Ecological Relevance of Pesticide Residues in Alberta Surface Waters: An Evaluation Based on Toxicity Testing

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SUMMARY

Low concentrations (ng/L to µg/L range) of many different pesticides are present in Alberta surface waters. The environmental significance of the presence of low levels of pesticides is unclear particularly because water quality guidelines (if available) apply only to single compounds and not mixtures. The objective was to assess the toxicity of pesticide mixtures to species representative of major trophic levels in aquatic systems. Pesticides were selected based on the frequency of detection in Alberta surface waters, relative concentration, and availability of information (toxicity data).

The study was conducted in three phases. The first phase involved range finding tests to compare species sensitivities and establish treatment levels for tests to derive endpoints based on measured pesticide levels (Phase II). The most sensitive species was carried forward to Phase III testing of pesticide mixtures.

The tests were done with nine technical or reagent grade chemicals (2,4-D, MCPA, MCPP, dicamba, bromoxynil, picloram, imazamethabenz, lindane, and diazinon) and four commercial formulations (MCPA, MCPP, diazinon and chlorpyrifos). The commercial formulations were not carried forward to Phase II and III. The testing included:

- Microbes
  - Estrogenic activity with the YES assay (Yeast Estrogen Screening assay)
  - Genetic induction potential (SOS-Chromotest)
  - Bacterial luminescence (light output at 15 minutes)
- Plants
  - 72 h algal growth inhibition test
  - 7 d duckweed growth inhibition test
- Invertebrate
  - 7 d *Ceriodaphnia dubia* survival and reproduction test
- Vertebrate (fish)
  - 7 d fathead minnow survival and growth test

The tests were conducted on a water-accommodated fraction (i.e., solution containing the compound in excess of the maximum water solubility).

The pesticides were not genotoxic and exhibited no estrogenic activity. These two assays were not carried forward into Phase II and III. The concentrations of each herbicide required to elicit a response in the other test species were substantially higher than the median and mean levels measured in surface waters (mg/L range as compared to ng/L and µg/L range). However, the effects of the two insecticides on *Ceriodaphnia* and fathead minnows were at concentrations near those levels found in surface waters.

The effects of the pesticides were also consistent amongst the test species. The insecticides lindane and diazinon and the herbicide bromoxynil were the most toxic. *Ceriodaphnia* was the most sensitive test species.

Mixtures of the seven herbicides had no adverse effects on survival and reproduction in *Ceriodaphnia* when administered at concentrations of 0.01 and 0.1 mg/L. Similar results were obtained for a mixture of the two insecticides (lindane and diazinon).

The findings suggest that the pesticide residues measured in surface waters of Alberta are at levels that have no adverse effects on species representative of major trophic levels in aquatic systems. It should be noted that the final tests were done with reagent and technical grade materials added to laboratory dilution water. Degradation products, metabolites, and differences in water quality conditions could affect the availability and toxicity of the chemicals. Further, there is a need to address other related issues such as the bioaccumulation and biomagnification of these substances in aquatic ecosystems, and impacts of peak runoff events.

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1.0 INTRODUCTION

Low levels of many different pesticides are regularly detected in samples from Alberta surface waters (AENV, unpublished data). The environmental significance of these findings is unclear particularly because water quality guidelines (if available) apply only to single compounds and not mixtures. There are few published studies on the environmental effects of pesticide mixtures administered at low concentrations (i.e., µg/L range).

The objective of this study was to assess the ecological relevance of pesticide mixtures in Alberta surface waters. An ecosystem approach was selected incorporating organisms representative of major trophic levels in aquatic systems (Table 1). The tests were conducted under defined laboratory conditions following standard Environment Canada test methods. These methods were designed to assess potential impacts of substances and conditions on selected life forms present in aquatic systems. Additional tests were included to assess potential genotoxicity and to screen for estrogenic effects.

The test species and endpoints are summarized in Table 1 and include microbes, plants, an invertebrate, and a fish. The approach includes both acute (survival) and chronic effects (growth and reproduction). Microbes are an integral component of aquatic ecosystems. They convert chemical energy into biomass (decomposition and cycling of nutrients) and serve as a major food source for invertebrates. Plants convert chemical energy and light into biomass and serve as a food source for invertebrates and fish. Invertebrates feed on plants, microbes, and detritus and are preyed upon by fish and other invertebrates. Effects detected at any one level could indicate potential impairment of ecosystem structure and function. This would warrant further analyses to determine what constituent or condition caused the effect.

The study was conducted in three phases as outlined in Figure 1.

I. Range Finding Tests
II. Derivation of Endpoints
III. Tests on Mixtures

Ten pesticides (7 herbicides and 3 insecticides) were included in the initial screening phase (Table 2). These compounds were selected based on the following criteria:

- Frequency of detection in surface waters,
- Concentration in surface waters versus guidelines for the protection of aquatic life, and
- Lack of available toxicity information.

The objective of Phase I was to determine the relative potencies of each compound to the selected test species. The pesticides and tests carried forward to Phase II were selected based on results from Phase I. The tests with mixtures were done with the most sensitive organism (Phase III).

2.0 METHODS

The methods apply to all three phases of the project where appropriate.

2.1 Test Substances

Seven herbicides and three insecticides were included in Phase I of the study (Table 2). The herbicides are listed based on the frequency of detection and concentration in surface waters. In other words, 2,4-D was detected more frequently and at higher concentrations than all other herbicides. The insecticides were included in Phase I largely because of a lack of information on the toxicity of these materials to aquatic life forms.

Reagent grade or technical grade material was obtained for all herbicides and two insecticides (lindane and diazinon). The percentage of active ingredient in the reagent or technical grade formulations is given in Table 2. . Table 3a and 3b present selected physical and chemical properties of each pesticide. Reagent grade 2,4-D, MCPA, bromoxynil, picloram, and lindane were purchased from Sigma. BASF Corporation provided technical grade MCPP and dicamba. Cyanamid Crop Protection, Canada (now BASF Corporation) provided a 5 g sample of technical grade imazamethabenz for the study. A sample of diazinon (technical grade) was provided by Novartis Crop Protection Inc. (now Syngenta). Clopyralid could not be obtained from a manufacturer and was not included in any of the testing.

Commercial formulations of two herbicides (MCPA, MCPP) and the insecticides diazinon and chlorpyrifos were also included in the initial range finding tests. These formulations contain solubilizing agents and the concentrations of the active ingredient far exceed the water solubility. These products were included in Phase I of the study for:

- Comparative purposes (formulation to reagent or technical grade material),
- Lack of access to the active ingredient, and
- Cost of the active ingredient.

The tests were conducted on water-accommodated fractions of the pesticides. The chemicals or commercial formulation were made up in litre volume with deionized water to a final nominal concentration of 1,000 mg/L (wt/vol). The solutions were shaken once or twice daily (bottles inverted and shaken) for one week and then allowed to settle for two days before test initiation. Reagent grade MCPA and MCPP, and technical grade dicamba went into solution (clear). Commercial formulations of diazinon and chlorpyrifos formed milky white suspensions. The other reagent and technical grade chemicals did not fully dissolve. The solid remained on the bottom of the bottle. The concentration in solution was believed stabilized by the presence of excess material on the bottom of the bottle (water-accommodated).

One stock solution was prepared for all tests in Phase I, and another set was prepared for Phases II and III.

Additional tests were conducted to assess the effects of a co-solvent on product solubility and the stability of the water-accommodated test solutions over time. These results are discussed in a separate section (3.1.4).

The stock and test solutions were submitted to the Alberta Research Council for analysis of the active ingredients. The results for all three Phases of the study were derived based on actual or measured concentrations.

2.2 Biological Tests

A total of seven biological tests were conducted on the pesticides in Phase I. Brief method descriptions follow. There are two types of endpoints derived in each test. The first type is interpolated from the dose and response relationship directly or with regression analyses (point estimates). These endpoints are expressed as the test concentration giving a defined change in the response variable (typically 20%, 25% or 50%). Responses measured include lethality (LCx), effect (ECx), and inhibition (ICx) concentrations.

The second type of endpoint is either estimated from the data or derived from a multiple comparison statistical method (ANOVA). All comparisons are made against a control. The NOEC is the highest concentration tested that had no observed significant effect on the response variable. The LOEC is the lowest concentration tested that had an observed significant effect or response. In most biological tests, a 20% to 30% change in the response variable is considered significant.

The tests were conducted following Environment Canada methods where applicable with some modifications. The objectives of Phase I were to assess relative potencies of the compounds, differences in species sensitivities, and to establish upper treatment levels for the definitive tests. The number of replicates was reduced in some of the tests to accommodate the large number of pesticides included in the Phase I assessment.

The YES assay and SOS-Chromotest were not carried forward to Phase II and III. All other tests were conducted following Environment Canada methods with the requirement that they met all criteria for a valid test.

2.2.1 Microbial Tests

There were three microbial tests, YES assay, SOS-Chromotest, and bacterial luminescence. These tests were conducted as full tests with no modification to the method.

2.2.1.1 YES Assay

The YES assay is a screening method for the detection of substances that have estrogenic activity (Routledge and Sumpter, 1996). The test is conducted with a genetically modified yeast (*Saccharomyces cerevisiae*) containing the human receptor for estrogen. The DNA sequence of the human estrogen receptor (hER) was integrated into the yeast genome. This sequence is linked to the lac-Z gene for ß-galactosidase. The hER is expressed in a form that binds with the

estrogen response elements (ERE) within a hybrid promoter on the expression plasmid. Estrogen binds to the estrogen receptor (expressed by hER) and this ligand complex binds to the ERE elements on the hybrid promoter linked to the gene for ß-galactosidase. The enzyme is synthesized and secreted into the medium and metabolizes the substrate chlorophenol red-ß-D-galactopyranoside (CPRG) producing a red colour (normally yellow).

There are two endpoints in the YES assay, cell viability and estrogenic induction potential. Cell viability is assessed by an increase in the turbidity of the test solutions compared to controls (growth). Induction is measured by the increase in red colour in the test solution. A positive result is considered equal to a level twice background absorbance at 540 nm (measured as an EC200, effective concentration giving a response equal to 200% of controls). A positive control is run with each test for quality assurance purposes and to assess culture sensitivity (17-β-estradiol).

2.2.1.2 SOS-Chromotest

The SOS-Chromotest is a test for the detection of genotoxins in aqueous and solid samples (Fish et al., 1989). Genotoxins are substances that can induce mutations and cause cancerous transformations of normal cells. These materials interact directly or indirectly with the genetic material of cells (DNA).

All cells have the ability to repair damage to their DNA. The SOS repair system in *Escherichia coli* is activated (expressed) when the cell detects a DNA lesion. The cell may be able to repair the lesion and continue to live or the damage may be too extensive and result in death of the cell. Incomplete repairs may result in transmissible changes to the genetic structure (mutations).

The strain of bacterium in the SOS-Chromotest is genetically altered to allow detection of potential genotoxins. The repair genes are replaced with a gene for β-galactosidase; an enzyme not normally present in the bacterium. As a result, β -galactosidase is produced when the SOS system is activated. This turns the test solution a blue colour. The amount of colour is related to the genotoxic inducing potential of the sample.

Certain kinds of compounds become more genotoxic following slight structural modifications, such as a change in a functional group. The liver produces enzymes that react with foreign compounds to break them down and to make it easier for the body to excrete. In some cases, the enzymes structurally modify the compound in such a fashion that it becomes more genotoxic. A variation of the test involves pre-treatment of the sample with a mixture of liver enzymes (S-9 fraction). Additional information on the presence and nature of genotoxins present in a sample is obtained by testing the sample with and without S-9 activation.

Two sets of endpoints are derived for the SOS-Chromotest, cytotoxicity and genotoxicity. Cytotoxicity is based on the synthesis and release of the enzyme alkaline phosphatase compared to controls. The results are expressed as an LC25 and LC50. Genotoxicity is expressed as an increase in β –galactosidase activity relative to positive controls with and without S9 activation (direct and indirectly acting genotoxins respectively). The EC200 is the value set equal to the maximal induction obtained with the positive control. The EC150 is one half of this value. These values are

derived by graphical interpolation. A NOEC and LOEC are derived for both cytotoxicity and genotoxicity.

2.2.1.3 Bacterial Luminescence

Vibrio fischeri is a marine bacterium that emits light as a metabolic by-product. Less light is produced when the organisms are exposed to substances that are stressful or lethal. In the test, light output is related to sample strength. Bacterial luminescence is a rapid, reproducible, and economical test for toxicity screening.

The stock solution was diluted as required in order to obtain an endpoint. Then the sample was osmotically adjusted and serially diluted three or more times with a final test volume of 1 mL. The final treatment levels in the basic test include 11%, 22%, 45% and 91% of the original sample plus a control. The test was conducted at 15 ^{o}C under controlled temperature conditions. The solutions were first allowed to acclimate to temperature before spiking with bacteria. Readings were taken after spiking and at 5 and 15 minutes.

The results were expressed as the effective concentration required to reduce light output by 20% and 50% after a fixed exposure period (IC20 and IC50 at 5 and 15 min).

All tests in Phases I and II were done following the Environment Canada method (1992a).

2.2.2 Plant Tests

Tests were conducted with the green alga, *Raphidocelis subcapitata* and duckweed (*Lemna minor*).

R. subcapitata (formerly *Selenastrum capricornutum*) is a crescent-shaped microscopic, unicellular, green alga. It is roughly 5 to 6 μm wide by 10 to 12 μm long. *Raphidocelis* is widely distributed in freshwater systems (found in eutrophic and oligotrophic surface waters), is well defined taxonomically, easy to culture, and is sensitive to many different types of toxic substances.

The tests were conducted in 96 well microplates following the Environment Canada test method (1992b). A sample of the water-accommodated fraction was spiked with nutrients and the alga, and then serially diluted with control water (which is spiked with nutrients and the alga). The solutions were then dispensed to the microplate in a predefined fashion. The plates were covered with lids, sealed in plastic bags and incubated under constant light for three days. The light intensity at the water surface was 4,000 lux.

Growth inhibition was assessed by changes in cell densities relative to controls. Optical density measurements at 430 nm are converted to cell densities with a factor derived for each plate. This factor was based on a correlation between absorbance and particle counts for a low, medium and high treatment level.

The initial range finding tests were done with one replicate for each pesticide as opposed to three replicates in the full test. The sample concentrations inhibiting growth by 25% and 50% were

derived from cell counts correlated to optical density measurements for each plate. The NOEC and LOEC were also estimated from the growth data.

Duckweed (*L. minor*) is a small vascular, aquatic macrophyte widely distributed in ponds, lakes and quiet streams. The plants grow by lateral branching, occurring singly or in small clusters (3 to 5 fronds). They are 2 to 4 mm in length, green to lime green in colour, and each frond has a single root emanating from the centre of the lower surface. Duckweed is small, has a simple structure, easy to culture and grows rapidly.

The tests were conducted in 200-mL clear plastic containers with clear lids (Environment Canada, 1998). Two three-frond, acclimated plants were placed into the vessel containing 150 mL of test solution. The vessels were capped with the lids and incubated at 25 $\pm$ 2 ^{o}C under continuous light for seven days. Growth effects were assessed based on the number of fronds and dry weight (biomass) compared to controls. The range finding tests were done with one replicate and the full test with three replicates. Endpoints are derived for both frond numbers and biomass (EC25 and EC50, NOEC and LOEC).

2.2.3 Invertebrate Test – Survival and Reproduction in Ceriodaphnia

The Cladoceran *Ceriodaphnia dubia* is a freshwater microcrustacean related to the waterflea *Daphnia magna*. This species is common to lakes, ponds, and slow moving regions of rivers throughout North America. *Ceriodaphnia* reproduce asexually under the right culture conditions, releasing their first brood within 3 to 4 days. They are sensitive to a broad range of aquatic contaminants and because of their small size they require only small sample volumes for testing.

The test is designed to measure effects on survival and reproduction over a seven-day exposure period (Environment Canada, 1992c). The tests were conducted in 30-mL plastic cups containing 15 mL of test solution. The test organisms were less than 24 h old and were released within an eight-hour period. One animal was placed into each of ten test cups per treatment level (range finding tests were done with three or six replicates). The solutions were replenished daily and the animals were fed a defined amount of algae and a fermented mixture of yeast, alfalfa powder, and trout chow.

Survival was scored daily along with the number of live young released. *Ceriodaphnia* will produce 3 broods (20 to 40 neonates in total) over a 7-day period. Sublethal effects were detected by a reduction in the total number of young produced over the 7-day test period. Other sublethal effects that were often observed include delays in brood development and release and maturation.

Endpoints were derived for effects on survival (LC25 and LC50) and reproduction (IC25 and IC50). The NOEC and LOEC are also determined for each endpoint.

2.2.4 Vertebrate Test – Survival and Growth of Fathead Minnow

Fathead minnows (*Pimephales promelas*) belong to the carp family and are native to most of North America. Male fathead minnows reach lengths of up to 10 cm. The females are smaller with lengths from 4 to 7 cm. They thrive in ponds, lakes, ditches, and slow moving streams feeding on small invertebrates and detritus.

This test is designed to measure sublethal effects on growth during one of the most sensitive stages of larval development (Environment Canada, 1992d). The tests were conducted in 0.5-L containers containing 250 mL of solution and 10 newly hatched larvae (<24 h old). There were 4 replicates per treatment level in full tests. For the range finding tests 2 replicates were used except for dicamba and imazamethabenz where only one replicate was used. Survival and signs of stress were recorded daily. The solutions were also replenished and the larvae fed a standard diet of brine shrimp twice daily (before and after replenishing). At the end of the test the larvae were dried and weighed to obtain a measure of growth (increase in dry weight).

Endpoints were derived for effects on survival (LC25 and LC50) and growth (IC25 and IC50). The NOEC and LOEC were also determined for each endpoint.

2.3 Full Tests and Tests on Mixtures

Full tests were conducted on seven herbicides (2,4 D, MCPA, MCPP, dicamba, bromoxynil, picloram, and imazamethabenz) and two insecticides (lindane and diazinon). The tests were done on reagent or technical grade chemical. No commercial formulations were carried forward to Phases II and III. The full tests were done with five or six treatment levels and a control following the Environment Canada methods. Samples of each test solution were archived for later chemical analyses.

The tests done on mixtures involved adding the compounds sequentially based on the frequency with which they were detected in surface waters. The seven herbicides were tested separately from the two insecticides. The concentration of each herbicide was initially set at 0.01 mg/L. If the mixtures have no effect on survival and reproduction, then the tests were repeated at 0.1 mg/L. Testing at higher concentrations was not done because levels in excess of 0.1 mg/L were not considered environmentally relevant.

The order of addition was:

1. 2,4 D / MCPA
2. 2,4 D / MCPA / MCPP
3. 2,4 D / MCPA / MCPP / dicamba
4. 2,4 D / MCPA / MCPP / dicamba / bromoxynil
5. 2,4 D / MCPA / MCPP / dicamba / bromoxynil / picloram
6. 2,4 D / MCPA / MCPP / dicamba / bromoxynil / picloram / imazamethabenz

The two insecticides (lindane and diazinon) were tested at 0.001 mg/L serially diluted to obtain five treatment levels and a control.

3.0 RESULTS AND DISCUSSION

3.1 Phase I: Range Finding Tests

The objective of Phase I was to assess the relative sensitivities of the test organisms to the selected pesticides. Only those substances that exerted an effect at concentrations within the range observed in surface waters were included in the Phase II assessment.

The Phase I assessment was based on results from range finding tests with treatment levels separated by an order of magnitude (factor of ten). The intent was to establish an upper treatment level for full or definitive tests (Phase II) and to provide enough information to assess relative sensitivities of the test species to the pesticides. However, full tests were done in Phase I with the microbial species. Microbial tests were relatively rapid and required little volume. Hence, both the range finding and definitive tests could be completed in a relatively short time period.

The test data are summarized in Tables 4 and 5. All endpoints are expressed in terms of weight of pesticide per volume of test solution based on measured levels (analyses of the water-accommodated stock solutions, Table 13a).

Results of tests on co-solvents and other observations of the pesticide stock solutions are presented in section 3.1.2.

3.1.1 Test Results

The test results are presented by trophic level.

3.1.1.1 Microbes

Reagent grade pesticides and commercial formulations did not exhibit any genotoxicity or estrogenicity at the levels tested (Tables 4, 5). The effects of the test substance on luminescent bacteria ranged over six orders of magnitude. The herbicides were less toxic or not toxic compared to the insecticides. Picloram and dicamba were not toxic as tested. The other herbicides had IC50 values ranging from 13 to 184 mg/L.

Commercial formulations of chlorpyrifos and diazinon were the most toxic to luminescent bacteria. The IC50 values were 0.02 and 1.5 mg/L and 0.005 and 0.15 mg/L respectively (two test solutions were prepared for each compound; Table 4). The reason for the large differences between the measured levels of active ingredient in each solution is unknown (Table 13a). However, it is consistent and could be due to solubility properties and the emulsion formed upon mixing with water.

3.1.1.2 Plants

Lemna and *Raphidocelis* were relatively insensitive to all of the test substances at concentrations less than 10 mg/L (Table 4). The effective dosages were two to five orders of magnitude above the levels detected in surface waters. The greater sensitivity to the commercial formulations was

likely a response to the carrier or to the solvents, alone or in combination with the active ingredients, rather than to the active ingredient alone.

3.1.1.3 Invertebrates

Two range finding tests were conducted with *Ceriodaphnia dubia*. The first test was terminated early because the selected concentrations of the test solutions were highly toxic (lethal) to *Ceriodaphnia*. This also caused some concern over the potential for cross contamination. The second test was done in isolation and special attention was paid to sample handling and preparation of the test solutions. The results from the second test were considered more reflective of the actual potencies of the test compounds.

Reagent grade 2,4-D and MCPP were not toxic at treatment levels greater than 5 mg/L (Table 4). The commercial formulation of MCPP was toxic at levels from 3 to 7 mg/L. MCPA was lethal at 1 mg/L and affected reproduction at 0.3 mg/L. The commercial formulation of MCPA was 3 to 4 orders of magnitude more toxic than the reagent grade product. This was likely due to the solubilizing agents and not the active ingredient.

Reagent grade dicamba and imazamethabenz and technical grade picloram were not toxic to *Ceriodaphnia* at the concentrations tested (Table 4). Bromoxynil (reagent grade) had a significant effect on survival and reproduction at 0.02 mg/L. Lindane (reagent grade), diazinon (technical grade and commercial formulations), and chlorpyrifos (commercial formulations) were lethal and affected reproduction at levels ranging from 0.003 to 0.00003 mg/L. These concentrations are within the range recorded in Alberta surface waters.

3.1.1.4 Vertebrates

The herbicides, in general, had no effect on survival and growth of fathead minnows at the concentrations tested. However, some effects were noted with the commercial formulation of MCPP and reagent grade bromoxynil. The insecticides were significantly more toxic than the herbicides. Lindane (reagent grade) was lethal at 0.004 mg/L. Chlorpyrifos (commercial grade) was lethal at 0.002 mg/L and reduced growth at 0.007 mg/L. The reagent grade of diazinon was lethal and reduced growth at 3 and 2 mg/L, respectively. The commercial formulation of diazinon was lethal and reduced growth at 0.1 mg/L. This was likely due to the toxicity of the solubilizing agents alone or in combination with the active ingredient.

3.1.2 Other Test Data

Observations on the water-accommodated stock solutions are summarized in Table 6. Compounds such as 2,4-D, bromoxynil, picloram, imazamethabenz, lindane, and diazinon did not dissolve fully. Hence, the amount added to one litre of water exceeded the solubility of the product. The commercial formulations of diazinon and chlorpyrifos formed emulsions after mixing with water (cloudy solutions). The stock solutions prepared with the commercial formulations of MCPA and MCPP were clear.

A number of test compounds had low water solubility (Table 3b). Attempts were made to first dissolve the substance in a co-solvent (methanol) and then into water. However, the solutions turned opaque (emulsion or precipitate) and there was some uncertainty about the concentration of material in solution and availability of the active ingredient.

The solubility of 2,4-D was not enhanced with a methanol co-solvent. A water-accommodated stock solution was more toxic than a similar solution prepared by first dissolving the compound in methanol (Table 7).

All tests were conducted with water-accommodated stock solutions of each compound. The intent was to exceed the water solubility of the material to insure a constant concentration in solution. The solutions were prepared by adding one gram of the active ingredient to one litre of deionized water. The solutions were mixed and allowed to settle for 48 hours. A volume of the stock solution was decanted for testing when required.

The solutions were stored at room temperature in darkness. Changes in potency over time of storage were evaluated with the bacterial luminescence test. The effective concentration of all pesticides except MCPA did not change over time (four months). The solution of MCPA has become progressively more toxic with storage (Table 8).

3.2 Phase II: Derivation of Endpoints

The objective of Phase II was to derive endpoints based on measured concentrations for pesticides carried over from Phase I. Nine pesticides were tested and included seven herbicides (2,4 D, MCPA, MCPP, bromoxynil, picloram, dicamba, and imazamethabenz) and two insecticides (lindane and diazinon). Water-accommodated solutions of the reagent grade or technical formulations were tested. The commercial formulations were not included in Phase II testing.

The tests conducted in Phase II included bacterial luminescence, inhibition of duckweed growth, algal growth inhibition, survival and reproduction of *Ceriodaphnia dubia*, and survival and growth of the fathead minnow. An upper limit was established for the each test species based on available water quality monitoring data (levels measured in the environment), test volume requirements, amount of chemical available, and Phase I results (10, 20 or 100 mg/L).

Samples of each water-accommodated stock solution were submitted to the Alberta Research Council for analysis of the active ingredient (Table 13b). The endpoints were derived based on measured levels of each pesticide present in the test solutions.

The concentrations giving a 50% change in the response variable are summarized in Table 9 and the NOEC in Table 10. The concentrations of the herbicides required to elicit a response were generally greater than levels measured in surface waters (mg/L compared to µg/L).

Bromoxynil was the most toxic herbicide tested. Effects ranged from 0.2 mg/L (EC50 for reproduction in *Ceriodaphnia*) to 16 mg/L (IC50 @ 15 min for bacterial luminescence; Table 9).

The test species were relatively insensitive to dicamba. Only one endpoint was obtained for the range of concentrations tested (LC50 of 44 mg/L for duckweed growth).

The luminescent bacterium was relatively insensitive to the pesticides. A response was only obtained with concentrations well in excess of those measured in the field (mg/L range as opposed to μg/L). The highest concentrations of dicamba and diazinon tested had no effect on light production (466 mg/L and 97 mg/L, respectively).

Duckweed growth was a more sensitive response variable than an increase in biomass. Endpoints for growth inhibition were obtained for 7 of the 9 pesticides. Only one endpoint was obtained for effects on biomass. It was interesting to note that the duckweed fronds enlarged but did not separate into daughter fronds (bud formation). In other words, the effect was on division (growth measured as the number of fronds) as opposed to growth through an increase in biomass. Longer exposure periods may provide greater resolution of effects on both growth and biomass due to the time required for the chemical to be taken up and exert an effect.

The alga, *Raphidocelis* was relatively insensitive to the nine pesticides compared to effects on duckweed growth. However, the effects on both species were consistent. 2,4-D, MCPA, MCPP, dicamba, picloram, and diazinon were the least toxic and bromoxynil, lindane, and imazamethabenz had the greatest effect on growth of both species. This suggests similar modes of action although duckweed was more sensitive.

Ceriodaphnia and fathead minnows were relatively insensitive to six of the seven herbicides at the concentrations tested. Bromoxynil affected reproduction in Ceriodaphnia (EC50 of 0.15 mg/L) and survival of fathead minnows (LC50 3.9 mg/L). No endpoint was obtained for an effect on growth of fathead minnows. The concentration of bromoxynil lethal to *Ceriodaphnia* was greater than the highest test concentration (0.8 mg/L).

The effects exerted by diazinon, bromoxynil, and in particular lindane were at levels near the concentrations measured in surface waters of Alberta. The concentrations of the other pesticides required to elicit responses were in excess of field values (low to high mg/L range).

3.3 Phase III: Tests on Mixtures

The objective of Phase III was to assess the effects of mixtures at concentrations near those measured in surface waters. Mixtures can be more potent than effects exerted by individual chemicals depending on the mode of action and interactions amongst the constituents. *Ceriodaphnia* was selected for Phase III because this organism was more sensitive to the individual pesticides than the other four test species.

The mixtures were prepared from the stock solutions from Phase II. The herbicides were tested in mixtures of equal concentrations (0.01 and 0.1 mg/L). In other words, the herbicides were added together to obtain a final individual concentration of 0.01 or 0.1 mg/L.

The test on the two insecticides was done by serially diluting a μg/L solution to obtain five treatment levels (0.0000625 to 0.001 μg/L).

The mixture of herbicides was not toxic to *Ceriodaphnia*. No effects were observed on survival and reproduction in any of the mixtures at both 0.01 and 0.1 mg/L (Table 11). The results were consistent with the findings from Phase II.

The mixture of lindane and diazinon had no effect on survival and reproduction of *Ceriodaphnia*. This was consistent with the Phase II results. Both the LC50 for survival and EC50 for reproduction for each insecticide were greater than the highest concentration tested in the mixture (0.001 mg/L; Table 12).

4.0 CONCLUSIONS AND RECOMMENDATIONS

This study was conducted to address the environmental relevance of pesticide residues present in surface waters of Alberta. The pesticides were selected based on the frequency of detection and relative concentrations in surface waters. The study was conducted in three phases. The first phase involved range finding tests to determine relative potencies of individual pesticides (reagent and technical grade chemicals and commercial formulations). The objective of Phase II was to obtain endpoints for the pesticide. The most sensitive species was carried forward to Phase III testing of mixtures. The results for all phases were expressed as measured values.

4.1 Phase I: Range Finding Tests

Range finding tests were conducted on nine pesticides (reagent or technical grade material) and four commercial formulations (for each commercial formulation two stock solutions were made). The commercial formulations were generally more toxic than the reagent grade materials. However, the concentrations required to elicit effects were three or more orders of magnitude above those measured in surface waters (Table 3b). The effect levels of the insecticides were lower than the herbicides but higher than levels detected in surface waters.

The order of increasing species sensitivities to the pesticides was:

green alga < duckweed < luminescent bacteria < fathead minnow < *Ceriodaphnia*

Plants were the least sensitive and some of the effects detected were believed a result of the solubilizing agents in the commercial formulations. The lack of a response from the duckweed and algae could be a direct result of the exposure/pathway for uptake and mode of action. The invertebrate, *Ceriodaphnia dubia*, was the most sensitive test species.

No genotoxic or estrogenic effects were detected. These tests were not carried forward into Phases II and III.

The water-accommodated stock solutions were a reliable method for preparing test solutions. These solutions were relatively stable over a four-month storage period in darkness at room temperature (Table 6).

4.2 Phase II: Derivation of Endpoints

Seven herbicides and two insecticides were carried forward into Phase II and III. The tests included bacterial luminescence, inhibition of algal growth and growth of duckweed, survival and reproduction of *Ceriodaphnia*, and survival and growth of fathead minnows.

One of the seven herbicides (bromoxynil) and both insecticides (lindane and diazinon) were the most toxic. The other herbicides were generally not toxic at the highest concentrations tested. The plants were more sensitive to the herbicides than the other species. *Ceriodaphnia* and

fatheads were more sensitive to the insecticides compared to the microbe and plants. These results were consistent with the Phase I findings.

4.3 Phase III: Tests on Mixtures

The objective of Phase III was to evaluate the effects of mixtures. The herbicides and insecticides were tested separately at 0.01 and 0.1 mg/L and 0.001 mg/L respectively. The tests were conducted with the most sensitive species, *Ceriodaphnia dubia*. No effects were detected on survival and reproduction for either the herbicide or the insecticide mixtures.

4.4 General Conclusions and Study Limitations

The effects of the herbicides were at concentrations well above the median and mean levels measured in surface waters of Alberta. These results suggest that pesticide residues measured in surface waters of Alberta are at levels that have no adverse effects on species representative of major trophic levels in the aquatic systems. However, it is important to note that the tests were conducted on the reagent or technical grade chemical in laboratory control water. Differences in water quality conditions could have some influence on the availability and action of pesticides in situ.

The test methods were designed by Environment Canada to provide a means to assess potential impacts on aquatic systems. The responses of the organisms were consistent. In other words, the most toxic pesticide was the one that had the greatest relative effect on each organism. The endpoints included both acute and chronic effects (growth, reproduction) across four trophic levels. This further supports the conclusion that low levels likely have no adverse impact on microbes, plants, invertebrates, and fish in aquatic systems.

This study did not address the issue of runoff events and peak levels. The test concentrations and interpretation were based on mean and median levels. Short term exposures to high concentrations can have equal or greater impacts than long term exposures to low concentrations. This is one area that requires additional study.

This study did not address bioaccumulation and biomagnification. It is possible that the accumulation of pesticides by organisms and biomagnification up through the food chain could adversely impact one or more trophic levels. However, the results suggest that a significant amount of chemical would need to be taken up in order to elicit a response. One way to assess this would be to analyze test organisms after exposure to low levels of the pesticides or tissue residue analyses on field collected material.

4.5 Recommendations

The following is recommended based on the findings from this study.

- Compare peak concentrations during runoff events to the results obtained for individual compounds to assess the potential for adverse effects. Additional work

could involve collecting samples during this period for biological testing and simulating the event under controlled conditions in the laboratory. Flow through tests can be done by shocking the test organism and then diluting out the chemical with clean water.

- Additional work is required on bioaccumulation and biomagnification through the food chain. The importance of this pathway can be evaluated by exposing organisms in the laboratory to low concentrations of pesticides and then analyzing tissue residues or collecting material from the field for analyses.

- Pesticide residues in surface waters of Alberta are at levels well below those that have an adverse effect on the test organisms employed in this study. Additional testing of mixtures at higher concentrations is not recommended.

- The water-accommodated stock solution prepared for testing was an effective and reliable method. Further work on pesticides should employ this method for generating test solutions.

5.0 REFERENCES

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Environment Canada, 1992b. Biological test method: Microplate growth inhibition test using *Selenastrum capricornutum*. 1992, EPS 1/RM/25. (amended 1997)

Environment Canada, 1992c. Biological test method: Test of reproduction and survival using the Cladoceran *Ceriodaphnia dubia*, EPS 1/RM/21. (amended 1997)

Environment Canada, 1992d. Biological test method: Test of larval growth and survival using fathead minnows. 1992, EPS 1/RM/22. (amended 1997)

Environment Canada 1998. Biological test method: Test for measuring the inhibition of growth using the freshwater macrophyte, *Lemna minor*. EPS 1/RM/37.

Fish, F., I. Lampert, A. Halachmi, G. Riesenfeld, and M. Herzberg, 1989. The SOS-Chromotest kit: A rapid method for the detection of genotoxicity. Tox. Assess. 2:135-147.

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6.0 TABLES

Table 1 Test Organisms and Endpoints

Trophic Level	Organism	Effect	Endpoints
microbe	*Escherichia coli*	cytotoxicity	LC50, LC25, NOEC, LOEC
		genotoxicity	EC200, EC150, NOEC, LOEC
	Saccharomyces cerevisiae	cytotoxicity	LC50, LC25, NOEC, LOEC
		estrogenicity	EC200, EC150, NOEC, LOEC
	Vibrio fischeri	light inhibition	IC50, IC20, NOEC, LOEC
plant	*Lemna minor*	growth	EC50, EC25, LOEC, NOEC
		biomass	EC50, EC25, LOEC, NOEC
	Raphidocelis subcapitata	growth	IC50, IC25, LOEC, NOEC
invertebrate	*Ceriodaphnia dubia*	survival	LC50, LC25, NOEC, LOEC
		reproduction	EC50, EC25, LOEC, NOEC
vertebrate	*Pimephales promelas*	survival	LC50, LC25, NOEC, LOEC
		growth	EC50, EC25, LOEC, NOEC

Note: LCx, ECx, and ICx, test concentrations giving an 'x' percent response in the test variable (L, lethal; E, effective; I, inhibitory); LOEC, lowest concentration tested that had an observed significant effect; NOEC, highest concentration tested that had no observed significant effect

Table 2 Test Substances

Common Name	Source	Product	Purity
2,4 D (acid)	Sigma	reagent grade (lot 155504PQ)	98%
MCPA (acid)	Sigma	reagent grade (lot 09309LU)	95%
	Home Depot	Later's Creeping Buttercup Weed Killer	40% w/v
MCPP (potassium salt)	BASF Coporation	technical grade (RS-MCPP-032596)	99.5%
	Home Depot	Later's Chickweed, Clover & Thistle Killer	15% w/v
dicamba	BASF Coporation	technical grade (RS-M36-100496)	99.16%
bromoxynil (octanoate ester)	Sigma	reagent grade (lot EU 50412TM)	*
picloram (amine salt)	Sigma	reagent grade (lot 02919PU)	*
imazamethabenz	Cyanamid Crop Protection Canada (BASF)	technical grade (AC12140-10)	95%
clopyralid		could not obtain material from manufacturer	
lindane	Sigma	reagent grade (lot 02517BQ)	97%
diazinon	Novartis Crop Protection, Inc.	technical grade (S96-1965)	87%
	Home Depot	Wilson - Diazinon (Batch A057B)	12.5% w/v
chlorpyrifos	Home Depot	Wilson - Ant and Grub Killer (Batch 80038)	5% w/v

Note: Sigma chemicals obtained from Aldrich Chemical Company, Inc.; *, no purity given - 100% assumed

Table 3a Selected Physical and Chemical Properties of Pesticides Included in the Assessment

Common Name	IUPAC Name	CAS	Mwt (g/mole)	Structure
2,4 D (acid)	(2,4-dichlorophenoxy)acetic acid	94-75-7	221	C8H6Cl2O3
MCPA (acid)	(4,chloro-2-methylphenoxy)acetic acid	94-74-6	200.6	C9H9ClO3
MCPP (potassium salt)	(2-(4-chloro-2-methylphenoxy)proprionic acid)	1929-86-8	214.6	C10H11ClO3
dicamba	3,6-dichloro-2-methoxybenzoic acid	1918-00-9	221.04	C8H6Cl2O3
bromoxynil (octanoate ester)	3,5-dibromo-4-hydroxybenzonitrile	1689-99-1	276.9	C7H3Br2NO
picloram (amine salt)	4-amino-3,4-trichloropicolinic acid	1918-02-1	241.5	C6H3Cl3N2O2
imazamethabenz	m-toluic acid,6-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)methyl ester & p-toluic acid,2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)methyl ester	81405-85-8	288.38	C16H2ON2O3
lindane	gamma-1,2,3,4,5,6-hexachlorocyclohexane	58-89-9	290.85	C6H6Cl6
diazinon	O,O-diethyl-O-(2-isopropyl-6-methyl-4-pyrimidinyl)-phosphorothioate	333-41-5	304.3	C12H21N2O3PS
chlorpyrifos	O,O-diethyl-O-3,5,6-trichloro-2-pyridyl-phosphorothioate	2921-88-2	350.6	C9H11C13NO3PS

Note: CAS, Chemical Abstracts Service registry number

Table 3b Selected Physical and Chemical Properties of Pesticides Included in the Assessment (cont'd)

Common Name	Water Solubility (mg/L)	Vapour Pressure (Pa)	Half Life (d)	Surface Water Concentrations[1] (ug/L)		Guideline[2] (ug/L)
				max	median	
2,4 D (acid)	682 to 900	1.10E-02	<30	4.0	0.027	4.0
MCPA (acid)	825	2.30E-05	na	2.5	0.015	2.6
MCPP (potassium salt)	620 to 920,000	3.10E-04	na	1.5	0.011	ng
dicamba	4,500 to 400,000	4.50E-03	<7	1.3	0.015	10
bromoxynil (octanoate ester)	0.08 to 130	<1.00E-03	4.6 to 34	0.2	0.009	5.0
picloram (amine salt)	430 to 200,000	8.20E-05	2.3 to 413	1.0	0.024	29
imazamethabenz	857 to 1,370	1.50E-06	na	3.5	0.098	ng
lindane	0.15 to 7	5.60E-03	4 to 113	0.1	0.010	0.01
diazinon	60	9.70E-05	180	0.1	0.007	ng
chlorpyrifos	1.4	2.70E-03	35 to 78	0.1	0.011	0.0035

Notes: 1, Data from Alberta Environment's surface water quality database for the period 1995-98; max, maximum recorded concentration in surface water; 2, CCME (2000) guidelines for the protection of aquatic life; ng, no guidelines.

Table 4 Range Finding Test Results Summary (Phase I): Test Concentrations (mg/L) Giving a 50% Change in the Response Variable (LC50, EC50, and IC50).

Common Name	Source	Microbe					Plant	
		bacterial luminescence ($IC50_{15\ min}$)	SOS-Chromotest viability	SOS-Chromotest genotoxicity	YES Assay viability	YES Assay estrogenic potential	duckweed growth (LC50)	duckweed biomass (EC50)
Reagents								
2,4 D (acid)	Sigma	84	> 1.8	> 1.8	> 0.31	> 0.31	> 93	> 93
MCPA (acid)	Sigma	89	> 1.6	> 1.6	> 0.28	> 0.28	75	41
MCPP (potassium salt)	BASF	66	> 2.0	> 2.0	> 0.35	> 0.35	24	> 106
dicamba	BASF	> 499	> 2.1	> 2.1	> 0.37	> 0.37	23	> 110
bromoxynil (octanoate ester)	Sigma	13	> 0.27	> 0.27	> 0.05	> 0.05	7.7	3.4
picloram (amine salt)	Sigma	> 234	> 1.0	> 1.0	> 0.17	> 0.17	33	> 51
imazamethabenz	BASF	184	> 1.0	> 1.0	> 0.17	> 0.17	> 52	> 52
lindane	Sigma	6	> 0.05	> 0.05	> 0.01	> 0.01	> 2.8	> 2.8
diazinon	Novartis	>107	not available				21	21
Commercial products								
MCPA (acid)	Home Depot	57 / 29	>3.3 / >0.87	>3.3 / >0.87	>0.58 / >0.15	>0.58 / >0.15	>174 / >46	> 174 / 21
MCPP (potassium salt)	Home Depot	57	>8.3 / >0.93	>8.3 / >0.93	>1.5 / >0.17	>1.5 / >0.17	>439 / >49	>439 / >49
diazinon	Home Depot	0.024 / 1.5	>1.4 / >0.11	>1.4 / >0.11	>0.25 / >0.02	>0.25 / >0.02	4.8 / 2.2	4.8 / 2.7
chlorpyrifos	Home Depot	0.005 / 0.15	>2.8 / >0.28	>2.8 / >0.28	>0.49 / >0.05	>0.49 / >0.05	1.2 / 1.1	1.2 / 1.1

Common Name	Plant	Invertebrate (*Ceriodaphnia*)		Vertebrate (fathead minnow)	
	algae growth (IC50)	survival (LC50)	reproduction (EC50)	survival (LC50)	growth (EC50)
Reagents					
2,4 D (acid)	> 46	> 4.6	> 4.6	> 46	> 46
MCPA (acid)	> 43	1.35	0.29	> 43	> 43
MCPP (potassium salt)	> 53	> 5.3	> 5.3	> 53	> 53
dicamba	40	> 5.5	> 5.5	> 55	> 55
bromoxynil (octanoate ester)	2.9	0.02	0.017	3.0	> 7
picloram (amine salt)	> 26	> 2.6	> 2.6	> 26	> 26
imazamethabenz	> 26	> 2.6	> 2.6	> 26	> 26
lindane	> 1.4	0.0024	0.0026	0.004	0.007
diazinon	>11	0.0019	0.0024	3	1.8
Commercial products					
MCPA (acid)	4 / 2	0.0003	0.0005	>87 / >23	>87 / >23
MCPP (potassium salt)	>219 / >25	6.9	2.8	41 / >25	68 / >25
diazinon	6.9 / > 7.3	0.00003	0.00003	0.2 / 0.23	0.1 / 0.16
chlorpyrifos	1.1 / 2	0.0002	0.0002	0.002 / 0.02	0.0073 / 0.074

Note: YES, yeast endocrine assay; LCx, lethal concentration; ECx, effective concentration; ICx inhibitory concentration

Note: two different stock solutions were made of each Home Depot product. One was made to have roughly 1000 mg/L of active ingredient and the other 1000 mg/L of the commercial formulations (results report, respectively).

Table 5 Range Finding Test Results Summary (Phase I): Highest Concentration (mg/L) Tested that had No Observed Effect (NOEC) on the Measured Response

Pesticide/Formulation	Source	Microbe					Plant	
		bacterial luminescence ($IC50_{15\ min}$)	SOS-Chromotest viability	SOS-Chromotest genotoxicity	YES Assay viability	YES Assay estrogenic potential	duckweed growth (NOEC)	duckweed biomass (NOEC)
Reagents								
2,4 D (acid)	Sigma	26	> 1.8	> 1.8	> 0.31	> 0.31	> 93	> 93
MCPA (acid)	Sigma	24	> 1.6	> 1.6	> 0.28	> 0.28	5.3	5.3
MCPP (potassium salt)	BASF	30	> 2.0	> 2.0	> 0.35	> 0.35	13	106
dicamba	BASF	125	> 2.1	> 2.1	> 0.37	> 0.37	3.4	110
bromoxynil (octanoate ester)	Sigma	4	> 0.27	> 0.27	> 0.05	> 0.05	4	2
picloram (amine salt)	Sigma	117	> 1.0	> 1.0	> 0.17	> 0.17	6	51
imazamethabenz	BASF	30	> 1.0	> 1.0	> 0.17	> 0.17	52	52
lindane	Sigma	0.8	> 0.05	> 0.05	> 0.01	> 0.01	2.8	2.8
diazinon	Novartis	24	not available				11	11
Commercial product								
MCPA (acid)	Home Depot	12 / 7	>3.3 / >0.87	>3.3 / >0.87	>0.58 / >0.15	>0.58 / >0.15	174 / 2.1	174 / 6
MCPP (potassium salt)	Home Depot	8 / 7	>8.3 / >0.93	>8.3 / >0.93	>1.5 / >0.17	>1.5 / >0.17	439 / 49	439 49
diazinon	Home Depot	0.008 / 0.3	>1.4 / >0.11	>1.4 / >0.11	>0.25 / >0.02	>0.25 / >0.02	< 4.5 / 2	< 4.5 / 2
chlorpyrifos	Home Depot	0.001 / 0.001	>2.8 / >0.28	>2.8 / >0.28	>0.49 / >0.05	>0.49 / >0.05	< 2.4 / 1	< 2.4 / 1

Pesticide/Formulation	Plant algae growth (NOEC)	Invertebrate (*Ceriodaphnia*) survival (NOEC)	Invertebrate (*Ceriodaphnia*) reproduction (NOEC)	Vertebrate (fathead minnow) survival (NOEC)	Vertebrate (fathead minnow) growth (NOEC)
Reagents					
2,4 D (acid)	46	4.6	4.6	46	46
MCPA (acid)	43	0.43	0.43	43	43
MCPP (potassium salt)	53	5.3	5.3	53	53
dicamba	28	5.5	5.5	55	55
bromoxynil (octanoate ester)	2	0.007	0.007	0.7	7
picloram (amine salt)	26	2.6	2.6	26	26
imazamethabenz	13	2.6	2.6	26	26
lindane	1.4	0.0014	0.0014	0.0014	0.0014
diazinon	11	< 0.0011	0.0011	1	0.1
Commercial product					
MCPA (acid)	1.4 / 0.7	< 0.00087	< 0.00087	87 / 23	87 / 23
MCPP (potassium salt)	55 / 25	2	2	22 / 25	22 / 25
diazinon	2.3 / 2	< 0.00073	< 0.00073	< 0.073 / 0.073	< 0.073 / 0.073
chlorpyrifos	< 0.6 / 0.8	< 0.00038	< 0.00038	<0.00038/0.003	<0.00038/0.003

Table 6 Observations on Water-accommodated Stock Solutions

Pesticide/Formulation	Source	Observation
Reagents		
2,4 D (acid)	Sigma	solids on bottom
MCPA (acid)	Sigma	solids on bottom
MCPP (potassium salt)	BASF	solids on bottom
dicamba	BASF	clear
bromoxynil (octanoate ester)	Sigma	solids floating and on bottom
picloram (amine salt)	Sigma	solids on bottom
imazamethabenz	BASF	solids on bottom
lindane	Sigma	solids on bottom
diazinon	Novartis	solids on bottom
Commercial products		
MCPA (acid)	Home Depot	clear
MCPP (potassium salt)	Home Depot	clear
diazinon	Home Depot	emulsion
chlorpyrifos	Home Depot	emulsion

Table 7 Effect of a Co-solvent on Toxicity of 2,4-D to Luminescent Bacteria

Method	IC50 @ 15 min (mg/L)
water accomodated	96
methanol co-solvent	169

Table 8 Changes in Potency of Stock Solutions Over Time (Bacterial Luminescence)

Pesticide/Formulation	Source	Result (IC50 @ 15 min in mg/L) 2000/08/30	2000/10/26	2001/01/05
Reagents				
2,4 D (acid)	Sigma	84	86	99
MCPA (acid)	Sigma	89	49	24
MCPP (potassium salt)	BASF	66	78	80
dicamba	BASF	>499 (127)	403 (46)	>549 (149)
bromoxynil (octanoate ester)	Sigma	13	10	13
picloram (amine salt)	Sigma	>254 (90)	>257	>257
imazamethabenz	BASF	184	92	99
lindane	Sigma	6.0	7.3	6.9
diazinon	Novartis	not available	not available	>107 (18)
Commercial products				
MCPA (acid)	Home Depot	57	22	nd
		29	40	nd
MCPP (potassium salt)	Home Depot	57	8.6	nd
		24	30	nd
diazinon	Home Depot	0.02	0.0087	nd
		1.50	0.26	nd
chlorpyrifos	Home Depot	0.005	0.00071	nd
		0.15	0.036	nd

Note: values in brackets are IC20 at 15 min in mg/L

Table 9 Full Test Results Summary (Phase II): Test Concentrations (mg/L) Giving a 50% Change in the Response Variable (LC50, EC50, and IC50)

Common Name	Source	Trophic Level								Guideline[1]
		Microbe	Plant			Invertebrate (*Ceriodaphnia*)		Vertebrate (fathead minnow)		
		bacterial luminescence ($IC50_{15\ min}$)	duckweed growth (LC50)	duckweed biomass (EC50)	algae growth (IC50)	survival (LC50)	reproduction (EC50)	survival (LC50)	growth (EC50)	
Reagents										
2,4 D (acid)	Sigma	87	12.4	> 92	73	> 18.9	> 18.9	> 9.4	> 9.4	0.004
MCPA (acid)	Sigma	72	17	> 82	> 85	> 17	> 17	> 8.5	> 8.5	0.0026
MCPP (potassium salt)	BASF	100	33	> 106	> 109	> 21.8	> 21.8	> 10.9	> 10.9	
dicamba	BASF	> 466	43.7	> 99	> 102	> 20.5	> 20.5	> 10.2	> 10.2	0.01
bromoxynil (octanoate ester)	Sigma	16	0.61	0.46	4	> 0.8	0.15	3.9	> 8	0.005
picloram (amine salt)	Sigma	> 196	> 84	> 84	62	> 20.9	> 20.9	> 8.6	> 8.6	
imazamethabenz	BASF	84	0.49	> 6.2	32	> 12.8	> 12.8	> 6.4	> 6.4	
lindane	Sigma	4.3	0.29	> 0.48	2.2	0.019	0.018	0.016	0.038	0.00001
diazinon	Novartis	> 97	> 52	> 52	> 107	0.0013	0.0024	3.7	3.2	

Note: 1, CCME guideline for the protection of aquatic life

Table 10 Full Test Result s Summary (Phase II): Highest Concentration (mg/L) Tested that had No Observed Effect (NOEC) on the Measured Response

Pesticide/Formulation	Source	Trophic Level							Guideline[1] (mg/L)
		Plant			Invertebrate (*Ceriodaphnia*)		Vertebrate (fathead minnow)		
		duckweed		algae	survival	reproduction	survival	growth	
		growth (NOEC)	biomass (NOEC)	growth (NOEC)	(NOEC)	(NOEC)	(NOEC)	(NOEC)	
Reagents									
2,4 D (acid)	Sigma	< 5.7	92	47	18.9	18.9	9.4	9.4	0.004
MCPA (acid)	Sigma	< 6.1	82	85	17	8.5	8.5	8.5	0.0026
MCPP (potassium salt)	BASF	6.6	26	54	21.8	21.8	10.9	10.9	ng
dicamba	BASF	6.4	99	102	20.5	20.5	10.2	10.2	0.01
bromoxynil (octanoate ester)	Sigma	0.2	0.2	1	0.8	0.2	2	2	0.005
picloram (amine salt)	Sigma	21	84	22	20.9	20.9	8.6	8.6	0.029
imazamethabenz	BASF	< 0.4	0.8	8	12.8	12.8	6.4	6.4	ng
lindane	Sigma	0.12	0.24	0.6	0.0125	0.0125	0.000625	0.025	0.00001
diazinon	Novartis	26	26	54	0.000625	0.0025	2.7	0.7	ng

Note: 1, CCME (2000) guidelines for the protection of aquatic life; ng, no guideline

Table 11 Test Results Summary (Phase III): Effects of Herbicide Mixtures on the Survival and Reproduction of *Ceriodaphnia*

	Herbicide Mixture Concentration (100 ug/L)				Herbicide Mixture Concentration (10 ug/L)			
	Survival (%)	% of Controls	Reproduction (Avr. # of young)	% of Controls	Survival (%)	% of Controls	Reproduction (Avr. # of young)	% of Controls
Mixtures of Herbicides[1]								
Contol	100	100	16.3	100	100	100	19.8	100
1	100	100	20.9	128	100	100	14.3	72
2	100	100	16.6	102	100	100	16.4	83
3	100	100	12.3	75	100	100	14.3	72
4	100	100	15.9	98	100	100	18.2	92
5	100	100	14.3	88	100	100	15.1	76
6	90	90	16.2	99	100	100	14.7	74

Note: See methods section for order of addition

Table 12 Test Results Summary (Phase III): Effects of Insecticide Mixtures on the Survival and Reproduction of *Ceriodaphnia*

	Invertebrate (*Ceriodaphnia*)			
	survival		reproduction	
	LC50 (ug/L)	NOEC (ug/L)	EC50 (ug/L)	NOEC (ug/L)
Insecticide Mixture	> 1	1	> 1	1

Table 13a Nominal and Actual Concentrations of Pesticide Stock Solutions for Phase I

Common Name	Source	Nominal (mg/L)	Actual (mg/L)	Purity (%)	Actual/Nominal (%)	Solubility (mg/L)	Recovery (%)	Comment
Reagent								
2,4 D (acid)	Sigma	1000	463	98	45	682	68	supersaturated
MCPA (acid)	Sigma	1000	425	95	40	825	52	supersaturated
MCPP (potassium salt)	BASF	1000	528	100	53	620	85	supersaturated
dicamba	BASF	1000	549	99	54	4500	12	clear
bromoxynil (octanoate ester)	Sigma	1000	70	100	7	65	108	supersaturated
picloram (amine salt)	Sigma	1000	257	100	26	430	60	supersaturated
imazamethabenz	BASF	1000	260	95	25	1114	23	supersaturated
lindane	Sigma	1000	14	97	1.4	3.6	392	supersaturated
diazinon	Novartis	1000	107	87	9.3	60	178	supersaturated
Commercial product								
MCPA (acid)	Home Depot	2500	870	40	14	825	105	clear
		1000	230	40	9	825	28	clear
MCPP (potassium salt)	Home Depot	6667	2194	15	5	620	354	clear
		1000	246	15	4	620	40	clear
diazinon	Home Depot	8059	726	13	1.13	60	1210	emulsion
		1000	73	13	0.91	60	122	emulsion
chlorpyrifos	Home Depot	18150	380	5	0.10	1.4	27143	emulsion
		1000	30	5	0.15	1.4	2143	emulsion

Note: recovery is the actual or measured concentration divided by the solubility of the pesticide
the lowest or average solubility reported for each compound was selected for deriving recoveries

Table 13b Nominal and Actual Concentrations of Pesticide Stock Solutions for Phase II and III

Common Name	Source	Nominal (mg/L)	Actual (mg/L)	Purity (%)	Actual/Nominal (%)	Solubility (mg/L)	Recovery (%)	Comment
Reagent								
2,4 D (acid)	Sigma	1000	472	98	46	682	69	supersaturated
MCPA (acid)	Sigma	1000	424	95	40	825	51	supersaturated
MCPP (potassium salt)	BASF	1000	544	100	54	620	88	supersaturated
dicamba	BASF	1000	512	99	51	4500	11	clear
bromoxynil (octanoate ester)	Sigma	1000	80	100	8	65	123	supersaturated
picloram (amine salt)	Sigma	1000	216	100	22	430	50	supersaturated
imazamethabenz	BASF	1000	160	95	15	1114	14	supersaturated
lindane	Sigma	1000	4.8	97	0.5	3.6	134	supersaturated
diazinon	Novartis	1000	107	87	9.3	60	178	supersaturated

Note: recovery is the actual or measured concentration divided by the solubility of the pesticide
the lowest or average solubility reported for each compound was selected for deriving recoveries

Table 13c Nominal and Actual Concentrations of Pesticide Mixture Solutions (Phase III)

Common Name	Source	Mixtures Herbicides (100 ug) Nominal (ug/L)	Herbicides (100 ug) Actual (ug/L)	Herbicides (10 ug) Nominal (ug/L)	Herbicides (10 ug) Actual (ug/L)	Insecticides (1 ug) Nominal (ug/L)	Insecticides (1 ug) Actual (ug/L)
Reagent							
2,4 D (acid)	Sigma	100	102	10	10.2		
MCPA (acid)	Sigma	100	100	10	10.0		
MCPP (potassium salt)	BASF	100	103	10	10.3		
dicamba	BASF	100	93	10	9.3		
bromoxynil (octanoate ester)	Sigma	100	114	10	11.4		
picloram (amine salt)	Sigma	100	84	10	8.4		
imazamethabenz	BASF	100	62	10	6.2		
lindane	Sigma					1	0.34
diazinon	Novartis					1	1

7.0 FIGURES

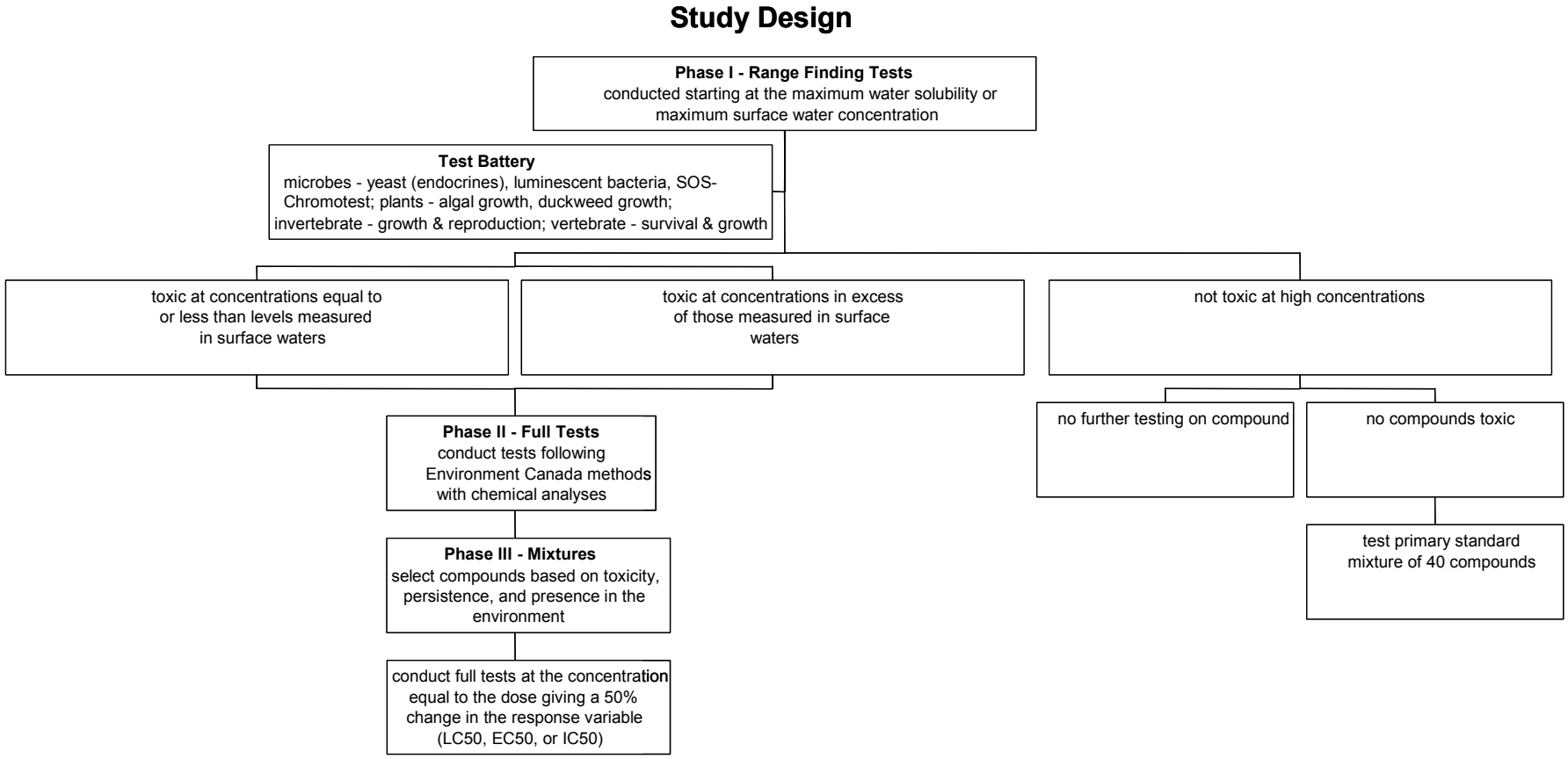

Figure 1 Project Overview