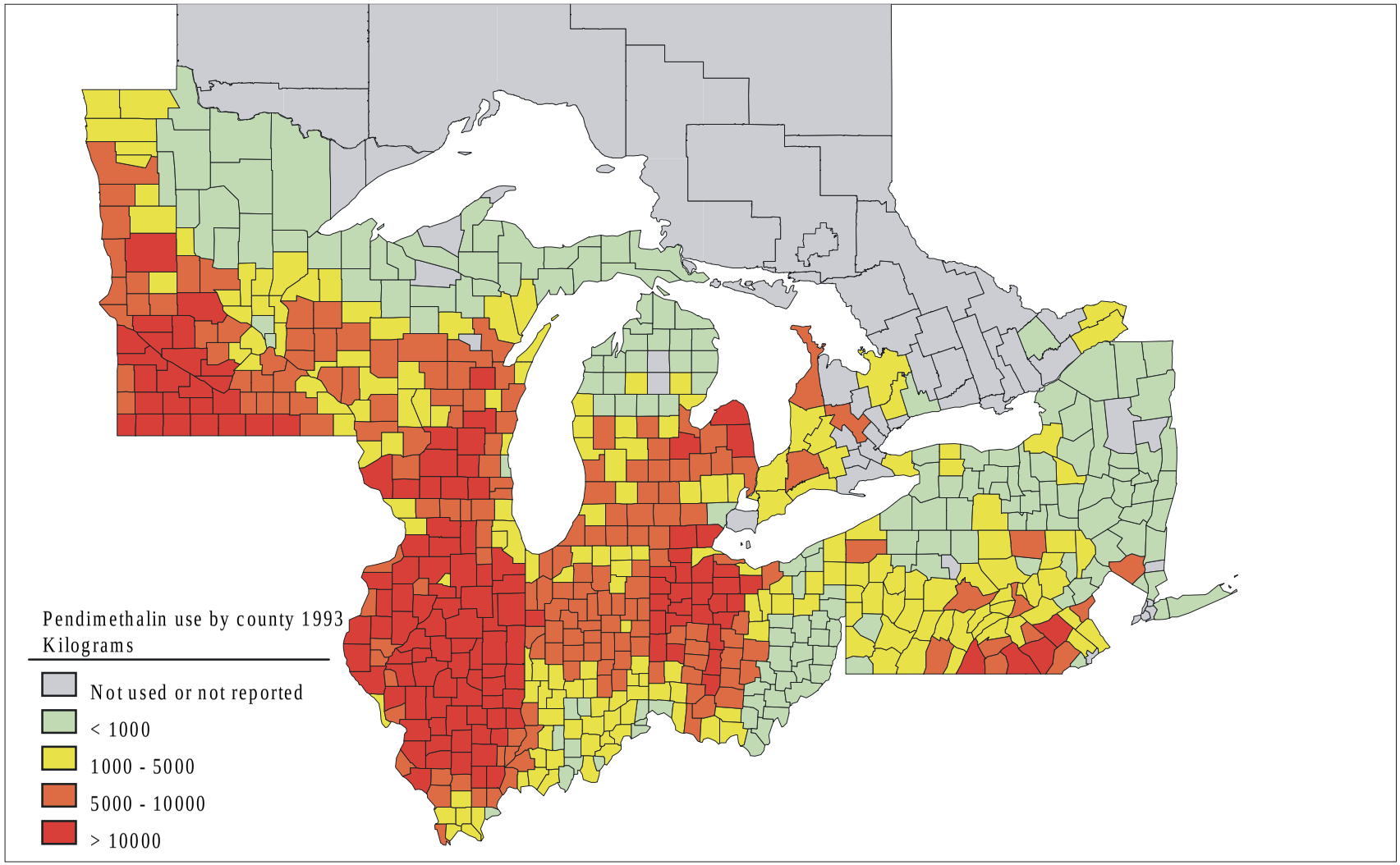

Figure 4.2 Pendimethalin use in theGreat Lakes Basin.

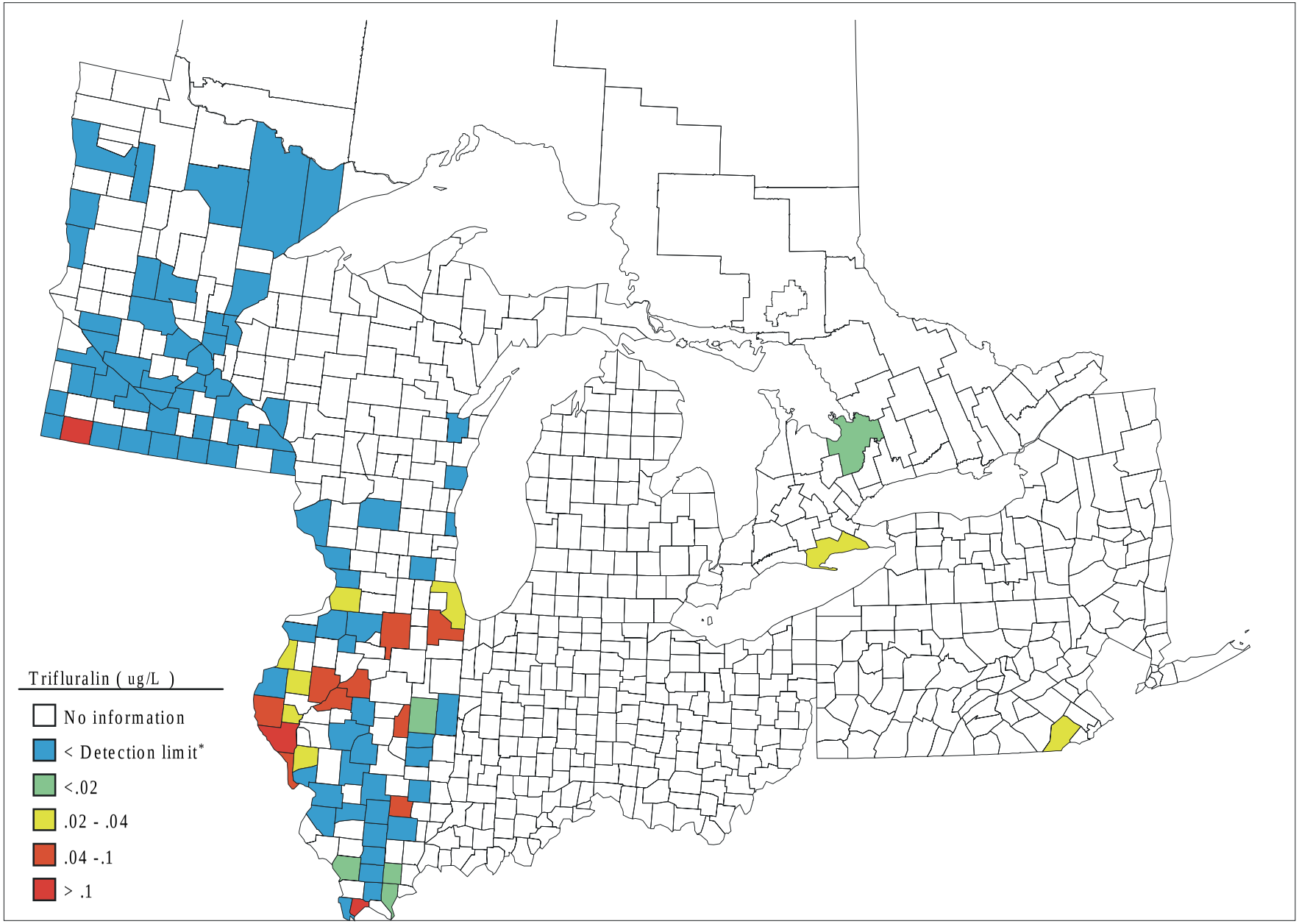

Figure 4.3 Maximum concentration of trifluralin in surface water by county. *Detection limits range from .01 -.1 ug/L.

Environmental concentrations of trifluralin documented in Ontario are shown in Figure 4.3. Similar data was not available for pendimethalin. Surface water concentrations of trifluralin in the Great Lakes basin parallels use patterns. Recorded aqueous concentrations have not exceeded 0.04 mg/L in Ontario, whereas as much as 0.3 mg/L has been found in some of the US states where use on a county basis exceeded 10 000 kg. However, detections in Ontario did not always coincide with high use areas, implying that higher surface water contamination may occur elsewhere in the province. Environmental sampling routinely takes place on particular streams or regions of interest from human health perspectives or unrelated environmental perspectives (i.e. in broad chemistry scans); this unsystematic approach to monitoring might, in this case, underestimate maximum environmental exposure. Sediment concentrations of trifluralin were only available for Ontario and ranged from 0.26 to 0.35 ng/g. Again, they were documented in a moderate use region of the province.

4.3 Metabolism and Bioconcentration in Biota

Trifluralin has a moderately high potential to bioconcentrate in aquatic biota, particularly fish. An estimated bioconcentration factor (BCF) of 1030 was derived for rainbow trout muscle, based on calculations using log K_{ow} values [14]. BCFs in five riverine fish species were evaluated *in situ* and found to range from 1800 for the golden redhorse (*Moxostoma erythrurum*) to 5800 for the sauger (*Stizostedion canadense*) [14]. The half-life for trifluralin in these larger species was calculated as ranging between 17 and 57 days. In this same study, laboratory experiments measured the BCF for fathead minnows as 3261, even though the half-life for excretion in the species was only 3 days.

A study of trifluralin toxicokinetics in rainbow trout estimated a BCF of 2280 for adults, based on a multi-compartment toxicokinetics model [15]. Wells and Cowan [16] estimated the half-life of trifluralin in Atlantic salmon (*Salmo salar*) tissue to be 40.5 days. Estimates of trifluralin bioaccumulation (tissue/water ratios) in snails (*Hellisoma sp*.) and mosquitofish (*Gambusia affinis*) in mesocosms with ^{14}C-spiked sediment were 878 and 3919, respectively [17].

Estimates based on radio-labelled herbicides should be interpreted with caution as these methods cannot differentiate between parent compounds and metabolites in either water or tissue. Similar mesocosm studies treating with pendimethalin indicated that the BCFs in mosquitofish and snails were low compared to those for trifluralin - approximately 250 and 150, respectively [18]. No other studies for pendimethalin accumulation in aquatic species were found.

Estimates of critical (lethal) body residues have not been made for fish or other aquatic biota. However, based on LC50 values (see below) and a conservative BCF value of 1000, the range of critical body residues for trifluralin would likely lie between 8.4 and 560 mg/g body weight. Environmental concentrations in fish tissue measured around the Great Lakes generally fall short of this range. A study of coho salmon (*Oncorhynchus kisutch*) captured in 12 Great Lakes tributaries all showed levels of trifluralin below 0.01 mg/g [19]. A subsequent study performed on fish resident in Lake Michigan tributaries found measurable levels of trifluralin in fish from all 14 streams. Values ranged from 0.004 to 0.126 mg/g trifluralin [20]. In Indiana, residues in several species of fish collected downstream of a trifluralin manufacturing plant were within the estimated critical body residue range; samples were taken prior to the implementation of effluent treatment [14].

Metabolism of trifluralin and pendimethalin occurs rapidly in rats, and specific transformation pathways have been studied in detail. Dinitroaniline herbicides in general are metabolized by a combination of alkyl oxidation, N-dealkylation and nitro-group reduction [21-23]. These initial reactions can proceed in any order leading to multiple metabolic pathways, but they are all ultimately followed by cyclization to a two-ring benzimidalole. After conjugation, excretion occurs by both biliary and renal routes. One study showed fish to have similar metabolic patterns [24]. In green sunfish (*Lepomis cyanellus*) the nonspecific cytochrome P450 inhibitor, piperonyl butoxide, inhibited the metabolism of trifluralin considerably [24], indicating that P450 enzymes are important in this metabolic pathway.

4.4 Toxic Mechanisms of Action

Although the mode of action of dinitroanilines in target plants is reasonably well understood, there is very little to suggest that these compounds behave similarly in animals. In weeds, they inhibit growth, primarily of the root system [1]. Intensive studies with trifluralin suggest that it inhibits cell division by binding to tubulin, thus impairing microtubule formation during mitosis [1]. Other investigators suggested that trifluralin affected plant microtubules by interfering with mitochondrial calcium accumulation [25]. The only evidence that these mechanisms may also operate in animals is a demonstrated inhibition of Ca^{2+} accumulation in rat liver mitochondria [26].

4.5 Acute Toxicity

Most of the published toxicity data for dinitroanilines report trifluralin acute lethality values. Only a handful of studies used pendimethalin in their evaluations; however, these few studies suggest that the acute toxicity of the two related compounds is within an order of magnitude. The crustacean *Daphnia magna* (Table 4.2) seemed much more sensitive to pendimethalin (48-hr EC50 = 0.017 mg/L) than to trifluralin (48-hr EC50 = 0.19-0.56 mg/L), but other tests with fish (rainbow trout, bluegill sunfish, channel catfish: Table 4.3), birds (quail) and mammals (mice, rats: Table 4.4) showed very similar toxicities within a species for the two chemicals.

With the notable exceptions of water fleas and one shrimp species (Table 4.2), fish appeared more sensitive to both compounds than aquatic invertebrates. Juvenile rainbow trout and bluegill sunfish, adult mosquitofish, and several larval marine fish species (Red Sea bream, herring, yellowtail, mullet, grunt) showed LC50s less than 0.05 mg/L (Table 4.3). The only invertebrate with an LC50 recorded in this range was the immature shrimp (*Palaemonetes kadiakensis*) with a 96-hr LC50 of 0.037 mg/L.

Nonetheless, many crustaceans and the one amphibian tested were only slightly less sensitive than these fish, with LC50s ranging 0.1 to 0.5 mg/L. Although differences in exposure conditions make comparisons difficult, most tests with birds and mammals found that LD50s were greater than the highest test concentration (Table 4.4), implying that these vertebrates were not very sensitive to either trifluralin or pendimethalin when administered orally.

When these herbicides were compared to the organochlorine, endosulfan, using toxicity values for a sensitive fish species, they were much less acutely toxic. Rainbow trout showed 96-hr LC50s of 0.00074, 0.03, and 0.491 mmol/L for endosulfan, trifluralin and pendimethalin, respectively. Unlike data available for other reviewed compounds such as EBDCs, indigenous invertebrate and fish species are fairly well represented in the published toxicity literature, at least for trifluralin, and the data in Tables 4.2 and 4.3 likely provide a reasonable range of sensitivities for wild populations in Ontario. There is, however, only one amphibian represented in the available literature and no data for reptiles. Given the consistency of the bird and mammal values for acute toxicity and the application methods for these compounds in Ontario fields (i.e. no granular forms), the risk to wild birds and small mammals posed by trifluralin and pendimethalin appears low. However, dinitroanilines do bioaccumulate to a moderate degree in fish, and the risk this exposure route poses to predators at the top of aquatic food chains has not been identified for either compound. The aquatic ecosystems once again appear to be most at risk from the environmental presence of these pesticides.

Table 4.2 Acute toxicity of dinitroanilines to aquatic invertebrates. Species indigenous to Ontario are identified by an (I) after the species name. Sample size (N) indicates the no. animals/replicate x no. replicates. LC50s represent active ingredient concentrations unless otherwise noted.

Group	Species	Common Name	Age	N	Chemical Form	Dose Method	Test Type	LC50[b] (mg/L)	R
Crustacea	*Simocephalus serrulatus (I)*	water flea	first instar	10 x -(2)	trifluralin (technical)[a]	static	48-hr EC50	0.45	27
Crustacea	*Simocephalus serrulatus (I)*	water flea	first instar	-	trifluralin (95.9%)	static	48-hr EC50	0.9	28
Crustacea	*Daphnia pulex (I)*	water flea	first instar	10 x -(2)	trifluralin (technical)[a]	static	48-hr EC50	0.24	27
Crustacea	*Daphnia pulex (I)*	water flea	first instar	-	trifluralin (95.9%)	static	48-hr EC50	0.625	28
Crustacea	*Daphnia magna (I)*	water flea	first instar	5 x 4	trifluralin (97%)[a]	static	48-hr	0.193	29
Crustacea	*Daphnia magna (I)*	water flea	first instar	-	trifluralin (95.9%)	static	48-hr EC50	0.56	28
Crustacea	*Daphnia magna (I)*	water flea	-	-	pendimethalin	flowthrough	48-hr EC50	0.0172	30
Crustacea	*Cypridopsis vidua*	ostracod	-	-	trifluralin (technical)	static	48-hr	0.25	31
Crustacea	*Procambarus clarkii*	crayfish	juvenile	10 x 10	Treflan	static	96-hr	13c	32
Crustacea	*Procambarus clarkii*	crayfish	adult	10 x 10	Treflan	static	96-hr	26c	32
Crustacea	*Procambarus clarkii*	crayfish	juvenile	10-12 x 3	trifluralin	static	24, 96-hr	13, 12	33
Crustacea	*Orconectes nais*	crayfish	-	-	trifluralin (technical)	static	48-hr	50.0	31
Crustacea	*Mysidopsis bahia*	mysid shrimp	juvenile	-	trifluralin	flowthrough	96-hr	>0.14[d]	34
Crustacea	*Asellus brevicaudus*	isopod	early instar	-	trifluralin (95.9%)	static	96-hr	>1.0	28
Crustacea	*Asellus brevicaudus*	isopod	-	-	trifluralin (technical)	static	48-hr		

Group	Species	Common Name	Age	N	Chemical Form	Dose Method	Test Type	LC50[b] (mg/L)	R
Crustacea	*Gammarus fasciatus*	amphipod	immature	-	trifluralin (95.9%)	static	24, 96-hr	8.7, 2.2	28
Crustacea	*Gammarus fasciatus*	amphipod	-	-	trifluralin (technical)	static	24, 48, 96-hr	3.2, 1.8, 1.0	31
Crustacea	*Palaemonetes kadiakensis*	shrimp	immature	-	trifluralin (95.9%)	static	24, 96-hr	0.21, 0.037	28
Crustacea	*Palaemonetes kadiakensis*	shrimp	-	-	trifluralin (technical)	static	48-hr	1.2	31
Insecta	*Chironomus riparius (I)*	midge	fourth instar larvae	-	trifluralin (95.9%)	-	48-hr EC50	1.0	35
Insecta	*Pteronarcys californica*	stonefly	2nd yr class	-	trifluralin (95.9%)	static	24, 96-hr	13, 2.8	28
Insecta	*Pteronarcys californica*	stonefly	nymph	-	trifluralin	static	48-hr	4.0	36

Table 4.2 Continued.

a - chemical was added in a solvent carrier; [27] - ethanol, [29] - acetone
b - LC50 unless otherwise stated in ‘test type’ column.
c - although test details were vague, these values likely represent the concentration of formulation and not active ingredient
d - species are marine and tests were conducted in salt water

Table 4.3 Acute toxicity of dinitroanilines to fish and amphibians. Species indigenous to Ontario are identified by an (I) after the species name. Sample size (N) indicates the no. animals/replicate x no. replicates.

Species	Common Name	Age	N	Chemical Form	Dose Method	Test Type	LC50 (mg/L)	R
Pimephales promelas (I)	Fathead minnow	44 days	- x 2	trifluralin (97%)a	flowthrough	12-day	0.115	29
Pimephales promelas (I)	Fathead minnow	juvenile	-	trifluralin (95.9%)	static	24, 96-hr	0.148-0.35, 0.105-0.16	28
Oncorhynchus mykiss (I)	Rainbow trout	-	-	pendimethalin	static	96-hr	0.138	12
Oncorhynchus mykiss (I)	Rainbow trout	juvenile	-	trifluralin (95.9%)	static	24, 96-hr	0.043-0.56, <0.014-0.33	28
Oncorhynchus mykiss (I)	Rainbow trout	juvenile	-	Treflan EC (46%)	static	24, 96-hr	0.0135-0.21, 0.01-0.098	28
Oncorhynchus mykiss (I)	Rainbow trout	fingerling	-	trifluralin (95.9%)	static	24, 96-hr	0.13, 0.086	28
Oncorhynchus mykiss (I)	Rainbow trout	swim-up fry	-	trifluralin (95.9%)	static	24, 96-hr	0.37->1.8, 0.083-0.17	28
Oncorhynchus mykiss (I)	Rainbow trout	yolk-sac fry	-	trifluralin (95.9%)	static	96-hr	1.6	28
Lepomis macrochirus (I)	Bluegill sunfish	-	-	pendimethalin	flowthrough	96-hr	0.199	12
Lepomis macrochirus (I)	Bluegill sunfish	juvenile	-	trifluralin (95.9%)	static	24, 96-hr	0.01->5.6, 0.0084-0.4	28
Micropterus salmoides (I)	Largemouth bass	juvenile	-	trifluralin (95.9%)	static	24, 96-hr	0.12, 0.075	28
Stizostedion vitreum (I)	Walleye	juvenile	-	trifluralin (95.9%)	static	24-hr	0.18	28
Carassius auratus	Goldfish	juvenile	-	Treflan EC (46%)	static	24, 96-hr	0.7, 0.145	28
Ictalurus punctatus (I)	Channel catfish	-	-	trifluralin (95.9%)	static	24, 96-hr	0.4-4.4, 0.21-2.2	28
Ictalurus punctatus (I)	Channel catfish	swim-up fry	-	trifluralin (95.9%)	static	96-hr	0.33	28
Ictalurus punctatus (I)	Channel catfish	yolk-sac fry	-	trifluralin (95.9%)	static	96-hr	0.66	28
Ictalurus punctatus (I)	Channel catfish	fingerling (1yr)	5 x -	trifluralin	static	96-hr	0.417	37
Ictalurus punctatus (I)	Channel catfish	-	-	pendimethalin	flowthrough	96-hr	0.418	38

Table 4.3 Continued.

Species	Common Name	Age	N	Chemical Form	Dose Method	Test Type	LC50 (mg/L)	R
Paralichthys olivaceus	Japanese flounder	larvae	7 x 1	trifluralin (45%)	static renewal	96-hr	0.056b	39
Gambusia affinis	Mosquitofish	adult	5-10 x 3	trifluralin	static	24, 96-hr	0.028, 0.012	39
Pagrus major	Red sea bream	larvae	6-10 x 1	trifluralin (45%)	static renewal	96-hr	0.021-0.026b	39
Clupea pallasi	Herring	larvae	7 x 1	trifluralin (45%)	static renewal	96-hr	<0.005b	39
Sebastes schlegeli	Jacopever	larvae	6 x 1	trifluralin (45%)	static renewal	96-hr	>0.074b	39
Seriola quinqueradiata	Yellowtail	larvae	6 x 1	trifluralin (45%)	static renewal	96-hr	<0.005b	39
Chasmichthys dolichognathus	Longchin goby	larvae	8 x 1	trifluralin (45%)	static renewal	96-hr	0.12b	39
Girella punctata	Girella	larvae	6 x 1	trifluralin (45%)	static renewal	96-hr	0.11b	39
Mugil cephalus	Mullet	larvae	8 x 1	trifluralin (45%)	static renewal	96-hr	0.032b	39
Parapristipoma trilineatum	Grunt	larvae	6 x 1	trifluralin (45%)	static renewal	96-hr	0.033b	39
Rasbora heteromorpha	Harlequin fish	-	-	Treflan (46%)	flowthrough	24-hr	0.6	40
Bufo woodhousii fowleri (I)	Fowlers toad	tadpoles	10 x -	trifluralin (technical)[a]	static	24, 48, 96-hr	0.18, 0.17, 0.10	41

chemical was added in a solvent carrier; [29, 41] - acetone
b - species are marine and tests were conducted in salt water

Table 4.4 Acute toxicity of dinitroanilines to birds and mammals. Species indigenous to Ontario are identified by an (I) after the species name. Sample size (N) indicates the no. individuals. LD50s represent active ingredient concentrations unless otherwis e noted.ive ingredient in mg/kg body weight unless otherwise stated

Species	Common Name	Age	N	Chemical Form	Dose Method	Test Type	Concentration[a]	R
Anas platyrhynchos (I)	Mallard[b]	3-4 mo	6	trifluralin (96.7%)	oral gavage	14-d LD50	>2000	42
Anas platyrhynchos (I)	Mallard	egg	30	Treflan	30 second immersion	18-d LC50	1.71[c]	43
Anas platyrhynchos (I)	Mallard	-	-	pendimethalin	diet	8-d LC50	10388	2
Phasianus colchicus (I)	Ring-necked pheasant[b]	3 mo	3	trifluralin (96.7%)	oral gavage	14-d LD50	>2000	42
Coturnix japonica	Japanese quail	14-d	15	Treflan EC (45%)[d]	diet	5-d LC50	>5000	44
Colinus virginianus (I)	Bobwhite quail	-	-	trifluralin	oral	LD50	>2000	2
Colinus virginianus (I)	Bobwhite quail	-	-	pendimethalin	diet	8-d LC50	4187	2
Mus muscalis (I)	Mouse	-	-	trifluralin	oral	LD50	5000	12
Mus muscalis (I)	Mouse	-	-	pendimethalin	oral	LD50	1340, 1620[e]	2
Rattus norvegicus (I)	Rat	-	-	trifluralin	oral	LD50	>5000	2
Rattus norvegicus (I)	Rat	-	-	pendimethalin	oral	LD50	1050, 1250[e]	12
Lapine sp. (I)	Rabbit	-	-	pendimethalin	oral	LD50	>5000	2

b - all test animals were female
c - active ingredient in mg/L/ha
d - chemical was added in a solvent carrier; [44] - corn oil
e - LD50s for females, males (respectively)

4.6 Chronic Effects

4.6.1 Chronic effects in mammals

Most of the chronic mammalian studies examined the mutagenic and carcinogenic potentials of trifluralin. The variability in test results among investigators suggests their results are conflicting.

In two independent studies, trifluralin was not found to be mutagenic in the Ames test [45-46]. The same assay detected no mutagenic activity with pendimethalin exposure [46]. Mutagenicity studies with fruit flies (*Drosophila melanogaster*) are often utilized for their ability to detect lesions that bacterial mutagenicity assays (like the Ames test) are incapable of detecting. However, studies of chromosomal effects in *Drosophila* exposed to dinitroanilines produced equivocal results. Murnik and associates [47-48] initially found that 100 mg/L trifluralin was capable of inducing chromosomal lesions, loss and nondisjunction in *Drosophila*.

Further studies verified that trifluralin induced paternal chromosomal loss and increases in deletion of the X:Y chromosome [49], but did not find corresponding maternal lesions [50]. Another study using two mutagenicity assays could neither implicate nor discount trifluralin as a mutagen in *Drosophila* [51]. Recent work by Kale et al. [52] showed that the Treflan formulation was mutagenic to fruit flies. However, this same group also determined that all of nine major herbicides were mutagenic, making the accuracy of their assay somewhat suspect.

Tests with the Olitref formulation of trifluralin found chromosomal mutations in mice at 33% of the LD50, but failed to show the same mutations at 1% of the LD50 [53]. However, prolonged treatment with the lower dose did increase the frequency of chromosomal aberrations [54]. A micronucleus assay with trifluralin in human lymphocytes was inconclusive since results varied with blood donor [55]. Tests with pendimethalin failed to show mutagenicity in the human sister chromatid exchange assay [56].

Mixed results with trifluralin and its formulations may have been due in part to the presence of dipropylnitrosamine (DPNA), a known carcinogen, in the trifluralin formulations. The US EPA required manufacturers to reduce levels of DPNA in formulations below 1 mg/kg in the early 1980s [57]. Subsequent 2-year dietary carcinogenicity testing revealed no benign or malignant neoplasms in mice [58-59].

A study with rats and rabbits observed that trifluralin has a low potential for developmental toxicity [60]. Lower fetal weight in rats was observed at 1000 mg/kg maternal dose. Similar effects were seen at 500 mg/kg in rabbits. Since substantial adult mortality occurred at lower doses than those inducing developmental effects, the authors concluded that trifluralin posed little risk as a developmental toxicant to these species. Pregnant mice exposed to 1000 mg/kg of trifluralin demonstrated a higher frequency of stillborn and atypical pups than controls [61]. Several skeletal deformities were observed in the *in utero* exposed pups.

4.6.2 Chronic effects in fish

The majority of studies on fish exposure to trifluralin have focused on the phenomenon of vertebral deformities. Couch et al. [62] first recorded vertebral dysplasia in sheepshead minnows (*Cyprinodon variegatus*) exposed to trifluralin concentrations ranging from 1.2 to 31 mg/L. Minnows exposed from the zygote stage showed vertebral dysplasia characterized by hyperostosis of acellular bone at concentrations above 5.5 mg/L.

No mechanism was determined, but deformities in adults exposed to 16.6 mg/L were associated with elevated levels of plasma calcium. Further study [63] showed that trifluralin-induced pituitary enlargement was related to compromised structural integrity of the organ and a striking occurrence of fluid-filled pseudocysts. These anomalies were observed both in individuals exposed to 1-5mg/L from zygote to 19 months and in a second group exposed from age 30 d to 19 months, suggesting that exposure of the zygote was not necessary for either vertebral dysplasia or pituitary anomalies.

Subsequent to Couch's studies, an accidental spill of trifluralin was recorded in Berwickshire, Scotland; it was implicated as the cause of death of over 200 brown trout (*Salmo trutta*) in the Eden Water [16]. Several months after the spill, anglers reported catching brown trout with spinal deformities. In order to determine if trifluralin was the cause of the observed deformities, a pulse exposure using the Treflan formulation was performed with Atlantic salmon. Parr were exposed to 500mg/L for 11 h and maintained in clean water for 12 months. Signs of vertebral fusion and dysplasia were observed as soon as 10 d post-treatment [16].

A recent study by Koyama [39] tested 10 species of marine fish for vertebral deformities after trifluralin exposure. Eight of the 10 species exhibited vertebral deformities, but one of these was at a concentration greater than the 96-h LC50. The lowest observed deformity concentration for the remaining species ranged from just slightly below the LC50 to 6-fold lower than the LC50. The most sensitive species was mullet (*Mugil cephalis*) with a no-observed-deformity concentration of 3 mg/L.

Long-term exposures of fathead minnow to trifluralin were conducted as part of an EPA study [29]. At 5.1 mg/L more than half of the fatheads died between 163-263 d. A delay in spawning behaviour was observed in the survivors at this concentration. Based on these data a maximum acceptable toxicant concentration (MATC) between 1.95 and 5.1 mg/L was suggested.

No information regarding the chronic toxicity of pendimethalin in fish could be found.

4.6.3 Chromic effects in invertebrates

Multi-generational studies with *Daphnia magna* showed decreased reproduction with each generation, and no survival in the third generation at 7.2 mg/L trifluralin [29]. The MATC for survival over three generations was between 2.3 and 7.2 mg/L. A subsequent study on this species using microcosms failed to demonstrate any effects on *Daphnia* reproduction or survival at concentrations between 10 and 1000 mg/L Treflan added to the sediment [35]. As this study did not measure resultant water concentrations while using a formulation, it is of no practical value with regard to chronic toxicity.

The trifluralin formulation Treflan has been used as a mycostatic agent in shrimp aquaculture. Studies to determine the sensitivity of shrimp found no effect on hatching at 200 mg/L trifluralin or at 10 mg/L Treflan [64]. The only effect observed was a reduction in nauplii 96 h survival at 10 mg/L Treflan.

4.7 Potential for Endocrine Disruption

Our working definition of an endocrine disrupting compound:

> *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*.

After examination of the relevant literature, we do not completely concur with the conclusion of Colborn et al.[65] that trifluralin has reproductive and endocrine-disrupting effects. Their categorization was founded on the single observation of histological pituitary anomalies in fish described above [63]. This paper on its own does not sufficiently demonstrate a pituitary-linked mechanism of action behind the major effect observed in fish; vertebral deformities.

Taken in the context of the overall data for fish, it seems more likely that vertebral deformities are caused by direct action of trifluralin on bony structures. Support for this hypothesis include the amazing rapidity with which vertebral deformities were manifested in test fish, and an unrelated observation that trifluralin appears to have a direct-acting effect on cellular calcium homeostasis [26].

The carcinogenic potential of trifluralin is also disputed in the literature, although it remains listed as a suspected carcinogen. Developmental toxicity studies in mammals have clearly demonstrated that trifluralin only has effects at concentrations approaching acute lethality [60-61]. Given these high concentrations and

the lack of mechanistic evidence for endocrine disruption, we would conclude that the probability of trifluralin acting as an endocrine-disrupter at environmental concentrations is exceedingly low.

4.8 Risk to Ontario Environments and Recommendations

Measurement of dinitroanilines in Ontario environments suggests that environmental levels are not sufficient to induce acute or chronic effects in tested animal species. The most sensitive fish species investigated showed deformity effects upon exposure to trifluralin as low as 1-3 mg/L, whereas the highest surface water concentration of trifluralin in the province was 0.04 mg/L. Also, tissue concentrations of trifluralin in Lake Michigan fish were well below the estimated critical (lethal) body burden.

However, exposure estimates have thus far appeared to result from incidental detections collected during surveys for other pesticides, and the measured concentrations in surface water and sediment may not be representative of maximum levels in the province. In addition and as was found with several other pesticides in this document, the empirical database for toxicity in several potentially high risk aquatic and semi-aquatic vertebrate groups is extremely limited. Only one paper discussed trifluralin effects in amphibians, and no literature of effects in reptiles or wild bird or small mammal species were found.

As such, the apparent low risk associated with dinitroaniline use in Ontario agriculture must be qualified by acknowledging the high degree of uncertainty surrounding responses of these wildlife groups. Given this uncertainty, the following rudimentary investigations are recommended:

- environmental concentrations of trifluralin and pendimethalin should be evaluated in high use areas such as Perth, Middlesex, Bruce and Wellington counties - surface water, sediment and fish tissue samples would provide a satisfactory basis for calculation of exposure estimates;
- the deformity effects observed in some fish should be expanded upon, using indigenous species and realistic exposure scenarios - amphibian and fish larvae may be the most sensitive test subjects to complete initial tests;
- given the frequency with which dinitroanilines are applied with other herbicides (particularly triazines) and with fertilizers, some examination of relevant mixture toxicity should be pursued.

4.9 References

1. Ashton, F.M. and A.S. Crafts. 1981. Dinitroanilines. In: Mode of Action of Herbicides (2nd ed.). John Wiley and Sons, New York, NY, USA. pp. 201-223.
2. Tomlin, C. 1994. The Pesticide Manual (Incorporating the Agrochemicals Handbook), 10th ed. British Crop Protection Council and The Royal Society of Chemistry. The Bath Press, Bath, England.
3. Hunter, C. and B. McGee. 1994. Survey of pesticide use in Ontario, 1993. Estimates of pesticides used on field crops, fruit and vegetable crops, provincial highway roadsides, and by licensed pesticide applicators. Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) Economics Information Report No. 94-01. OMAFRA Policy Analysis Branch, Toronto, ON, Canada.
4. Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). 1998. Guide to Weed Control, 1998. OMAFRA Publication No. 75. OMAFRA, Toronto, Canada.
5. Niemczyk, H.D. and A.A. Krause. 1994. Behaviour and mobility of preemergent herbicides in turfgrass: a field study. J. Environ. Sci. Health 29B:507-539.
6. Pedersen, H.J., P. Kudsk, and A. Helweg. 1995. Adsorption and ED50 values of five soil-applied herbicides. Pestic. Sci. 44:131-136.
7. North American Compendiums Ltd. 1996. Pest Control Canada - A Reference Manual (11th ed.). North American Compendiums Ltd., Hensall, ON, Canada.
8. Kent, R.A., M. TachJ, P-Y. Caux, and B.D. Pauli. 1992. Canadian Water Quality Guidelines for Trifluralin. Environment Canada Scientific Series No. 190. Ecosystem Sciences and Evaluation Directorate, Environment Canada, Ottawa, ON, Canada.
9. Hoff, R.M., D.C.G. Muir, and N.P. Grift. 1992. Annual cycle of polychlorinated biphenyls and

organohalogen pesticides in air in southern Ontario. 1. Air concentration data. Environ. Sci. Technol. 26:266-275.
10. Gaynor, J.D. 1985. Dinitroaniline herbicide persistence in soil in southwestern Ontario. Can. J. Soil Sci. 65:587-592.
11. Jensen, K.I.N., J.A. Ivany, and E.R. Kimball. 1983. Efficacy of three dinitroaniline herbicides in processing peas and their residues in soils. Can. J. Plant Sci. 63:687-693.
12. Montgomery, J.H. 1993. Agrochemicals Desk Reference - Environmental Data. Lewis Publishers, Boca Raton, FL, USA.
13. Isensee, A.R., P.C. Kearney, and G.E. Jones. 1979. Modeling aquatic ecosystems for metabolic studies. In: M.A.Q. Khan, J.J. Lech, and J.J. Menn, eds. Pesticide and Xenobiotic Metabolism in Aquatic Organisms. American Chemical Society (ACS) Symposium Series No. 99. ACS, Washington, D.C., USA. pp. 195-216.
14. Spacie, A. and J.L. Hamelink. 1979. Dynamics of trifluralin accumulation in river fishes. Environ. Sci. Technol. 13:817-822.
15. Schultz, I.R. and W.L. Hayton. 1993. Toxicokinetics of trifluralin in rainbow trout. Aquat. Toxicol. 26:287-306.
16. Wells, D.E. and A.A. Cowan. 1982. Vertebral dysplasia in salmonids caused by the herbicide trifluralin. Environ. Pollut. 29A:249-260.
17. Yockim, R.S., A.R. Isensee, and E.A. Walker. 1980. Behaviour of trifluralin in aquatic model ecosystems. Bull. Environ. Contam. Toxicol. 24:134-141.
18. Isensee, A.R. and P.S. Dubey. 1983. Distribution of pendimethalin in an aquatic microecosystem. Bull. Environ. Contam. Toxicol. 30:239-244.
19. Clark, J.R., A. DeVault, R.J. Bowden, and J.A. Weishaar. 1984. Contaminant analysis of fillets from Great Lakes coho salmon, 1980. J. Great Lakes Res. 10:38-47.
20. Camanzo, J., C.P. Rice, D.J. Jude, and R. Rossmann. 1987. Organic priority pollutants in nearshore fish from 14 Lake Michigan tributaries and embayments, 1983. J. Great Lakes Res. 13:296-309.
21. Nelson, J.O., P.C. Kearney, J.R. Plimmer, and R.E. Menzer. 1977. Metabolism of trifluralin, profluralin, and fluchloralin by rat liver microsomes. Pestic. Biochem. Physiol. 7:73-82.
22. Erkog, F.g. and R.E. Menzer. 1985. Metabolism of trifluralin in rats. J. Agric. Food Chem. 33:1061-1070.
23. Zulalian, J. 1990. Study of the absorption, excretion, metabolism, and residues in tissues in rats treated with carbon-14-labelled pendimethalin, PROWL herbicide. J. Agric. Food Chem. 38:1743-1754.
24. Reinbold, K.A. and R.L. Metcalf. 1976. Effects of the synergist piperonyl butoxide on metabolism of pesticides in green sunfish. Pestic. Biochem. Physiol. 6:401-412.
25. Quader, H., I. Wagenbreth, and D.G. Robinson. 1978. Structure, synthesis, and orientation of microfibrils V. On the recovery of *Oocystis solitaria* from microtubule inhibitior treatment. Eur. J. Cell Biol. 18:39-51.
26. Hertel, C., H. Quader, D.G. Robinson, I. Roos, E. Carafoli, and D. MarmJ. 1981. Herbicides and fungicides stimulate Ca^{2+} efflux from rat liver mitochondria. FEBS Letters 127:37-39.
27. Sanders, H.O. and O.B. Cope. 1966. Toxicities of several pesticides to two species of cladocerans. Trans. Am. Fish. Soc. 95:165-169.
28. Mayer, F.L.Jr. and M.R. Ellersieck. 1986. Manual of acute toxicity: interpretation and data base for 410 chemicals and 66 species of freshwater animals. United States Department of the Interior (USDI) Resource Publication No. 160. USDI, Fish and Wildlife Service, Washington, D.C., USA.
29. Macek, K.J., M.A. Lindberg, S. Sauter, K.S. Buxton, and P.A. Costa. 1976. Toxicity of four pesticides to water fleas and fathead minnows: acute and chronic toxicity of acrolein, heptachlor, endosulfan, and trifluralin to the water flea (*Daphnia magna*) and the fathead minnow (*Pimephales promelas*). US Environmental Protection Agency (EPA), Duluth, MN, USA. EPA-600/3-76-099.
30. Montague, B. 1995. Environmental Effects Branch Oneliner Toxdata Electronic File (1991-1995). US Environmental Protection Agency (EPA), Washingon, D.C.

31. Sanders, H.O. 1970. Toxicities of some herbicides to six species of freshwater crustaceans. J. Water Pollut. Control Fed. 24:1544-1550.
32. Naqvi, S.M., R. Hawkins, and N.H. Naqvi. 1987. Mortality response and LC50 values for juvenile and adult crayfish, *Procambarus clarkii* exposed to Thiodan[7] (insecticide), Treflan[7], MSMA, Oust[7] (herbicides) and Cutrine-Plus[7] (algicide). Environ. Pollut. 48:275-283.
33. Naqvi, S.M. and T-S. Leung. 1983. Trifluralin and oryzalin herbicides toxicities to juvenile crawfish (*Procambarus clarkii*) and mosquitofish (*Gambusia affinis*). Bull. Environ. Contam. Toxicol. 31:304-308.
34. Nimmo, D.R., T.L. Hamaker, E. Matthews, and J.C. Moore. 1981. An overview of the acute and chronic effects of first and second generation pesticides on an estuarine mysid. In: F.J. Vernberg, A. Calabrese, F.P. Thurberg, and W.B. Vernberg, eds. Biological Monitoring of Marine Pollutants. Academic Press, New York, NY, USA. pp. 3-19.
35. Johnson, B.T. 1986. Potential impact of selected agricultural chemical contaminants on a northern prairie wetland: a microcosm evaluation. Environ. Toxicol. Chem. 5:473-485.
36. Cope, O.B. 1966. Contamination of the freshwater ecosystem by pesticides. J. Appl. Ecol. 3:33-44.
37. McCorkle, F.M., J.E. Chambers, and J.D. Yarbrough. 1977. Acute toxicities of selected herbicides to fingerling channel catfish, *Ictalurus punctatus*. Bull. Environ. Contam. Toxicol. 18:267-270.
38. Montague, B. 1995. Environmental Effects One-liner Toxdata Electronic File (1991-1995). US Environmental Protection Agency, Washington, D.C., USA.
39. Koyama, J. 1996. Vertebral deformity susceptibilities of marine fishes exposed to herbicide. Bull. Environ. Contam. Toxicol. 56:655-662.
40. Alabaster, J.S. 1969. Survival of fish in 164 herbicides, insecticides, fungicides, wetting agents and miscellaneous substances. Int. Pest Control 11:29-35.
41. Sanders, H.O. 1970. Pesticide toxicities to tadpoles of the western chorus frog *Pseudacris triseriata* and Fowlers toad *Bufo woodhousii fowleri*. Copeia 1970:246-251.
42. Hudson, R.H., R.K. Tucker, and M.A. Haegele. 1984. Handbook of toxicity of pesticides to wildlife. United States Department of the Interior (USDI) Resource Publication No. 153. USDI, Fish and Wildlife Service, Washington, D.C., USA.
43. Hoffman, D.J. and P.H. Albers. 1984. Evaluation of potential embryotoxicity and teratogenicity of 42 herbicides, insecticides, and petroleum contaminants to mallard eggs. Arch. Environ. Contam. Toxicol. 13:15-27.
44. Hill, E.F. and M.B. Camardese. 1986. Lethal dietary toxicities of environmental contaminants and pesticides to *Coturnix*. United States Department of the Interior (USDI) Fish and Wildlife Technical Report No. 2. USDI, Fish and Wildlife Service, Washington, D.C., USA.
45. Andersen, K.J., E.G. Leighty, and M.T. Takahashi. 1972. Evaluation of herbicides for possible mutagenic properties. J. Agric. Food Chem. 20:649-656.
46. Eisenbeis, S.J., D.L. Lynch, and A.E. Hampel. 1981. The Ames mutagen assay tested against herbicides and herbicide combinations. Soil Sci. 131:44-47.
47. Murnik, M.R. 1978. Mutagenicity of the herbicide trifluralin in *Drosophila melanogaster*. Mutat. Res. 53:235-236 (abstract only).
48. Bryant, M.L. and M.R. Murnik. 1979. Mutagenicity of the herbicide Trifluralin in *Drosophila melanogaster*. Genetics 91:s15 (abstract only).
49. Foureman, P.A. 1981. Identification of aneuploidy inducing chemicals in *Drosophila*. Environ. Mutat. 3:319 (abstract only).
50. Foureman, P.A. 1988. The TX;Y test for the detection of nondisjunction and chromosome breakage in *Drosophila melanogaster*. Mutat. Res. 203:309-316.
51. Yoon, J.S., J.M. Mason, R. Valencia, R.C. Woodruff, and S. Zimmering. 1985. Chemical mutagenesis testing in *Drosophila*. IV. Results of 45 coded compounds tested for the National Toxicology Program. Environ. Mutat. 7:349-367.
52. Kale, P.G., B.T. Petty, S. Walker, J.B. Ford, N. Dehkordi, S. Tarasia, B.O. Tasie, R. Kale, and Y.R. Sohni. 1995. Mutagenicity testing of nine herbicides and pesticides currently used in agriculture.

Environ. Molec. Mutat. 25:148-153.
53. Nehjz, M., A. Paldy, A. Selypes, M. Krsealvi, L. Lrinczi, and G. Berencsi. 1979. The mutagenic effect of trifluralin-containing herbicide on mouse bone marrow *in vivo*. Ecotoxicol. Environ. Saf. 3:454-457.
54. Nehjz, M., A. Selypes, A. Plady, E. Mazzag, G. Berencsi, and K. J. Rmay. 1982. The effects of five weeks treatment with dinitro-*o*-cresol- or trifluralin-containing pesticides on the germ cells of male mice. J. Appl. Toxicol. 2:179-180.
55. Surralljs, J., N. Xamena, A. Creus, and R. Marcos. 1995. The suitability of the micronucleus assay in human lymphocytes as a new biomarker of excision repair. Mutat. Res. 342:43-59.
56. Dunkelberg, H., J. Fuchs, J.G. Hengstler, E. Klein, F. Oesch, and K. Strhder. 1994. Genotoxic effects of the herbicides alachlor, atrazine, pendimethaline, and simazine in mammalian cells. Bull. Environ. Contam. Toxicol. 52:498-504.
57. US Environmental Protection Agency (EPA). 1979. Trifluralin (Treflan) - position document ½/3. EPA Special Pesticide Review Division, Washington, D.C., USA.
58. US Environmental Protection Agency (EPA). 1984. Health and environmental effects profile for trifluralin. EPA Environmental Criteria and Assessment Office, Cincinnati, OH, USA. EPA/600/X-84/234.
59. US Environmental Protection Agency (EPA). 1987. Guidance for the registration of pesticide products containing trifluralin as the active ingredient. EPA Office of Pesticide Programs, Washington, D.C., USA.
60. Byrd, R.A., J.K. Markham, and J.L. Emmerson. 1995. Developmental toxicity of dinitroaniline herbicides in rats and rabbits. I. Trifluralin. Fund. Appl. Toxicol. 26:181-190.
61. Beck, S.L. 1981. Assessment of adult skeletons to detect prenatal exposure to 2,4,5-T or trifluralin in mice. Teratology 23:33-55.
62. Couch, J.A., J.T. Winstead, D.J. Hansen, and L.R. Goodman. 1979. Vertebral dysplasia in young fish exposed to the herbicide trifluralin. J. Fish Dis. 2:35-42.
63. Couch, J.A. 1984. Histopathology and enlargement of the pituitary of a teleost exposed to the herbicide trifluralin. J. Fish Dis. 7:157-163.
64. Lio-Po, G.D. and E.G. Sanvictores. 1986. Tolerance of *Penaeus monodon* eggs and larvae to fungicides against *Lagenidium* sp. and *Haliphthoros philippinensis*. Aquaculture 51:161-168.
65. Colborn, T., F.S. vom Saal, and A.M. Soto. 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ. Health Perspect. 101:378-384.

5.0 1,3-DICHLOROPROPENE

5.1 Description and Use

1,3-Dichloropropene was introduced as a commercial fumigant nematicide in 1955 (1). It is widely used in agriculture as a soil fumigant for control of parasitic plant nematodes on a multitude of crops, including tobacco, tomatoes, potatoes, peaches, strawberries and any crop that is susceptible to nematodes [2,3].

1,3-Dichloropropene is marketed in a variety of commercial formulations in Ontario (Table 5.1). 1,3-Dichloropropene, a mixture of *cis* and *trans* isomers, is a clear, straw coloured liquid that quickly disperses from the liquid form to the vapour phase, it has a penetrating, irritating, chloroform-like odour [4]. Large areas of subsoil can be fumigated from applications along a narrow path. However, the performance and effective radius of 1,3-dichloropropene is dependent on the vapour pressure, diffusion coefficient, soil type, moisture content, temperature and organic content of the soil [5].

Use of 1,3-dichloropropene in Ontario is shown on a county-wide basis in Figure 5.2. Application was highest in south western portions of the province ie. Haldimand-Norfolk, Brant, Elgin, and Oxford counties. This reflects its frequent use on tobacco pests. Across Ontario, 1,3-dichloropropene is used to combat root-lesion nematode *Pratylenchus penetrans* that causes brown-root rot of tobacco and lesions in strawberries and raspberries [6,7]. It is also an important pesticide when planting a new orchard or when replanting an orchard on an old tree site [6]. In 1993, use estimates for 1,3-dichloropropene in Ontario were 35,2709 kg for field crops (mainly tobacco) and 951 kg for fruits (8). 1,3-Dichloropropene is typically applied to crops using a subsoil injection technique [9]. Application of 1,3-dichloropropene takes place in late April or early May in Ontario.

5.2 Environmental Concentrations

Very little information exists on surface water concentrations in Ontario because surface water monitoring has been limited. Only one study has looked for it in surface waters in Ontario [10]. It is not expected to be found in surface water mainly due to its high volatility and low solubility in water, losses are more likely to occur from volatilization than from leaching. However, Merriman et al. [10], found detectable quantities of 1,3-dichloropropene in six surface water samples collected at four stations in Big Creek Watershed downstream of tobacco growing areas between 9 May and 18 May. The concentrations of 1,3-dichloropropene range from 0.18 mg/L to 4.12 mgL (Figure 5.3). The evaporation of 1,3-dichloropropene from water has been reported to have a half-life of less than 5 h and in a soil/water slurry, it was rapidly converted to 3-chloropropononic acid [11].

The half-life of 1,3-dichloropropene in soil has been estimated to be between 3 days and 37 days depending on various environmental conditions [12]. In a more recent study, the half-lives of the *cis* and *trans* isomers of 1,3-dichloropropene in four water-saturated sandy subsoil materials collected between 2 and 4 m below the soil surface, were between 16 and 64 days [13]. On a tomato field in Florida soil concentrations ranged from 0.61 mg/kg 3 days after application to 0.01 mg/kg at 90 days [14]. In soils *cis* and *trans* isomers of 1,3-dichloropropene degrade primarily to 3-chloroallyl alcohol [15].

After application of 1,3-dichloropropene appreciable amounts of the chemical can be measured in the air around the applied area [5,16]. These concentrations in the air can remain quite high (up to 10 ppm) at ground level on the application site for many days [5]. However, concentrations disperse as the distance from the site increases [16]. The concentration of 1,3-dichloropropene in the air in Simcoe, Ontario on 3 May, 1995 was 164.96 mg/m^3 (Tom Dann, unpublished data). Simcoe is located in a tobacco agricultural area.

Table 5.1 Commercial Fumigant Formulations Used in Ontario.

Trade name	Formulation
Telone II - *Dow Chemical*	94% 1,3-dichloropropene 6% inert ingredients
Telone - C17 - *Dow Chemical*	78% 1,3-dichloropropene 16.5% chloropicrin
Vorlex Plus - *Nor-Am Chemical*	40% 1,3-dichloropropene 20% methly isothiocyanate
Vorlex Plus CP - *Nor-Am Chemical*	34% 1,3-dichloropropene 17% methly isothiocyanate 15% chloropicrin

5.3 Bioconcentration and Metabolism of 1,3-dichloropropene

The main metabolic pathway of 1,3-dichloropropene is a glutathione-dependent reaction in which 1,3-dichloropropene is conjugated with glutathione which is further metabolized to mercapturic acid and excreted in urine [17,18,19,20]. 1,3-Dichloropropene is not believed to bioaccumulate because of its low octanol water partition coefficient (K_{ow}= 1.82) [21,10].

5.4 Toxic Mechanisms of Action

In nematodes, 1,3-dichloropropene is believed to act by chemically combining with a nucleophilic centre of an essential enzyme [22]. In non-target organisms, it depletes tissue nonprotein sulfhydryl by binding to it and binds to macromolecules which causes tissue damage [23].

5.5 ACUTE TOXICITY

Very little information exists on the acute toxicity of 1,3-dichloropropene to non-target wildlife. However, the data available indicates 1,3-dichloropropene is low to moderately toxic (Table 5.2). Furthermore, the concentrations found to be acutely toxic to aquatic organisms have not been found in aquatic environments in Ontario.

Inhalation studies with rats and other small mammals have found that single inhalation exposures of 1,3-dichloropropene at concentration between 400 and 1000 ppm for short periods of time (two hours to seven hours) resulted in severe lung, nasal, liver and kidney injury as well as death [24,25].

Small terrestrial organisms, such as, mammals, retiles and amphibians may potentially be exposed to toxic concentrations of 1,3-dichloropropene in the air when moving through or around fields where 1,3-dichloropropene was recently applied. However, the lack of 1,3-dichloropropene inhalation studies on wildlife limits making conclusions on the risk associated with this type of exposure.

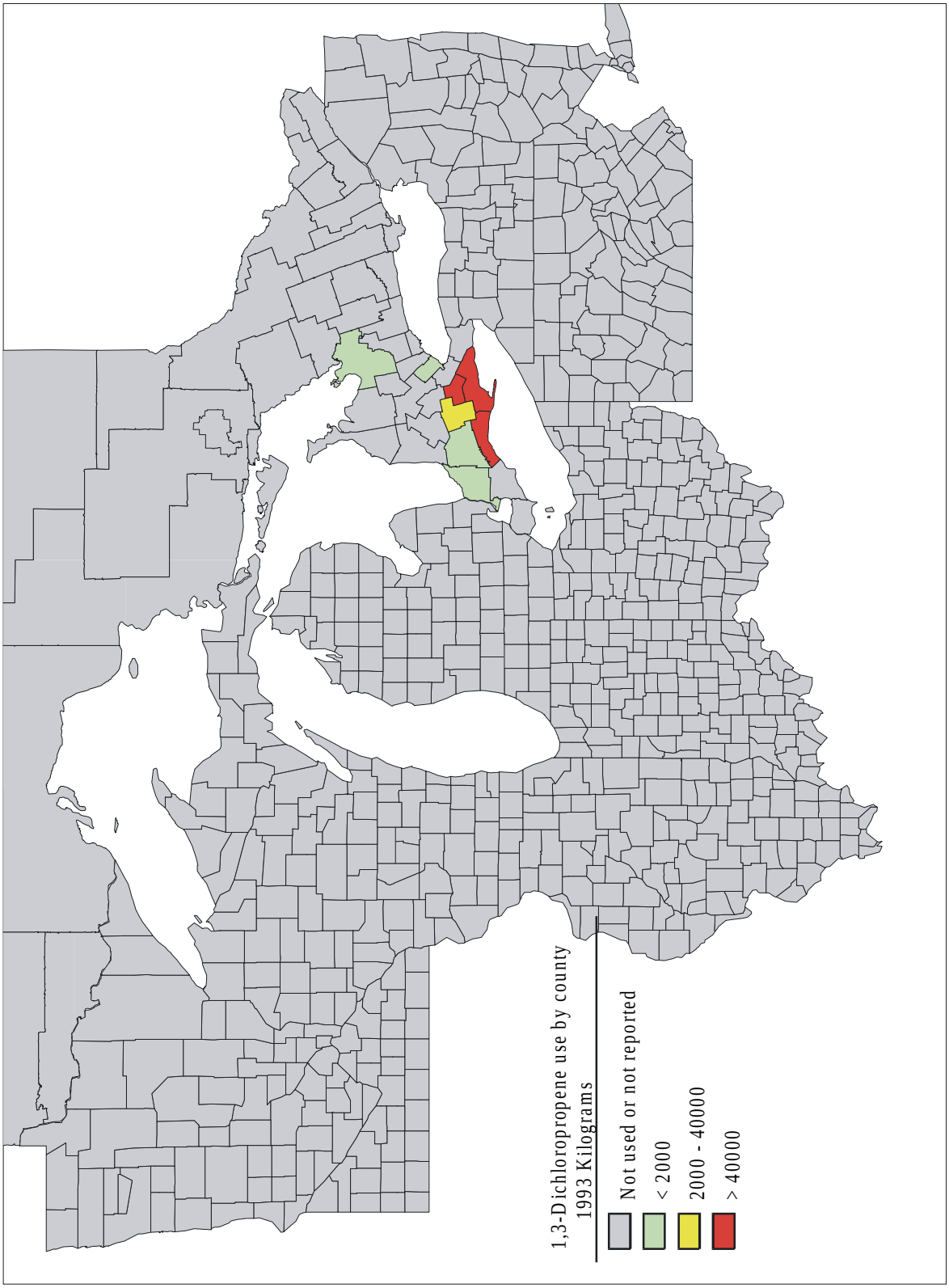

Figure 5.1 1,3-dichloropropene use in theGreat Lakes Basin.

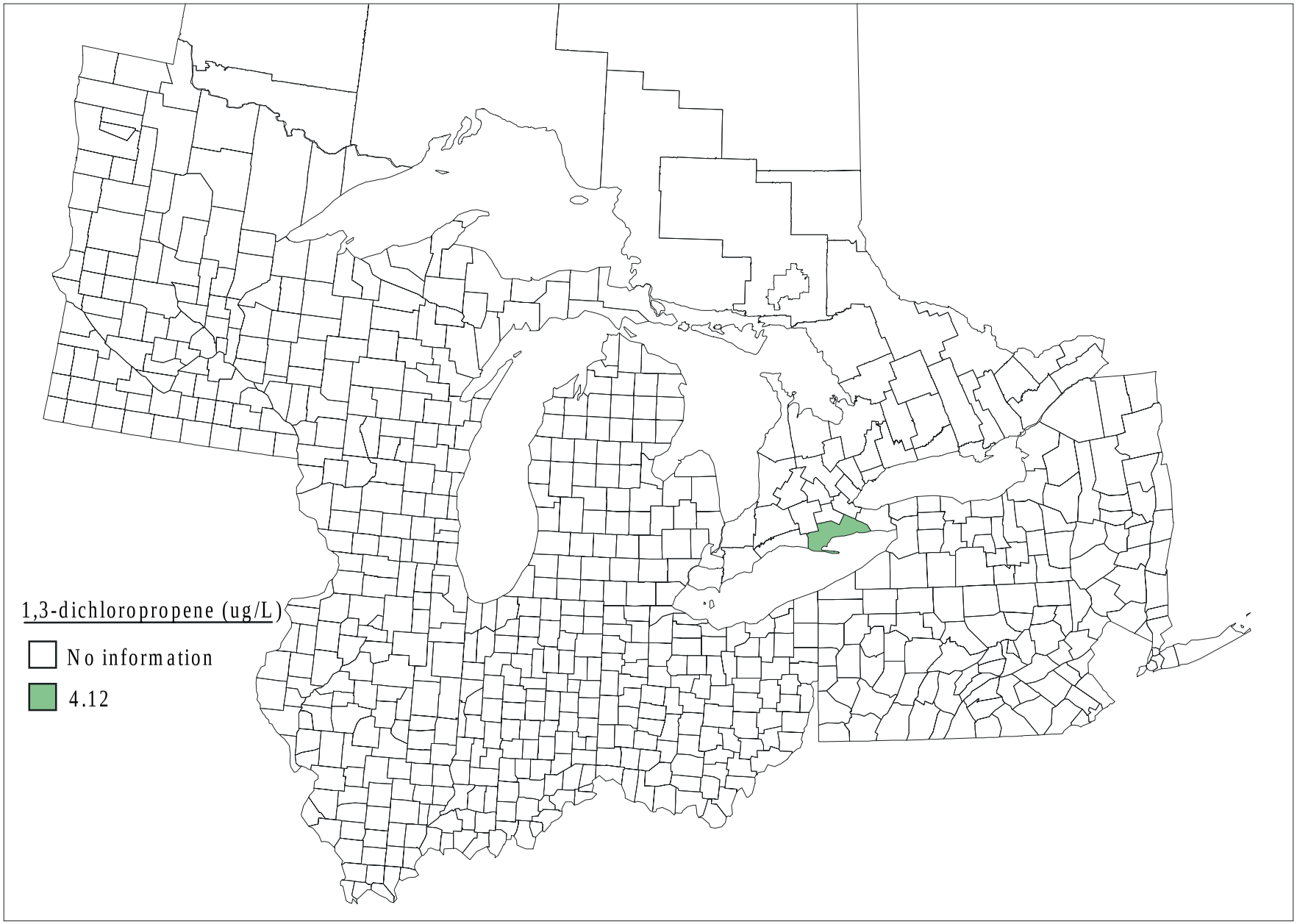

Figure 5.2 Maximum concentration of 1,3-dichloropropene found in surface water by county.

5.6 Chronic Effects

The amount of information on the chronic toxicity of 1,3-dichloropropene to wildlife is extremely limited. A flow through 28 day chronic toxicity study on fathead minnows (*Pimephales promelas*) exposed to 1,3-dichloropropene a no observable adverse effect level of 0.18 mg/L, a lowest observable effect level of 0.33 mg/L and a maximum allowable toxic concentration of 0.29 mg/L [26].

A chronic inhalation study on male and female rats and mice exposed for 13 weeks found depressed growth rates in animals exposed to 90 or 150 ppm. The primary target tissues were identified as the nasal mucosa in both species and the urinary bladder in female mice. No treatment-related effects were observed in rats or mice exposed to 10 ppm [27].

1,3-dichloropropene is a suspected carcinogen [28,29] and mutagen [30,31]. 1,3-dichloropropene was found to be mutagenic in strain TA1535 of *Samonella tryphimurium*; with the addition of rat liver the mutagenicity and cytotoxicity were reduced [31]. However, Talcott and King [32] believe impurities in the formulation, mainly two known mutagens, epichlorhydrin (CAS: 106-89-8; 1-chloro-2,3- epoxypropane) and 1,3-dichloro-2-propanol (CAS: 96-23-1) were the cause of observed mutagenicity. A dose-related amount of DNA fragmentation was observed at 62.5 to 250 mg/kg body weight in liver and gastric mucosa of rats. Evidence of DNA fragmentation was absent in lung, bone marrow, and brain [28]

5.7 Potential for 1,3-dichloropropene to Act as an Endocrine Disrupter

Our working definition of an endocrine disrupting compound:

> *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.*

1,3-dichloropropene is not suspected to be a endocrine disrupting compound. No evidence in the literature indicates that it affects reproduction or endocrine function. However, few studies examined endpoints such as reproductive success or endocrine disruption.

5.8 Risk to Ontario Environments and Recommendations

No definite conclusions on risk to wildlife can be made at this time because of the extremely limited nature of toxicity and environmental concentration information. There may be a potentially toxic inhalation exposure to wildlife in terrestrial ecosystems near areas of recent use. The following issues should be priorities for further evaluation of the extent and degree of risk posed by 1,3-dichloropropene in Ontario.

- a comprehensive survey of air and surface water concentrations during times of application in the areas where 1,3-dichloropropene is heavily used should be conducted;
- a number of field and laboratory inhalation toxicity studies on indigenous amphibian, reptile and small mammal species must be completed;
- more precise use information would help narrow the geographic focus for monitoring in high-risk counties.

Table 5.2 Acute toxicity of 1,3-Dichloropropene to various species. Species indigenous to Ontario are identified by an (I) after the common name.

Species	Common Name	Age	SampleSize	Dose Method	Test Type	Concentration (mg/L)	R
Lepomis macrochirus	Bluegill (I)	adults	-	Aqueous, static	24-hr LC50	6.8	[33]
Lepomis macrochirus	Bluegill (I)	adults	-	Aqueous, static	96-hr LC50	6.1	[33]
Oncorhunchus mykiss	Rainbow Trout (I)	adults	10	Aqueous, static	24-hr LC50	8.8	[34]
Oncorhunchus mykiss	Rainbow Trout (I)	adults	10	Aqueous, static	48-hr LC50	7.2	[34]
Oncorhunchus mykiss	Rainbow Trout (I)	adults	10	Aqueous, static	96-hr LC50	5.9	[34]
Cyprinodon variegatus	Sheepshead minnow	juveniles	-	Aqueous, static	24-hr LC50	6.8 to 9.2	[35]
Cyprinodon variegatus	Sheepshead minnow	juveniles	-	Aqueous, static	48-hr LC50	3.3	[35]
Cyprinodon variegatus	Sheepshead minnow	juveniles	-	Aqueous, static	72-hr LC50	2.2	[35]
Cyprinodon variegatus	Sheepshead minnow	juveniles	-	Aqueous, static	96-hr LC50	1.8	[35]
Daphnia magna	Water flea (I)	neonates	-	Aqueous, static	24-hr LC50	7.2	[36]
Daphnia magna	Water flea (I)	neonates	-	Aqueous, static	48-hr LC50	6.15	[36]
Colinus virginianus	Bobwhite quail (I)	adult	-	Oral	LD50	152 mg/kg	[9]
Anas platyrhynchos	Mallard duck (I)	adult	-	Diet	5-day LC50	>10000mg/kg	[9]
Rattus norvegicus	Rat (I)	adult	-	Oral	LD50	150 mg/kg	[9]
Rattus norvegicus	Rat (I)	adult	-	inhalation	LC100 (<2hr)	1000 mg/L	[4]

5.9 Reference List

1. Berry, D., W. Campbell, J. Stirt, and D. Salunkhe. 1980. Uptake and metabolism of 1,3-dichloropropene in plants. J. Food. Saf. 4:247-255.
2. Flessel, P., J. Goldsmith, E. Kahn, J. Wesolowski , K. Maddy, and S. Peoples. 1978. Acute and possible long term effects of 1,3-dichloropropene -California. Morb. Mort. Week. Rep. 27:55
3. Maddy, K.T., H.R. Fong, J.A. Lowe, D.W. Conrad, and A.S. Fredrickson. 1982. A study of well water in selected California communities for residues of 1,3-dichloropropene, chloroallyl alcohol and 49 organophosphate or chlorinated hydrocarbon pesticides. Bull. Environ. Contam. Toxicol. 29:354-359.
4. Yang, R.S. 1986. 1,3-Dichloropropene. Residue. Rev. 97:19-35.
5. Albrecht, W.N. and K. Chenchin. 1985. Dissipation of 1,2-dibromo-3-chloropropane (DBCP), cis-1,3-dichloropropene (1,3-DCP), and dichloropropenes from soil to atmosphere. Bull. Environ. Contam. Toxicol. 34:824-831.
6. Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). 1998. Fruit production recommendations, 1998-1999. OMAFRA Publication No. 360. OMAFRA, Toronto, ON, Canada.
7. Tu, C.M. 1993. Effect of nematicides, Telone registered II and Vorlex registered , on microflora and nitrification in tobacco soil. Bull. Environ. Contam. Toxicol. 50:43-48.
8. Hunter, C. and B. McGee. 1994. Survey of pesticide use in Ontario, 1993. Estimates of pesticides used on field crops, fruit and vegetable crops, provincial highway roadsides, and by licensed pesticide applicators. Ontario Ministry of Agriculture, Food and Rural Affairs, Economics Information Report No. 94-01. Policy Analysis Branch, OMAFRA, Toronto, ON, Canada.
9. Tomlin, C. 1994. The Pesticide Manual: Incorporating the Agrochemicals Handbook (10th ed.). The British Crop Protection Council and the Royal Society of Chemistry. The Bath Press, Bath, England.
10. Merriman, J.C., J. Struger, and R.S. Szawiola. 1991. Distribution of 1,3-dichloropropene and 1,2-dichloropropane in Big Creek watershed. Bull.Environ.Contam.Toxicol. 47:572-579.
11. Yon, D.A., G.A. Morrison, and A.S. McGibbon. 1991. The dissipation of 1,3-dichloropropene in ditch bottom sediment and associated aerobic ditch water. Pest. Sci. 32:147-159.
12. Van Dijk, H. 1974. Degradation of 1,3-dichloropropene in the soil. Agro-Ecosystems 1:177-184.
13. Boesten, J.I., L.T. Pas, J.H. Smelt, M. Leistra, and L.T. Van-der-Pas. 1991. Transformation rate of methyl isothiocyanate and 1,3-dichloropropene in water-saturated sandy subsoils. Nether. J. Agri. Sci. 39:179-190.
14. Obreza, T.A. and E.O. Onterman. 1991. Small-scale ground water monitoring for 1,3-dichloropropene in southwest Florida. Proc. Soil Crop Sci. Soc. Florida. 50:94-98
15. Ou, L.T., K.Y. Chung, J.E. Thomas, T.A. Obreza, and D.W. Dickson. 1995. Degradation of 1,3-dichloropropene (1,3-D) in soils with different histories of field applications of 1,3-D. J. Nemat. 27:249-257.
16. Berg, F.V., A.H. Roos, L.T. Tuinstra, and M. Leistra. 1994. Measured and computed concentrations of 1,3-dichloropropene and methyl isothiocyanate in air in a region with intensive use of soil fumigants. Water Air Soil Pol. 78:247-264.
17. Brouwer, D.H., E.J. Brouwer, J.A. de-Vreede, R.T. van-Welie, N.P. Vermeulen, and J.J. Van-Hemmen. 1991. Inhalation exposure to 1,3-dichloropropene in the Dutch flower-bulb culture. Part I. Environmental monitoring. Arch. Environ. Contam. Toxicol. 20:1-5.
18. Climie, I.J., D.H. Hutson, B.J. Morrison, and G. Stoydin. 1979. Glutathione conjugation in the detoxication of (Z)-1,3-dichloropropene (a component of the nematocide D-D) in the rat. Xenobiotica 9:149- 156.
19. Fisher, G.D. and W.W. Kilgore. 1988a. Mercapturic acid excretion by rats following inhalation exposure to 1,3- dichloropropene. Fundam. Appl. Toxicol. 11:300-307.
20. Fisher, G.D. and W.W. Kilgore. 1988b. Tissue levels of glutathione following acute inhalation of 1,3-dichloropropene. J. Toxicol. Environ. Health 23:171-182.
21. Krijgsheld, K.R. and A. van-der-Gen. 1986. Assessment of the impact of the emission of certain organochlorine compounds on the aquatic environment. Part 2. Allylchloride, 1,3- and

2,3-dichloropropene. Chemosphere 15:861-880.
22. Metcalf, R. 1978. Fumigants. In Pesticides in the Environment. White-Stevens J., editor. MarcelDekker, New York. 120-130.
23. Stott, W.T. and P.E. Kastl. 1986. Inhalation pharmacokinetics of technical grade 1,3-dichloropropene in rats. Toxicol. Appl. Pharmacol. 85:332-341.
24. Fisher, G.D. and W.W. Kilgore. 1989. Pharmacokinetics of S-(3-chloroprop-2-enyl)glutathione in rats following acute inhalation exposure to 1,3-dichloropropene. Xenobiotica 19:269-278.
25. Torkelson, T.R. and F. Oyen. 1977. The toxicity of 1,3-dichloropropene as determined by repeated exposure of laboratory animals. Am. Ind. Hyg. Assoc.J. 38:217-223.
26. USEPA. 1978. In depth studies on the health and environmental impacts of selected water pollutants. 68-01- 4646, Washington D.C.
27. Stott, W.T., J.T. Young, L.L. Calhoun, and J.E. Battjes. 1988. Subchronic toxicity of inhaled technical grade 1,3-dichloropropene in rats and mice. Fundam. Appl. Toxicol. 11:207-220.
28. Ghia, M., L. Robbiano, A. Allavena, A. Martelli, and G. Brambilla. 1993. Genotoxic activity of 1,3-dichloropropene in a battery of *in vivo* short-term tests. Toxicol. Appl. Pharmacol. 120:120-125.
29. Yang, R.S., J.E. Huff, G.A. Boorman, J.K. Haseman , M. Kornreich, and J.L. Stookey. 1986. Chronic toxicology and carcinogenesis studies of Telone II by gavage in Fischer-344 rats and B6C3F1 mice. J. Toxicol. Environ. Health 18:377-392.
30. De Lorenzo, F., S. Degl'Innocenti, A. Ruocco, L. Silengo, and R. Cortese. 1977. Mutagenicity of pesticides containing 1,3-dichloropropene. Cancer Res. 37:1915-1917.
31. Neudecker, T., A. Stefani, and D. Henschler. 1977. *In vitro* mutagenicity of the soil nematicide 1,3-dichloropropene. Experientia 33:1084-1085.
32. Talcott, R.E. and J. King. 1984. Mutagenic impurities in 1,3-dichloropropene preparations. J. Natl. Cancer Inst. 72:1113-1116.
33. Buciafusco, R.J., S.J. Ells, and G.A. LeBlanc. 1981. Acute toxicity of priority pollutants to bluegill (*Lepomis macrochirus*). Bull. Environ. Contam. Toxicol. 26:446-452.
34. Schneider, B.A. 1979. Toxicology Handbook of Mammalian and Aquatic Data. US EPA, Bettsville, MD.
35. Heitmuller, P.T., T.A. Hollister, and P.R. Parrish. 1981. Acute toxicity of 54 industrial chemicals to sheepshead minnows (*Cyprinodon variegatus*). Bull. Environ. Contam. Toxicol. 27:596-604.
36. LeBlanc, G.A. 1980. Acute toxicity of priority pollutants to water flea (*Daphnia magna*). Bull. Environ. Contam. Toxicol. 24:684-691.

6.0 AZINPHOS-METHYL

Organophosphorus (OP) pesticides have replaced more persistent organochlorine pesticides in the treatment of agricultural pesticides [1,2]. The choice to switch to the use of organophosphorus pesticides was based on their properties of low bioaccumulation or bioconcentration and increased rate of biodegradation (relatively low environmental persistence) as well as governmental restrictions on the use of organochlorines. However, several field studies revealed that organophosphorus pesticides can cause adverse effects in some non-target wildlife by means of a variety of nuerotoxic and biochemical mechanisms including the inhibition of cholinesterase enzyme activity, behavioral effects, embryo-toxicity and teratogenicity [2-7].

6.1 Description and Use

Azinphos-methyl (CAS No. 85-50-0; O,O-Dimethyl S-[(4-oxo-1,2,3-benzo-triazin-3(4H)-yl) methyl] phosphorodithioate) was introduced as a broad spectrum insecticide in 1956 [8]. It is used primarily as a foliar application against phytophagous insect pests on fruit, field and to smaller extent, vegetable crops (Table 6.1). It works as both a contact insecticide and a stomach poison [8-11]. In one season it may be applied on multiple occasions by both power-operated ground sprayers and aircraft.

Azinphos-methyl is sold in a variety of formulations in Ontario, predominantly as wettable powders, emulsifiable concentrates and sprayable concentrates. The principal commercial products are Guthion 50WP and Guthion 240SP, which contain 50% and 240 g/L of azinphos-methyl respectively [12]. The inert ingredients are proprietary and thus, not reported.

Use of azinphos-methyl in the Great Lakes basin is shown on a county-wide basis in Figure 6.2. Agricultural surveys conducted in 1993 suggest that total azinphos-methyl used in Ontario was 71,983 kg with the highest use in southwestern Ontario and major fruit and vegetable, growing areas [15].

6.2 Environmental Concentrations

Azinphos-methyl adsorbs strongly to soil particles and exhibits relatively low water solubility. Therefore, it is reasonably immobile in soil and has low leaching potential [16,17,18]. Azinphos-methyl dissipates from soil more rapidly in the surface layers (0-2.5 centimeters deep) than in deeper layers (2.4-7.5 cm), and may be degraded by microbial actions, ultraviolet (UV) light and hydrolytic decomposition. It volatilizes when exposed to air. Biodegradation and volatilization are the primary routes of dissipation in soil. High levels of soil moisture and the presence of UV light may make photodecomposition an important degradation pathway as well [19]. Estimates vary on how quickly it disappears from the soil, one estimated half-life in sandy loam is five days [19]. Its reported half-life in nonsterile soil is 21 days under aerobic conditions or 68 days under anaerobic conditions [11]. Field studies on the break-down and dissipation of azinphos-methyl in soil demonstrate that it is not persistent and is degraded by 90% within 30 days [16]. Azinphos-methyl is also not persistent in alkaline surface waters, but is more persistent at acidic pH [20].

In water, azinphos-methyl is subject to degradation by sunlight and microorganisms with a half-life between two and three days [8]. Biodegradation is probably the most important degradation process for azinphos methyl in natural waters. Concentrations of azinphos-methyl in Ontario surface waters have occurred at levels that are lethal to some aquatic organisms. Azinphos-methyl at concentrations of 6.5 and 7.9 mg/L were found in surface waters from two streams (2nd order and 3rd order) collected in the summer of 1996 and 1997 in the Niagara Peninsula, down stream from a mixed orchard agricultural region [21]. However, most of the samples collected in the summer were usually below 1 mg/L at both locations. Azinphos-methyl was not found in surface water at these two locations in early spring or in the fall, indicating that azinphos-methyl is not persistent in the environment. Harris et al.[22], found azinphos-methyl in surface waters in orchards in southern Ontario at concentrations up to 1 mg/L (Figure 6.2).

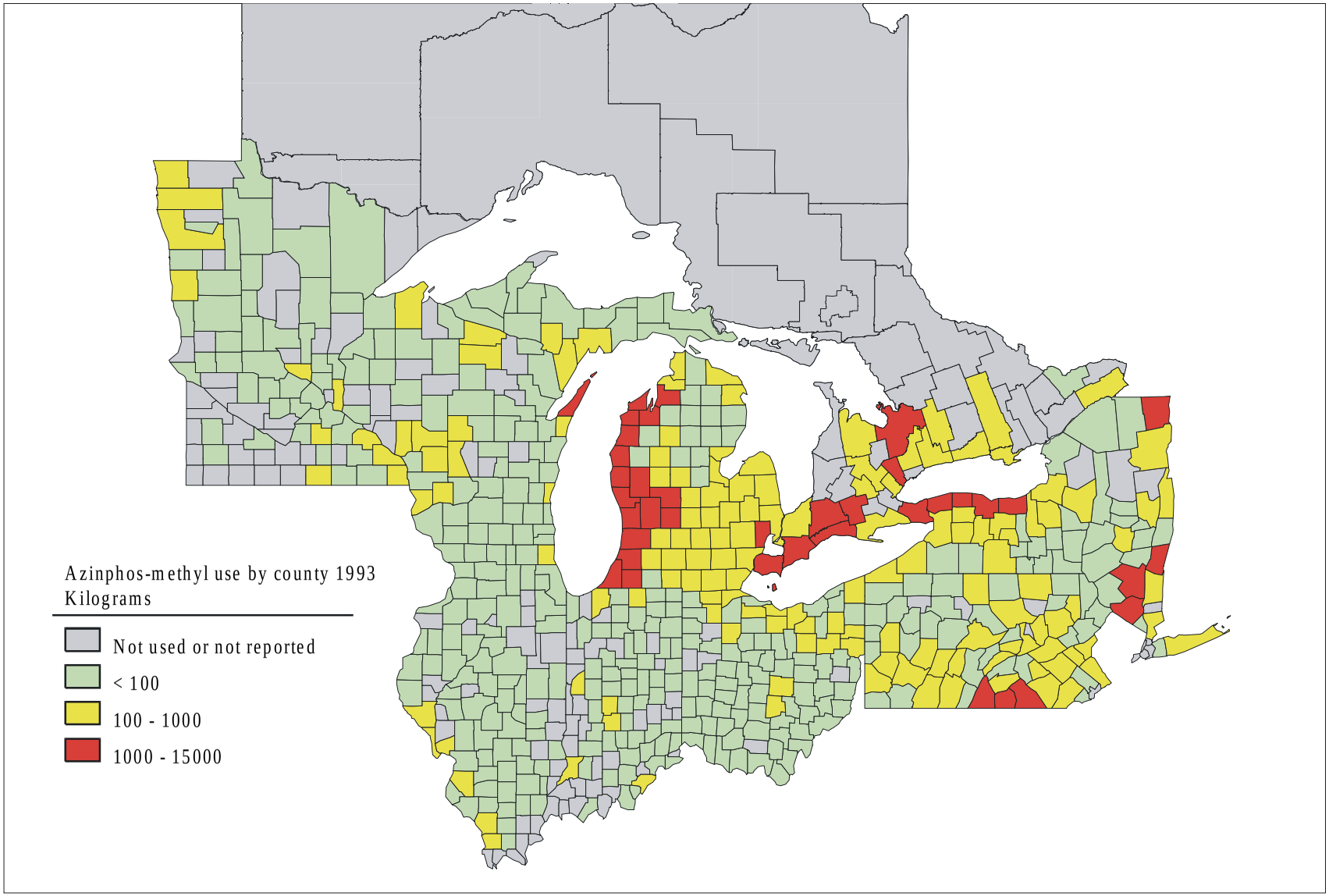

Figure 6.1 Azinpos-methyl use in theGreat Lakes Basin.

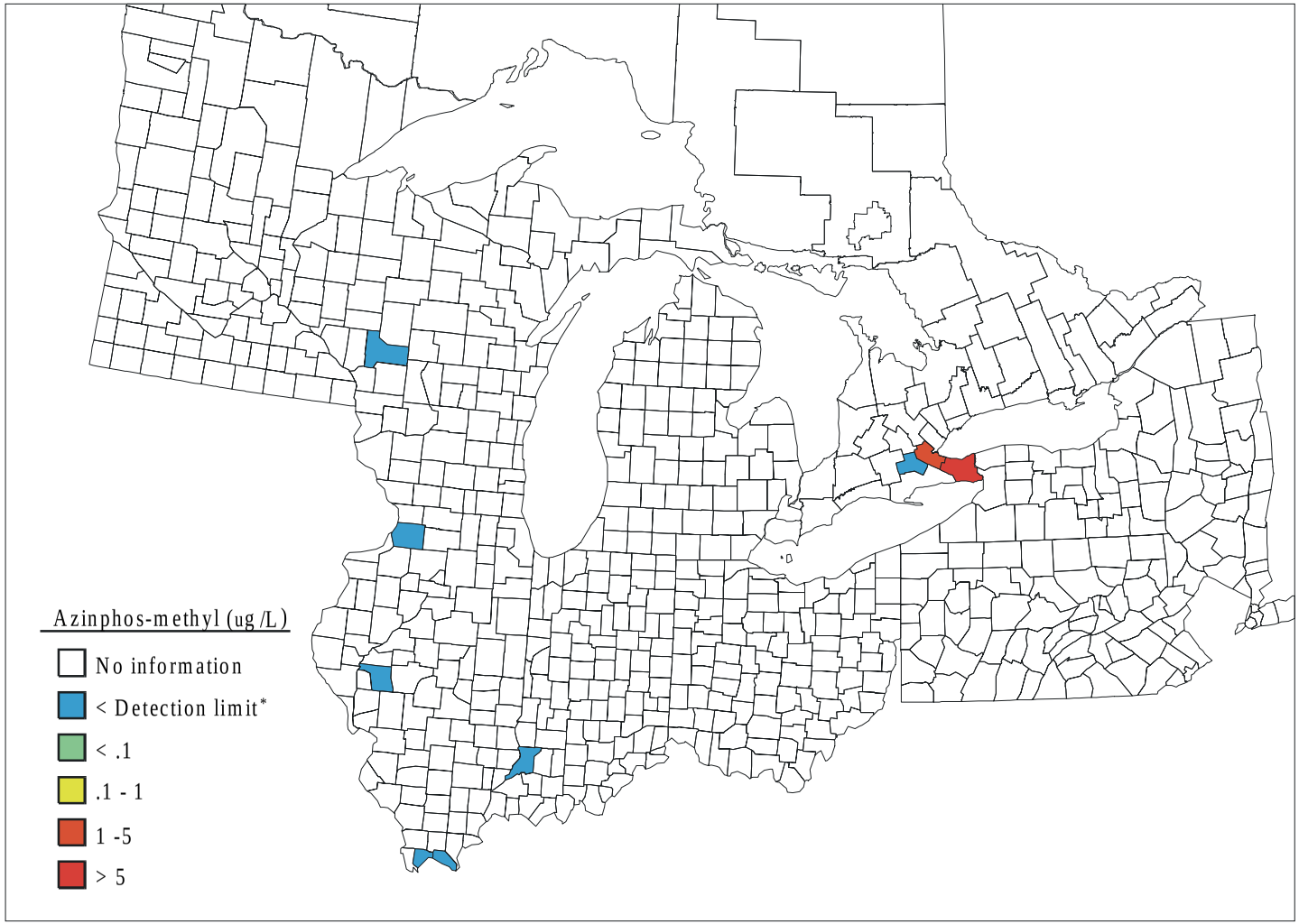

Figure 6.2 Maximum concentration of azinphos-methyl found in surface water by county. *Detection limits range from .01 -.038 ug/L.

Table 6.1 Recommended [8,19,20] applications of azinphos-methyl in 1998 Ontario agriculture.

Crop(s) Protected	Insect(s) Controlled	Application Rate[a]
beans (green, wax, lima)	leafhoppers, Mexican bean beetle, green cloverworm, corn borer	2.25 L/ha
cabbage, cauliflower, broccoli, brussels sprouts	cabbageworm, cabbage looper, diamondback moth caterpillar, ahids	2.25 L/ha
kale	cabbageworm, cabbage looper, diamondback moth caterpillar	2.25 L/ha
cucumber	cucumber beetle	1.5 L/ha
Potato	Colorado potato beetles, flea beetles, tarnished plant bug, leafhoppers	2.25 L/ha
spinach	leaf miner, aphids	1.8 L/ha
tomato	aphids	2.25 kg/ha
apple	Spring feeding caterpillars, Green fruitworm, leafrollers, oystershell scale, European apple sawfly, codling moth, apple maggot,	2.10 kg/ha
blueberries	Cherry fruitworm, Cranberry fruitworm	1.10 kg/ha
cherry (sour),cherry (sweet), plum	Plum curculio, Cherry fruit fly	2.00 kg/ha
grape	grape berry moth, leafhoppers	1.75 kg/ha
peach	tarnished plant bug	2.00 kg/ha
pear	pear psylla, plum curculio	2.10 kg/ha
strawberry	Strawberry leafroller, potato leafhopper	1.50 kg/ha

[a] - Two or more forms of azinphos-methyl were usually listed in OMAFRA publications [8,19,20] (primarily Guthion 50WP, 240 SC, APM 50WP), but only one was chosen as an example for this table. Note that recommendations for vegetables listed an applied mass (kg) or volume (L) only [19]; it was assumed that these values were to be applied on a 'per hectare' basis. Fruit tree 'per hectare' recommendations were based on 4.5-5.5 m high trees.

6.3 Bioconcentration and Metabolism of azinphos-methyl

Both the route and rate of metabolism of azinphos-methyl is highly species-specific. Azinphos-methyl undergoes an oxidative desulfuration resulting in an increase in toxicity of the biotransformation product [8]. This product is an oxygen analog, which can be readily hydrolyzed in mammals but not usually in insects [8].

The metabolism of a single oral dose of radio labelled azinphos-methyl was investigated in the rat. The majority of the radioactivity was eliminated in the expired air within 48 hours as radioactive carbon dioxide. The organs of the rat did not contain significant radioactive residues. Multiple radioactive metabolites were detected in the urine. The authors concluded that most of the identified metabolites were formed via cleavage of P-O methyl ester bonds [23].

6.4 Toxic Mechanisms of Action

Azinphos-methyl is highly toxic by inhalation, dermal absorption, ingestion, and eye contact (24,25). Like all organophosphorus chemicals, azinphos methyl is referred to as a cholinesterase inhibitor. Upon binding to the serine-OH active site of acetylcholinesterase (AChE) (which is phosphorylated in the process) the normal functioning of the enzyme is blocked. AChE is responsible for the neutralization of the biological activity of the neurotransmitter acetylcholine (ACh), which is essential to the proper functioning of the nervous system [11]. With the accumulation of free, unbound ACh at the nerve ending of all cholinergic nerves, there is a continual stimulation of electrical activity.

All organophosphorus insecticides have the same principal mode of action. The reaction between the insecticide and the active site in the AChE protein results in the formation of a transient intermediate complex that hydrolyzes with the loss of a substituent group. This reaction results in a stable, unreactive phosphorylated inhibited enzyme that can only be reactivated at a very slow rate. With many organophosphorus insecticides, enzyme inhibition is irreversible, thus the symptoms of intoxication are prolonged and the toxicity will persist until new AChE are synthesized. However, the specificity for the enzyme and the rate at which the phosphorylated enzyme dissociates to produce free AChE varies between the different organophosphorus insecticides.

Severe poisoning will affect the central nervous system, producing loss of coordination, loss of reflexes, weakness, fatigue, involuntary muscle contractions, twitching, tremors and eventually paralysis of the body extremities and the respiratory muscles. There may also be involuntary defecation or urination, psychosis, irregular heartbeats, unconsciousness, convulsions and coma. Death may be caused by respiratory failure or cardiac arrest [25].

6.5 Acute Toxicity

There is wide variation in the recorded LD50s for azinphos-methyl depending on the route of exposure and the test animal.

Azinphos-methyl is moderately toxic to mammals. The oral LD_{50} for azinphos methyl in rats is 4.4 to 16 mg/kg body weight [8,26,11,19], in guinea pigs is 80 mg/kg [8,27], and in mice is 8 to 20 mg/kg [26]. The dermal LD50 in rats is 88 to 220 mg/kg [10,28;27,11], and in mice is 65 mg/kg [25].

Azinphos-methyl is also moderately toxic to birds (Table 6.2). Acute symptoms of azinphos-methyl poisoning in birds include pupil dilation, clenched talons, regurgitation, wing drop, wing spasms, diarrhea and lack of movement [29]. When chickens were fed azinphos-methyl at a dosage of 40 mg/kg, they developed leg weakness. The acute toxicity data for fish indicate that azinphos-methyl is moderately to very highly toxic depending on the species tested (Table 6.3). Fish poisoned with azinphos-methyl exhibit impairment of the central nervous system; a response pattern that is typical of organophosphorus toxicity. This includes erratic swimming, accompanied by uncontrolled convulsions at varying intervals, rapid gill movements, paralysis, and death [8].

Azinphos methyl is highly toxic to aquatic invertebrates (Table 6.4). A microcosm experiment found concentration as low as 0.8 mg/L reduced cladoceran populations slightly [30]. The study also found that greater than 95% of the amphipods were killed at a concentration of 8 mg/L.

Azinphos-methyl is moderately toxic to amphibians for the species tested (Table 6.5). The No Observed Adverse Effect Level (NOAEL) values for Guthion and Guthion 2S ranged from 0.48 to 7.96 mg/L for the African clawed frog (*Xenopus laevis*) [31,32].

Table 6.2 Avian toxicity of azinphos-methyl. Species indigenous to Ontario are identified by an (I) after the species name.

Species	Common name	Test Type	Concentration	R
Agelaius phoeniceus (I)	Red-winged blackbird	LD50	8.5 mg/kg	11
Sturnus vulgaris (I)	European starling	LD50	27.0 mg/kg	11
Phasianus colchicus (I)	Ring-neck pheasant	LD50	283 mg/kg	11
Colinus virginianus (I)	Bobwhite quail	LD50	32.2 mg/kg	29
Anas platyrhynchos (I)	Mallard duck	LD50	136 mg/kg	11

6.6 Chronic Effects

Studies suggest that the effects of sublethal multiple organophosphorus insecticide exposures exhibit cumulative toxicity, in decreasing ChE activity. As well, exposure to a mixture or a number of different organophosphorus insecticides may be additive in toxicity because the mechanism of action is the same. There is also some evidence that suggests that organophosphorus pesticides may cause reproductive effects.

6.6.1 Chronic Effects in Mammals

A number of studies examining chronic mammalian toxicity of azinphos-methyl have utilized laboratory rats and mice; unfortunately only a few studies measuring acute or chronic toxicity in the field exist.

A study in which rats were fed 2 and 5 mg/kg body weight of azinphos-methyl for 60 days showed no adverse effects, however concentrations of 10 mg/kg or greater did [27]. In another study rats exhibited no cholinesterase inhibition when fed 0.25 mg/kg/day for 60 days. However, 1 mg/kg/day resulted in decreased growth and slight inhibition of brain and red blood cell cholinesterase [8]. Two studies did not show increases in the incidence of tumours in mice or rats [11,26]. A study on pine voles in apple orchards found azinphos-methyl decreased AChE activity which correlated with reduced food consumption and decreased weight. The same study also found that the voles became less aggressive after exposure to azinphos-methyl [43].

6.6.2 Chronic Effects in Birds

A number of studies have described testicular dysfunction in a wild psittacine bird (*Psittacula krameri*) exposed to a variety of organophosphates [44,45]. Maitra and Sarka [45] found that 10 mg/100g body mass/day of methyl parathion, an organophosphorus insecticide with a similar mode of toxicity as azinphos-methyl, caused testicular injury in a wild passerine, white-throated munia (*Lonchura malabarica*). They found decreases in seminiferous tubular diameter and testicular weight, which may be attributed to impairment of hypophysial (pituitary) function. Significant negative correlation was found between the number of degenerated germ cells containing tubules and the rate of AChE activity in the testes as well as in the brain. A field study found sertoli cell development was either delayed or unusual in male tree swallow (*Tachycineta bicolor*) chicks in apple orchards that were sprayed with a number of different pesticides including organophosphorus insecticides [46].

Multiple studies have shown decreased reproductive success in birds from orchards sprayed with organophosphorus insecticides among other chemicals. The effects included reduced fertility, reduced egg production, hatchability of eggs and fledging of young [47, 7, 48]. Although chemicals other than

Table 6.3 Fish toxicity of azinphos-methyl. Species indigenous to Ontario are identified by an (I) after the species name.

Species	Common Name	Age	Dose Method	Test Type	LC50 ug/L	R
Salmo salar	Atlantic salmon	juvenile	flowthrough	96-hr	2.5	33
Salmo salar	Atlantic salmon	fry	static	96-hr	1.8	33
Ictalurus melas	Black bullhead	-	static	96-hr	3500	34
Promoxis	Black crappie	-	static	96-hr	3	11
Lepomis macrochirus (I)	Bluegill sunfish	-	-	96-hr	4.6	35
Lepomis macrochirus (I)	Bluegill sunfish	-	-	96-hr	4	34
Lepomis macrochirus (I)	Bluegill sunfish	-	static	96-hr	40.4	33
Lepomis macrochirus (I)	Bluegill sunfish	-	static	96-hr	22	34
Lepomis macrochirus (I)	Bluegill sunfish	-	static	96-hr	4.1	32
Lepomis macrochirus (I)	Bluegill sunfish	-	static	96-hr	5.2	37
Lepomis macrochirus (I)	Bluegill sunfish	-	flowthrough	96-hr	4.8	33
Lepomis macrochirus (I)	Bluegill sunfish	-	-	48-hr	25	36
Salvelinus fontinalis (I)	Brook trout	-	static	96-hr	3.5	33
Salvelinus fontinalis (I)	Brook trout	-	static	96-hr	1.2	33
Salmo trutta (I)	Brown trout	-	static	96-hr	4	34
Cyprinus carpio (I)	Carp	-	static	96-hr	695	34
Ictalurus puncatatus (I)	Channel catfish	-	static	96-hr	3290	34
Ictalurus puncatatus (I)	Channel catfish	-	static	96-hr	3220	38
Oncorhynchus kisutch	Coho salmon	-	static	96-hr	4.2	37
Oncorhynchus kisutch	Coho salmon	-	static	96-hr	6.1	33
Pimephales promelas (I)	Fathead minnow	-	static	96-hr	93	37
Leuciscusidus melan	Golden orfe	-	static	96-hr	120	33
Carassius auratus	Goldfish	-	static	96-hr	4270	34
Mi cropterus salmoides (I)	Largemouth bass	-	static	96-hr	52	34
Micropterus salmoides (I)	Largemouth bass	-	static	96-hr	48	33
Carassius auratus	Goldfish	-	-	96-hr	1.4	20
Esox lucius (I)	Northern pike	-	static	96-hr	0.36	39
Oncorhynchus mykiss (I)	Rainbow trout	-	-	96-hr	2	35
Oncorhynchus mykiss (I)	Rainbow trout	-	static	96-hr	14	34
Oncorhynchus mykiss (I)	Rainbow trout	-	static	96-hr	3.2	37
Oncorhynchus mykiss (I)	Rainbow trout	-	static	96-hr	6	33
Cryprinodon variegatus	Sheepshead minnow	-	static	96-hr	1.86	33
Cryprinodon variegatus	Sheepshead minnow	-	static	96-hr	2.3	33
Leiostomus xanthuru	Spot	-	flowthrough	48-hr	28	33
Mugil cephalus	Striped mullet	-	flowthrough	48-hr	3.2	36
Perca flavescens (I)	Yellow perch	-	static	96-hr	2.4	33
Perca flavescens (I)	Yellow perch	-	static	96-hr	20	33
Gasterosteus aculeatus	3-spine stickleback	-	-	96-hr	8.5	33
Lepomis cyanellus	Green sunfish	-	static	96-hr	52	33
Micropterus salmoides (I)	Largemouth bass	-	-	48-hr	25	36

Table 6.4 Invertebrate toxicity of azinphos-methyl. Species indigenous to Ontario are identified by an (I) after the species name.

Species	Common name	Dose Method	Test Type	LC50 ug/L	R
Procambarus sp. (I)	Crayfish	static	96-hr	56	33
Callinectes sapidus	Blue crab	flowthrough	48-hr	320	33
Chironomus tentans (I)	midge	-	96-hr	0.37	33
Mysidopsis bahia	opossum shrimp	-	96-hr	0.24	33
Gammarus fasciatus (I)	Scud	static	96-hr	0.1	40
Gammarus lacustris (I)	Scud	static	96-hr	0.14	33
Palaemonetes kadiakensis	Glass shrimp	static	96-hr	0.2	40
Penaeus aztecus	Brown shrimp	flowthrough	48-hr	2.4	33
Crassostrea virginica	Eastern osyter	static	96-hr	4700	33
Daphnia magna (I)	Water flea	-	48-hr	1.6	41

Table 6.5 Amphibian toxicity of azinphos-methyl. Species indigenous to Ontario are identified by an (I) after the species name.

Species	Common name	Age	Chemical	Dose method	Test type	Conc. mg/L	R
Xenopus laevis	African clawed frog	embryo	Guthion	renewal	96-hr	1660	32
Xenopus laevis	African clawed frog	tadpole	Guthion	renewal	96-hr	2940	32
Xenopus laevis	African clawed frog	embryo	Guthion2S	renewal	96-hr	1600	32
Xenopus laevis	African clawed frog	tadpole	Guthion 2S	renewal	96-hr	420	32
Pseudacris triseriata (I)	Chorus frog	tadpole	Guthion	static	96-hr	3200	41
Pseudacris regilla	Chorus frog	tadpole	Guthion	renewal	96-hr	4140	32
Pseudacris regilla	Chorus frog	tadpole	Guthion2S	renewal	96-hr	460	32
Bufo woodhouseii fowleri (I)	Fowlers toad	tadpole	Guthion	static	96-hr	130	40
Bufo woodhouseii fowleri (I)	Fowlers toad	tadpole	Guthion	static	96-hr	109	41
Rana clamitans	Green frog	tadpole	Guthion 50	stat. renewal	96-hr	>5000	42
Rana clamitans	Green frog	tadpole	Guthion 50	stat. renewal	13-d	2610	42

OPs were sprayed in these orchards, studies implicated cholinesterase inhibiting pesticides such as OPs and carbamates insecticides because they have the highest known acute and chronic toxicity to avianspecies in toxicity tests as compared to other compounds used in orchards such as EBDCs, fungicides, synthetic-pyrethroids insecticides, endosulfan and dicofol (Canadian Wildlife Service database, unpublished).

Patnode and White [47] also found that daily egg and chick survival rates decreased as pesticide toxicity increased in three species of birds, northern mockingbird (*Mimus polyglottus*), northern cardinal (*Cardinalis cardinalis*), and brown thrasher (*Toxostoma rufum*) in pecan orchards.

Graham and DesGranges [49] examined the affect of azinphos-methyl on the biology and physiology of birds inhabiting sprayed apple orchards and potato fields in Quebec. They found decreases in cholinesterase activity for 3 species of birds; Chipping sparrow (*Spizella passerina*), American robin (*Turdus migratorius*), and Song sparrow (*Melospiza melodia*) in apple orchards, but no statistical effect was demonstrated in birds from potato fields. They feel that lack of a measurable impact in potato fields was largely attributed to the lower dose rate and to the behavior of resident birds, which led them to avoid sprayed areas. They found no reproductive effects in either the orchards or the fields when compared to controls, however sample size of monitored nests was small (<20 in exposure area).

Fluetsch and Sparling [7] examined the effects of pesticides on avian species inhabiting apple orchards in Pennsylvania. Nests of two species, Mourning dove (*Zenaida macroura*) and American robin were

monitored in 3 organic and 3 conventional apple orchards during 1990 and 1991. Organophosphorus pesticides (including azinphos-methyl, phosphamidon, parathion, dimethoate), carbamate and organochlorine pesticides were sprayed individually or in mixtures as many as 19 times each year.

Spray card tests found that organophosphorus insecticides were deposited on 86% of the nests in conventional orchards. Daily survival rates for nests and hatching success of both species were higher in the organic orchards than in the conventional orchards for the two years combined. Fluetsch and Sparling believe that repeated applications of pesticides within the conventional orchards reduced the reproductive success of doves and robins and may have lowered avian species diversity compared with organic orchards. Unfortunately, no medical pathological (endopathology, histopathology, cytopathology, etc) investigations were undertaken in this study, thus no information was obtained pertaining to mechanisms of action in the dove and robin.

The egg and chick survival and pesticide exposure of tree swallows and eastern bluebirds (*Sialia sialis*) were annually monitored using nest boxes in sprayed and non-sprayed apple orchards in southern Ontario during 1988-1994 [48]. Associations were determined between reproductive success of the nests and organochlorine residues in eggs and the degree of exposure and toxicity of pesticides applied during the study period. Because many pesticides in current use are not persistent in wildlife tissues, a toxicity score was developed to describe the exposure in each nest. The toxicity score was calculated as the product of the extent of the orchard sprayed and the application rate of the chemicals divided by an acute toxicity index of each chemical. The toxicity scores for cholinesterase inhibiting chemicals such as OPs, including azinphos-methyl, and carbamate insecticides were the highest among all the compounds applied in orchards.

Bishop et al. [48] also found total organochlorine concentrations in tree swallow eggs ranged from 0.74 to 3.5 mg/g and in eastern bluebird eggs ranged from 0.47 to 106.3 mg/g wet weight. There was a significant increase in infertile and early embryonic death and unhatched eggs in eastern bluebirds as organochlorine concentrations increased in eggs. At the gradient of contamination found in tree swallow eggs, there were no trends between reproduction and organochlorine levels.

In more than half the study years and over the entire study period, egg fertility and daily survival rates of eggs and chicks of tree swallows declined with increasing toxicity scores of pesticides used during 1988-1994 [48]. Fewer years and reproductive parameters were appear affected in eastern bluebirds. Reduced egg fertility was detected in eastern bluebirds as toxicity scores increased but this only occurred in two years and there was no overall trend for 1988-1994. Daily egg and chick survival was not associated with pesticides in the clutches of eastern bluebirds initiated prior to 1st June in each year.

The bluebird nests produced after that date had significantly lower daily chick or egg survival rates as pesticide exposure and toxicity increased in four study years. Although these findings are consistent with those of Patnode and White [47] and Fleutsch and Sparling [7] in which OPs and carbamates are implicated in decreased reproduction in birds nesting in orchards, we cannot discount the possibility that organochlorine chemicals contributed to these effects in bluebirds nor can we discount the possible impact of other chemicals such as fungicides, synthetic pyrethroids, and acaricides that are often applied to orchards alone or with OP and carbamate chemicals.

Taken together, the studies indicate that birds nesting in orchards are lethally and sublethally affected at every critical stage of reproduction and development by pesticides used in orchards. The studies mainly indicate that the use of OP and carbamate insecticides are the main concern but other chemicals including organochlorine residues may also be affecting reproduction.

6.6.3 Chronic effects in fish

In a study conducted by Tanner and Knuth [50] in northern Minnesota, adult Bluegill sunfish (*Lepomis macrochirus*) were exposed to a single application of azinphos-methyl in 12 littoral enclosures. The responses measured were adult behavior and spawning, embryo hatchability, larval survival until swim-up, young-of-year, young-of-year growth and total biomass. They treated 4 enclosures with 1.0 and 4.0 mg/L and 4 remained untreated. Quantifiable residues remained in the water for 8 days. Concentrations of 4.0 or 1.0 mg/L did not cause any significant long-term (63 day) effects on reproduction, embryo hatchability, larval survival, growth or biomass. They concluded that the apparent lack of significant long-term effects on reproductive success can be partially explained by the relatively short half-life of azinphos-methyl in littoral enclosures.

Adelman et al. [51] found azinphos-methyl reduced fecundity in fathead minnow (*Pimephales promelas*) at concentrations as low as 0.51 mg/L in chronic tests. The concentration was maintained over the course of the test (250 days).

6.7 Potential for Endocrine Disruption

Our working definition of an endocrine disrupting compound:

> *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.*

After examination of relevant literature, we conclude that cholinesterase inhibiting pesticides have the potential to affect reproduction and the endocrine system. However, the lack of studies on azinphos-methyl and its affects pertaining to endocrine endpoints makes it very difficult to determine if azinphos-methyl is a true endocrine disrupter. The reproductive effects in recently reported in orchard-nesting birds do not specifically link azinphos-methyl with endocrine disruption, however they do imply a possible interactive effect of OPs with other compounds on the endocrine system. More research on azinphos-methyl effects on reproduction is essential to determine if it is disrupting the endocrine system. The documented research to support endocrine disruption in fish and amphibians for azinphos-methyl is not strong. Thus, more studies are necessary before conclusions on aquatic organisms can be made.

6.8 Risk to Ontario Environments and Recommendations

Azinphos-methyl does pose an acute risk to wildlife in Ontario. Aquatic ecosystems are at the highest risk after spray events, during this time azinphos-methyl, as well as other OPs (Diazinon) can run off into water bodies at concentrations that are lethal to a number of aquatic species.

- high use regions need to be identified, water samples taken at appropriate times (after spray events) and residues of a number of organophosphorus pesticides measured.
- evaluation of the toxicity of mixtures of OPs with other compounds such as fungicides, in aquatic and terrestrial systems needs to be conducted.
- laboratory and field studies using endocrine disruption endpoints must be completed on terrestrial and aquatic species which are found in ecosystems that are considered to be in high-risk areas.
- endocrine-disruption effects found in wild birds need further evaluation to determine long-term impacts of organophosphorus pesticide alone or in mixtures.
- similar studies to those on reproduction of birds should be performed on organisms such as fish in aquatic ecosystems.

6.9 References

1. Fowler D.L. and J.N. Mahan. 1980. The pesticide review. Agric. Stabilization Conservation Service. USDA, Washington, DC.
2. Maitra, S.K. and R. Sarkar. 1996. Influence of methyl parathion on gametogenic and acetylcholinesterase activity in the testis of white-throated munia (*Lonchura malabarica*). Arch. Environ. Contam. Toxicol. 30:384-389.
3. Hill, E.F., R.G. Heath, J.W. Spann, and J.D. Williams. 1975. Lethal dietary toxicities of environmental pollutants to birds. US Fish and Wild. Ser. Spec. Sci. Rep. Wildlife, # 191, Washington DC, p61.
4. Grue C.E. and B.K. Shipley. 1984. Sensitivity of nestlings and adult starlings to dicrotophos, an organophosphate pesticide. Environ. Res. 35:454-465.
5. Stone, W.B. and P.B. Gradoni. 1985. Recent poisonings of wild birds by diazinon and carbofuran. Northeastern Environ. Sci. 4:160-164.
6. Custer, T.W. and C.A. Mitchell. 1987. Exposure to insecticides of bushland wildlife within the lower Rio Grande Valley, Texas, USA. Environ. Pollut. 45:207-220.
7. Fluetsch, K.M. and D.W. Sparling. 1994. Avian nesting success and diversity in conventionally and organically managed apple orchards. Environ. Toxicol. Chem. 13:1651-1659.
8. Chemagro Division Research Staff. 1974. Guthion (azinphos methyl): Organophosphorus insecticide. Res. Rev. 51:123-180.
9. McEwen, F. L. and G. R. Stephenson. 1979. The use and significance of pesticides in the environment. NY: John Wiley and Sons, Inc.
10. Berg, G. L., ed. 1986. Farm chemicals handbook. Willoughby, OH: Meister Publishing Company.
11. U. S. Environmental Protection Agency. 1985. EPA chemical profile (on azinphos-methyl). Washington, DC.
12. Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). 1998. Vegetable production recommendations, 1998-1999. OMAFRA Publication No. 363. OMAFRA, Toronto, ON, Canada.
13. Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). 1998. Fruit production
14. Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). 1998. Pest management recommendations for greenhouse crops. OMAFRA Publication No. 365. OMAFRA, Toronto, ON, Canada.
15. Hunter, C. and B. McGee. 1994. Survey of pesticide use in Ontario, 1993. Estimates of pesticides used on field crops, fruit and vegetable crops, provincial highway roadsides, and by licensed pesticide applicators. Ontario Ministry of Agriculture, Food and Rural Affairs, Economics Information Report No. 94-01. Policy Analysis Branch, OMAFRA, Toronto, ON, Canada.
16. U. S. Environmental Protection Agency. 1986. Pesticide fact sheet for azinphos methyl. Fact sheet no. 100. Office of Pesticide Programs. Washington, D.C.
17. Howard, P.H. ed. 1989. Handbook of Environmental Fate and Exposure Data for Organic Chemicals, Vol. III: Pesticides. Lewis Publishers, Chelsea, Michigan.
18. Occupational Health Services, Inc. 1991. MSDS for Azinphos Methyl. OHS Inc., Secaucus, NJ.
19. Worthing, C. R., ed. 1983. The pesticide manual: A world compendium. Croydon, England: The British Crop Protection Council.
20. Pickering , Q.H., C. Henderson, and A.E. Lemke. 1962. The toxicity of organic phosphorus insecticides to different species of warmwater fishes. Trans. Am. Fish. Soc. 94:175.
21. Struger J. Organophosphorus insecticides and endosulfan in surface waters of the Niagara fruit belt, Ontario, Canada. IAGLR Conference. Cleveland, Ohio. May 1999.
22. Harris, M.L., C.A. Bishop, J. Struger, M.R. van den Heuvel, G.J. Van Der Kraak, D.G. Dixon, B. Ripley, and J.P. Bogart. 1998. The functional integrity of northern leopard frog (*Rana pipiens*) and green frog (*Rana clamitans)* populations in orchard wetlands. I. Genetics, physiology, and biochemistry of breeding adults and young-of-the year. Environ. Toxicol. Chem. 17:1338-1350.
23. Fakhr, I.I., S.D. Zayed, and N.A. Hamdy. 1996. Fate and metabolism of radio labelled insecticide azinphos-methyl in rat. J. Pest. Sci. 21:1-5.

24. Meister, R.T., ed. 1992. Farm Chemicals Handbook '92. Meister Publishing Company, Willoughby, OH.
25. US Environmental Protection Agency. 1990. Azinphos methyl; Deletion of certain uses and directions for use for agricultural crops. Fed. Reg. 55: 45846.
26. Hayes, W. J. 1982. Pesticides studied in man. Baltimore, MD: Williams and Wilkins.
27. Hartley, D. and H. Kidd, eds. 1983. The agrochemicals handbook. Nottingham, England: Royal Society of Chemistry.
28. Clayton, G. D. and F. E. Clayton, eds. 1981. Patty's industrial hygiene and toxicology. Third edition. Vol. 2: Toxicology. NY: John Wiley and Sons.
29. U. S. Department of Agriculture, Soil Conservation Service. 1990. SCS/ARS/CES Pesticide Properties Database: Version 2.0 (Summary). USDA - Soil Conservation Service, Syracuse, NY.
30. Stay, F.S. and A.W. Jarvinen. 1995. Use of microcosm and fish toxicity data to select mesocosm treatment concentrations. Arch. Environ. Contam. Toxicol. 28:451-458.
31. Schuytema, G.S., A.V. Nebeker, and W.L. Griffis. 1994. Toxicity of Guthion and Guthion 2S to *Xenopus laevis* embryos. Arch. Environ. Contam. Toxicol. 27:250-255.
32. Schuytema, G.S., A.V. Nebeker, and W.L. Griffis. 1995. Comparative toxicity of Guthion and Guthion 2S to *Xenopus laevis* and *Pseudracris regilla*. Bull. Environ. Contam. Toxicol. 54:382-388.
33. Montaque, B. 1995. Environmental Effects Branch Oneliner Toxdata Electronic File (1991-1995). US Environmental Protection Agency (EPA), Washington, DC.
34. Macek, K.J and W.A. McAllister. 1970. Insecticide susceptibility of some common fish family representatives. Trans. Am. Fish. Soc. 99:20-27.
35. Kidd, H. and D.R. James. 1991. Agrochemicals Handbook. 3rd ed. Royal Society for Chemistry, England.
36. Meyer, J.R. 1965. The experimental use of Guthion as a selective fish eradicator. Trans. Am. Fish. Soc. 94:203.
37. Katz, M. 1961. Acute toxicity of some organic insecticides to three species of salmonoids and to threespine stickleback. Trans. Am. Fish. Soc. 90:264-268.
38. Holcombe, G.W., G.L. Phipps, A.H. Sulaiman, and A.D. Hoffman. 1987. Simultaneous multiple species testing: acute toxicity of thirteen chemicals to twelve diverse freshwater amphibian, fish and invertebrate families. Arch. Environ. Contam. Toxicol. 16:697-710.
39. Johnson, W.W. and M.T. Finley. 1980. Handbook of acute toxicity of chemicals to fish and aquatic invertebrates. Resource Publication 137, Fish and Wildlife Service, USDI, Washington, DC, p 98.
40. Sanders, H.O. 1970. Pesticide toxicities to tadpoles of the western chorus frog (*Pseudracris triseriata*) and Fowler's toad (*Bufo woodhousii fowleri*). Copia 1970:246-251.
41. Mayer, F.L., Jr., and M.R. Ellersieck. 1986. Manual of acute toxicity: Interpretation and data base for 410 chemicals and 66 species of freshwater animals. US Fish and Wildlife Service, Resource Publication # 160. Washington D.C.
42. Harris, M.L., C.A. Bishop, J. Struger, B. Ripley, and J.P. Bogart. 1998. The functional integrity of northern leopard frog (*Rana pipiens*) and green frog (*Rana clamitans*) populations in orchard wetlands.II . Effects of pesticides and eutrophic conditions on early life stage development. Environ. Toxicol. Chem. 17:1351-1363.
43. Durda, J.L., R.A. Powell, and G.T. Barthalmus. 1989. Physiological and behavioral effects of Guthion on pine voles, *Microtus pinetorum*. Bull. Environ. Contam. Toxicol. 43:80-86.
44. Maitra, S.K. and R. Sarkar.1991. Histopathological changes in the testes after oral administration of quinalphos, an organophosphorus pesticide, in a subtropical wild bird *Pssitacula krameri*. Eur. Arch. Biol. 102:413-429.
45. Maitra, S.K. and R. Sarkar.1993. Evaluation of testicular responsiveness to ingestion of methyl-parathion in roseringed parakeets (*Pssitacula krameri*). Pest. Res. J. 5:60-67

46. Bishop, C.A., G.J. Van Der Kraak, P. Ng, J.E.G. Smits, and A. Hontela. 1998. Health of tree swallows (*Tachycineta bicolor*) nesting in pesticide-sprayed apple orchards in Ontario, Canada. II. Sex and thyroid hormone concentrations and testes development. J. Toxicol. Environ. Health, Part A, 55:561-581.
47. Patnode, K.A., and D.H. White. 1991. Effects of pesticides on songbird productivity in conjunction with pecan cultivation in southern Georgia: a multiple-exposure experimental design. Environ. Toxciol. Chem. 10:1479-1486.
48. Bishop, C,A., B.T. Collins, P. Mineau, N.M. Burgess, W.F. Read, and C. Risley. 1999. Impacts of pesticides on cavity-nesting birds in apple orchards. In: Adams, N.J. and R.H. Slotow (eds) Proc. 22 Int. Ornithol. Congr., Burban: 1043-1057. Johannesburg: BirdLife South Africa.
49. Graham, D.J. and J.L. DesGranges. 1993. Effects of the organophosphate azinphos-methyl on birds of potato fields and apple orchards in Quebec, Canada. Agric. Ecosyst. Environ. 43:183-199.
50. Tanner, D.K. and M.L. Knuth. 1995. Effects of azinphos-methyl on the reproductive success of the bluegill sunfish, Lepomis macrochirus, in littoral enclosures. Ecotoxi.Environ. Safety 32:184-193.
51. Adelman, I.R., L.L. Smith, and G.D. Siesennop. 1976. Chronic toxicity of guthion to the fathead minnow (*Pimephales promelas*). Bull. Environ. Contam. Toxicol. 15:726-733.

7.0 PESTICIDE MIXTURES

The practice of mixing pesticides during manufacturing and field application is quite common in agriculture, yet the toxicological properties of such mixtures are often unknown. The following discussion serves to emphasize the lack of and need for relevant research on herbicide, fungicide and insecticide mixes.

7.1 Description of Mixtures Relevant to Ontario

Pesticide mixes used in Ontario may be divided into three broad categories: 1. those combined during the manufacture of a formulation (i.e. coformulation); 2. those recommended [1-4] for simultaneous application in one spray tank to combat a particular pest; and 3. those commonly applied by farmers in one spray tank to combat multiple pests that are present in crops at the same time. The first two are easily identified in agricultural publications, but the third group is difficult to identify without a more intimate knowledge of regional farming practices.

There are several examples of formulations containing more than one active ingredient. Of the 36 available to Ontario farmers (Table 7.1), 26 (72%) are herbicide combinations, mostly containing more than one class of chemical. The phenoxy and s-triazine classes predominate in mixes designed to control multiple species of grasses, thistles and weeds. The only strictly insecticidal formulation (malathion plus pyrethrin) is registered for the relatively small provincial mushroom industry. The nematocides listed are all fumigants used primarily on tobacco, but also on a variety of fruits and vegetables.

Herbicides and fungicides also dominate the list of tank mixes recommended [1-4] by the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) in fruit and vegetable production (Table 7.2). With the exception of one mix of two s-triazines (atrazine and cyanazine), all involve combinations of two or more pesticide classes. The s-triazines, acetanilides, dinitroanilines and ethylenebisdithiocarbamates (EBDCs) occur frequently in spray tank mixes.

Although there has been no comprehensive, province-wide assessment of the pesticide combinations farmers routinely apply to crops, some information on tank mixes used in Ontario apple orchards has been gathered during amphibian and avian research studies (C. Bishop, Canadian Wildlife Service, unpublished data). Aside from the frequent use of myclobutanil with an EBDC (as outlined in Table 7.2), some other combinations found in 1993-94 spray schedules were as follows:

- an EBDC (mancozeb or metiram) and myclobutanil with one or more of streptomycin sulphate, deltamethrin, chinomethionat, simazine, azinphos-methyl, phosmet and clofentezine;
- myclobutanil plus diazinon;
- metiram plus phosmet;
- captan plus phosmet and dicofol;
- dicofol plus deltamethrin;
- diazinon plus carbaryl;
- Dikar (no common name) plus a miticide and azinphos-methyl;
- captan plus an organophosphate (azinphos-methyl or diazinon); and
- captan plus endosulfan and propargite or dicofol.

Since apple production recommendations closely parallel those for other fruit crops like apricots, cherries, peaches and pears (see Table 7.2), we might expect similar combinations to be applied to other fruit orchards. It is unclear whether the frequency and form of tank mixes could also be extrapolated to estimate spray practices in vegetable and field crop production. Nonetheless, existing information would suggest that the application of insecticide and fungicide mixes is more common than agricultural publications indicate.

7.2 Toxicity of Pesticide Mixtures

A comprehensive review of all pesticide mixture toxicity studies is beyond the scope of this report. Thompson [5] provides an excellent recent review. An overview of her discussion, along with reference to other literature relevant to those compounds discussed in chapters two to six will be conducted here.

The greatest concern with pesticide mixtures in natural environments is the characterization of their interactive toxicity. Most risk assessments assume that combinations exhibit additive toxicity, but synergism and antagonism have also been seen in some studies. For example, over one-third of binary pesticide combinations tested in one laboratory with bluegill sunfish showed greater than additive toxicity [6].

The majority of studies testing interactions among pesticides have investigated the most common insecticide classes, e.g. organochlorines, organophosphates, pyrethroids and carbamates. Four references to endosulfan mixtures were discovered [7-10], and two of these detected greater than additive toxicity. When rainbow trout were exposed to endosulfan mixed with the diethyl-phosphorodithionate disulfoton, acute toxicity increased by two orders of magnitude and chronic effects were also dramatically enhanced compared to singular exposures [7].

Similarly, an *in vitro* examination of estrogen receptor activation showed greater than additive effects of binary combinations of endosulfan with dieldrin, toxaphene and chlordane [8]. Conversely, an organophosphate (azinphos-methyl)/organochlorine (endosulfan) mixture produced simply additive acute effects in the mummichog (*Fundulus heteroclitus*) [9]. The fourth endosulfan study assessed an organochlorine (endosulfan)/ pyrethroid (deltamethrin) formulation used to control the tsetse fly in Botswana; unfortunately, although significant acute and chronic effects were detected, no comparisons were made to effects produced by either compound administered independently [10].

Although a great deal of speculation has surrounded the effects of EBDCs in mixture, few investigators have attempted to quantify EBDC interactions using relevant combinations. Thompson [5] reports that EBDCs are of interest from the perspective of mixture toxicity because they have an inhibitory effect on the principal vertebrate detoxification pathway - the cytochrome P450 enzyme system. Inhibition may prolong or delay the metabolism and excretion of other compounds (e.g. organochlorines) present in the mixture that usually induce cytochrome P450.

However, such studies would be further complicated by the fact that EBDCs affect the P450 system in a time-dependent manner; they produce inhibition during the first few hours after exposure, then induction for up to 3 days post-exposure [5]. Two relevant EBDC mixture studies were found in the recent literature [11,12]. When mancozeb was fed in combination with deltamethrin (a pyrethroid) to rats, the toxicity associated with mancozeb was dramatically increased [11]. In another study testing the combined effects of a variety of dithiocarbamates and copper, strong synergistic toxic effects were observed with maneb and zineb, but not with mancozeb [12]. The authors suggested that the metals associated with the different EBDCs were responsible for the discrepancies in synergistic behavior.

Despite the fact that most of the pesticide mixtures used in agriculture involve combinations of herbicides, the literature on herbicide interactions is sparse. This may be because, based on a small number of studies [13,14] along with described similarities in modes of action, the assumption is made that many herbicide mixtures will show simple toxic additivity. When crayfish (*Procambarus clarkii*) were exposed to a mixture of two dinitroaniline herbicides (trifluralin and oryzalin), there was a 20-fold decrease in associated toxicity compared to single compound tests [15]. However, when the synergist piperonyl butoxide was added to test solutions of trifluralin and methoxychlor, the metabolism of both pesticides by the green sunfish was greatly inhibited [16]. Piperonyl butoxide has been routinely used by manufacturers for years to increase the efficacy of pyrethrin insecticides. Apparently, its synergistic capabilities extend to at least one class of herbicide as well. Given the complexity of pesticide mixtures present in aquatic environments, it would be reasonable to expect that influxes of herbicides and insecticides would coincide. Thompson [5] emphasizes the varying effects of different compounds on drug-metabolizing enzymes and their relevance in creating synergism. While her arguments are valid, the most relevant mixtures are not always those most commonly studied nor those known to inhibit or induce cytochrome P450 or esterase enzyme systems. It would seem more relevant to identify the most common combinations found in Ontario surface waters and use them as a basis for mixtures research.

Table 7.1 Formulations composed of two or more active ingredients.

Group[a]	Pesticide Classes	Active Ingredients	Formulation Name(s)
H	phenoxy/benzoic acid	2,4-D/dicamba	Kil-mor, Tricep, Premium 3-way Turf, Par III, Killex, Dycleer 24
H	phenoxy/s-triazine	2,4-D/atrazine	Shotgun
H	s-triazine/benzothiadiazine	atrazine/bentazon	Laddok
H	phenylcarbamate (2)	desmedipham/ phenmedipham	Betamix
H	triazolopyrimidine sulfonanilide/acetanilide	flumetsulam/ metolachlor	Broadstrike Dual
H	triazolopyrimidine sulfonanilide/dinitroaniline	flumetsulam/ trifluralin	Broadstrike Treflan
H	uracil/phenoxy	bromacil/2,4-D	Calmix Pellets
H	uracil/substituted urea	bromacil/diuron	Krovar
H	hydroxybenzonitrile/ phenoxy	bromoxynil/MCPA	Buctril M
H	imidazolinone/s-triazine	imazethapyr/ metribuzin	Conquest
H	benzoic acid/s-triazine	dicamba/atrazine	Marksman
H	benzoic acid/phenoxy	dicamba/MCPA	Dyvel
H	benzoic acid/phenoxy (2)	dicamba/MCPA/ mecoprop	Target
H	phenoxy (2)	dichlorprop/2,4-D	Dichlorprop-D, Weedone CB, Diphenoprop BK 700, Estaprop, Diphenoprop See
H	acetanilide (2)	metolachlor/ benoxacor	Dual II
H	phenoxy (2)	mecoprop/2,4-D	Expedite Broadleaf, Meco-D, Premium 2-way Turf. Turf-Rite 2+2
H	triazolopyrimidine sulfonanilide/pyridine/phenoxy	flumetsulam/clopyralid/2,4-D	Striker
H	imidazolinone/dinitroaniline	imazethapyr/ pendimethalin	Valor
H	acetanilide/s-triazine	metolachlor/atrazine	Primextra Light
H	sulfonylurea (2)	nicosulfuron/rimsulfuron	Ultim, Ultim DF
H	bipyridylium (2)	paraquat/diquat	Weed & Grass Killer
H	bipyridylium/s-triazine	paraquat/simazine	Terraklene
H	pyridine/phenoxy	picloram/2,4-D	Tordon 101 Mixture

Table 7.1 Continued.

Group[a]	Pesticide Classes	Active Ingredients	Formulation Name(s)
H	acetanilide/phenoxy	propanil/MCPA	Stampede CM
H	sulfonyl urea (2)	thifensulfuron-methyl/ tribenuron methyl	Refine Extra
F	morpholine/EBDC	dimethomorph/ mancozeb	Acrobat
F	acylalanine/EBDC	metalaxyl/ mancozeb	Ridomil MZ
F	acylalanine/copper	metalaxyl/copper hydroxide	Ridomil/Copper
F	carbamate/acid/chlorophenyl	propamocarb/HCl/ chlorothalonil	Tattoo C
F/N	halogenated hydrocarbon/ chloroalkene	dichloropropene/chloropicrin	Telone C17R
F/I	organophosphate/ dithiocarbamate	ethion/thiram	Ethion 5/Thiram G
I	organophosphate/ pyrethroid	malathion/pyrethrin	Mushroom Fly Dust
H/F/N/I	isothiocyanate/halogenated hydrocarbon	methyl isothiocyanate/ dichloropropene	Vorlex Plus
H/F/N/I	isothiocyanate/halogenated hydrocarbon/chloroalkene	methyl isothiocyanate/ dichloropropene/ chloropicrin	Vorlex Plus
H/F/N/I	organohalogen/chloroalkene	methyl bromide/ chloropicrin	Terr-O-Gas 67

[a] - H = herbicide, F = fungicide, N = nematocide, I = insecticide.

Table 7.2 Tank mixes of pesticides recommended [1-4] for Ontario fruit and vegetable production.

Pesticide Classes in Mix	Active Ingredients	Crop(s) Protected
s-triazine + dinitroaniline	metribuzin+trifluralin, atrazine/ cyanazine+pendimethalin	apple, apricot, cherry, plum, peach, pear, tomato, field corn
s-triazine + dinitroaniline + acetanilide	metribuzin+trifluralin+metolachlor	tomato
s-triazine + acetanilide	simazine/atrazine/cyanazine/metribuzin+meto-lachlor	apple, apricot, cherry, plum, peach, pear, sweet corn, tomato, potato
s-triazine + acetanilide + amino acid	atrazine+metolachlor+glyphosate	sweet corn
s-triazine + amino acid	simazine+glyphosate	apple, grape
s-triazine + s-triazine	cyanazine+atrazine	sweet corn
s-triazine + substituted urea	atrazine/metribuzin+linuron	sweet corn, potato
s-triazine + substituted urea + acetanilide	metribuzin+linuron+metolachlor	potato
s-triazine + hydroxybenzonitrile	atrazine+bromoxynil	sweet corn
s-triazine + phenyl pyridazine	atrazine/metribuzin+pyridate	tomato, sweet corn
s-triazine + amide	simazine+napropamide	apple, apricot, cherry, plum, peach, pear, asparagus
s-triazine + bipyridylium	simazine/atrazine+paraquat	apple, pear, blueberry, raspberry, sweet corn
s-triazine + thiocarbamate	metribuzin/atrazine/cyanazine+ EPTC	sweet corn, potato
s-triazine + uracil	metribuzin+terbacil	apple, apricot, cherry, plum, peach, pear
acetanilide + substituted urea	metolachlor+linuron/monolinuron/ metobromuron	potato
acetanilide + thiocarbamate	metolachlor+EPTC	sweet corn
dinitroaniline + thiocarbamate	trifluralin+EPTC	snap bean, lima bean
dinitroaniline + imidazolinone	trifluralin/pendimethalin+ imazethapyr	field corn
dinitroaniline + benzoic acid	pendimethalin+dicamba	field corn
dinitroaniline + sulfonylurea	pendimethalin+rimsulfuron	field corn

Tab;e 7.2 Continued.

Pesticide Classes in Mix	Active Ingredients	Crop(s) Protected
amide + uracil	napropamide+terbacil	apple, apricot, cherry, plum, peach, pear
ethofumesate + thiocarbamate	ethofumesate+cycloate	sugar beet
pyridazinone + trichloroacetic acid	pyrazon+TCA	red beet
EBDC + conazole	mancozeb/metiram+myclobutanil	apple
EBDC + carbamate	mancozeb/metiram+benomyl	apple, pear
EBDC + oil	mancozeb/metiram + Superior oil	apple
EBDC + copper	mancozeb + copper sulphate	greenhouse, bean, cole & vine crops, potato, pepper, tomato
captan + conazole	captan+myclobutanil	apple
captan + benzimidazole	captan+thiophanate-methyl	apple
captan + carbamate	captan+benomyl	apple, cherry, raspberry, blackberry
captan + organophosphate + organochlorine	captan+diazinon+lindane	fruit & vegetable seed
dithiocarbamate + organophosphate + organochlorine	thiram+diazinon+lindane	fruit & vegetable seed

7.3 Risk to Ontario Environments and Recommendations

- an ongoing program of environmental monitoring of pesticides in Great Lakes tributaries needs to implemented in addition to existing periodic monitoring that is carried out in agro-ecosystems of concern, to continue to assess the mixtures of pesticides that occur in the environment.
- analytical methods for the analysis of fungicides and ultralow volume pesticides such as the imidazolinone and sulfonylurea herbicides and other newly registered compounds must be established as part of an environmental monitoring program.
- Laboratory or mesocosm studies must be undertaken to experimentally evaluate the synergistic effects of environmentally relevant mixtures.

7.4 References

1. Ontario Ministry of Agriculture, Food and Rural Affairs 1997. 1997-1998 Ontario mushroom pesticide recommendations. OMAFRA Publication No. 367.Toronto, ON, Canada.
2. Ontario Ministry of Agriculture, Food and Rural Affairs. 1998. Fruit production recommendations 1998-1999. OMAFRA Publication No. 360. Toronto, ON, Canada.
3. Ontario Ministry of Agriculture, Food and Rural Affairs. 1998. Vegetable production recommendations 1998-1999. OMAFRA Publication No. 363. Toronto, ON, Canada.
4. Ontario Ministry of Agriculture, Food and Rural Affairs. 1998. Guide to weed control, 1998. OMAFRA Publication No. 75. Toronto, ON, Canada.
5. Thompson, H.M. 1996. Interactions between pesticides; a review of reported effects and their implications for wildlife risk assessment. Ecotoxicol. 5:59-81.
6. Macek, K.J. 1975. Acute toxicity of pesticide mixtures to bluegills. Bull. Environ. Contam. Toxicol. 14:648-652.
7. Arnold, H., H-J. Pluta, and T. Braunbeck. 1995. Simultaneous exposure of fish to endosulfan and disulfoton *in vivo*: ultrastructural, stereological and biochemical reactions in hepatocytes of male rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 33:17-43.
8 Arnold, S.F., D.M. Klotz, B.M. Collins, P.M. Vonier, L.J. Guillette Jr., and J.A. McLachlan. 1996. Synergistic activation of estrogen receptor with combinations of environmental chemicals. Science 272:1489-1492.
9. Fulton, M.H. and G.I. Scott. 1991. The effect of certain intrinsic and extrinsic variables on the acute toxicity of selected organophosphorus insecticides to the mummichog, *Fundulus heteroclitus*. J. Environ. Sci. Health 26B:459-478.
10. Knauer, J., J.R. Duncan, and G.S. Merron. 1993. Sublethal effects of an organochloride/ synthetic pyrethroid insecticide cocktail on *Tilapia rendalli rendalli* (Pisces: Cichlidae). S. Afr. J. Sci. 89:249-251.
11. SzJpv‘lgyi, J., K. Nagy, M. Bed\, A. Reg‘ly-MJrei, M. Szerletics, K. So\s, and M. Antal. 1988. Examination of the interaction of Decis and Dithane in rats. Toxicol. 53:107-111.
12. Bonnemain, H. And D. Dive. 1990. Studies on synergistic toxic effects of copper and dithiocarbamate pesticides with the ciliate protozoan *Colpidium campylum* (Stokes). Ecotoxicol. Environ. Saf. 19:320-326.
13. Faust, M., R. Altenburger, W. Boedecker, and L.H. Grimme. 1993. Additive effects of herbicide combinations on aquatic non-target organisms. Sci. Total Environ. Suppl. 1993:941-952.
14. Faust, M., R. Altenburger, W. Boedecker, and L.H. Grimme. 1994. Algal toxicity of binary combinations of pesticides. Bull. Environ. Contam. Toxicol. 53:134-141.
15. Naqvi, S.M. and T-S. Leung. 1983. Trifluralin and oryzalin herbicides toxicities to juvenile crawfish (*Procambarus clarkii*) and mosquitofish (*Gambusia affinis*). Bull. Environ. Contam. Toxicol. 31:304-308.
16. Reinbold, K.A. and R.L. Metcalf. 1976. Effects of the synergist piperonyl butoxide on metabolism of pesticides in green sunfish. Pest. Biochem. Physiol. 6:401-412.

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 > 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.

Neuro-endocrine 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, algicides, 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.

GLOSSARY OF ACRONYMS

a.i. - Active Ingredient

ACh - Acetylcholine

AChE - Acetylcholinesterase

AhR - Aryl Hydrocarbon Receptor

ATP - Adenosine Triphosphate

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

EAC - Endocrine Active Chemical

EBDC - Ethylene Bisdithiocarbamate

EBIS - Ethylene Bisisothiocyanate Sulfide

EC - Emulsifiable Concentrate

EC_{50} - effective Concentration Causing a Response in 50% of a Test Population

ED_{50} - Effective Dose Causing a Response in 50% of a Test Population

EDA - Ethylene Diamine

EDC - Endocrine Disrupting Chemical

EEC - Expected Environmental Concentration

ETU - Ethylene Thiourea

EU - Ethylene Urea

FSH - Follicle Stimulating Hormone

GABA - Gamma-amino Butyric Acid

K_{ow} - Octanol/Water Partition Coefficient

LC_{50} - Concentration (In Water) Causing 50% Mortality

LD_{50} - Oral Dose Causing 50% 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

TBT - Tributyltin

TRH - Thyroid Releasing Hormone

TSH - Thyroid Stimulating Hormone

US EPA - United States Environmental Protection Agency

WHO - World Health Organization

WP - Wettable Powder