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Predicting Effects of Pesticides in Freshwater Aquatic Ecosystems – using field data validation.

by Lucina Singh

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements of the Degree of Master of Science.

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Your file Votre référence
ISBN: 978-0-494-27024-0
Our file Notre référence
ISBN: 978-0-494-27024-0

ABSTRACT

The plausibility of using single-species acute laboratory toxicity data to predict the effects of pesticide contamination of freshwater aquatic ecosystems was investigated, using single application studies. Toxicity units were developed using the geometric mean *Daphnia* spp. values or hazard concentrations for 5% of species derived from species sensitivity distributions for crustacea, insecta or algae, and peak pesticide water concentration. Pesticide physico-chemical (Kow, Koc) and fate (hydrolysis, water photolysis, aerobic aquatic biotransformation and aerobic soil biotransformation half-lives) properties were used along with the toxicity units and structural properties of the experimental systems (volume, surface area to volume ratio) to produce models capable of explaining the effects seen in the experiments. Akaike's Information Criterion was used to select the best model combinations, which were then generated using linear regressions. The hazard concentration is a better predictor than *Daphnia* spp. and fate parameters are necessary to produce better predictions.

ACKNOWLEDGEMENTS

Funding for this research was made possible by a partial scholarship from the Organisation of American States (OAS) administered through Latin American Scholarship Program of American Universities (LASPAU) and Public Service Ministry, Government of Guyana; and grants from the National Agro-Environmental Standards Initiative through Environment Canada. The research was conducted at the National Wildlife Research Centre of the Canadian Wildlife Service, Environment Canada.

I would like to thank my supervisor Dr. Pierre Mineau for all his patience and guidance, in addition to financial support. I am also grateful for the invaluable suggestions made by my committee members – Drs. Peter Delorme, Scott Findlay and Mark Forbes.

Clare Morrison and Melanie Whiteside provided vital laboratory data, while Katie Harding gave helpful tips on the use of the statistical software. I also like to thank Brian Collins for his assistance with statistical and mathematical matters.

Finally, I would like to thank my family, friends and co-workers at the Pesticides and Toxic Chemicals Control Board (Guyana) who offered support, encouragement and advice that made the undertaking and completion of this research possible.

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AAB - Aerobic aquatic biotransformation half-life
AFs - Application Factors
AIC - Akaike's Information Criterion
AICc - Akaike's Information Criterion correction estimates for small samples
ASB - Aerobic soil biotransformation half-life
EC50 - Effect Concentrations for 50% of the test population
EU - European Union
EXTOXNET - EXtention TOXicology NETwork
HC - Hazard Concentration
HC5 - Hazard Concentration for five percent of the species
HC5- A - Hazard concentrations for 5% of the algal species
HC5-C - Hazard concentration for 5% of crustacean species
HHL - Hydrolysis half-life
Koc - Organic carbon absorption coefficient
Kow – Octanol-water partition coefficient
L AAB – Log-transformed aerobic aquatic biotransformation half-life
L ASB – Log-transformed aerobic soil biotransformation half-life
LC50 - Lethal Concentrations for 50% of the test population
L(E)C50 - Lethal or Effect Concentration for 50% of the test population
L HHL – Log-transformed hydrolysis half-life
L TU Daphnia - Log-transformed TUs based on the geometric means of *Daphnia* species
L TU HC5-A - Log-transformed TUs based on HC5-A
L TU HC5-C - Log-transformed TUs based on HC5-C
L WPHL – Log-transformed water photolysis half-life
L volume - Log-transformed volume
L TSA/V R - Log-transformed total surface area to volume ratio
Log koc – log-transformed organic carbon absorption coefficient
log Kow - Log-transformed octanol-water partition coefficient
MPC - The Maximum Permissible Concentration
NOEC - No Observable Effect Concentration
OECD – Organisation for Economic Co-operation and Development
PAN - Pesticide Action Network
PMRA - Pest Management Regulatory Agency – Government of Canada
PNEC - Predicted No Effect Concentration
R^2 - coefficient of determination
SSDs - Species Sensitivity Distributions
TSA/V R - Total surface area to volume ratio
TU - Toxicity Units
UP - Uniform Principles
USDA NRCS-
USEPA - United States Environmental Protection Agency
WPHL - water photolysis half-life

CHAPTER ONE - INTRODUCTION

1.1 Background

Pesticides are widely used in agriculture today as a means of increasing the quality and quantity of production in order to meet the consumption needs of a growing global population. These substances by their nature are intended to harm some organism, and as a result have the potential to induce and have in many instances caused unwanted impacts in the environment.

Risk managers are faced with the challenge of deriving ecological thresholds that are protective of complex and dynamic ecosystems. Efforts to develop ecological hazard and risk assessment methods capable of evaluating chemicals in the aquatic environment began in the 1970s (Selck *et al.* 2002) and consequently, a variety of tools and methods are available. However, there continues to be much debate pertaining to the most appropriate parameters to be used, statistical strengths and validation.

Prior to registering a chemical, regulatory agencies require single species laboratory toxicity data. Typically, these data are compared to predictive exposure levels and thresholds of acceptability are applied to the resulting ratio of exposure to toxicity. Other relevant information requested such as the physico-chemical and fate characteristics of the pesticide are used to help estimate exposure levels; however they are typically not factored into the toxicity determination of the compound.

The main objective of this research is to investigate how well these single species laboratory data can predict real world effects and to offer a simple field-validated (but not site-specific) tool which can assist risk managers in better predicting the effects of pesticides on the aquatic environment.

This research constructs models using lethal or effect concentrations for single species, as well as Hazard Concentration values from Species Sensitivity Distributions (SSDs), which is an extrapolation method used to derive protective levels. To date, most regulatory assessments, whether in Europe or North America, still rely on extrapolating from a single species rather than SSDs. It is important to assess whether the extra effort required to generate SSDs results in a better prediction of impacts.

The models are based on data from studies that use model ecosystems to determine the effects of pesticides on freshwater aquatic environments. These types of data are normally applied on a single pesticide basis; however in this study a large body of such model ecosystem research on several pesticides was used to build predictive models.

The influence physico-chemical and fate characteristics of pesticides exert on toxicity is often qualitatively reported in analyses; however there are few studies that quantify that effect or attempt to use it in a regulatory framework.

1.2 Literature Review

1.2.1 Extrapolation Methods

Extrapolation methods, which use known data to make predictions, are being used by risk assessors to determine the ecological thresholds for chemicals within the aquatic environment. There are two major groups of extrapolation methods: Application Factors (AF) and Species Sensitivity Distribution (SSD). It should be noted that the SSD and AF are not necessarily exclusive. The AF can be applied to the output from an SSD.

1.2.1.1 Application Factors

The AFs (also called safety factors or uncertainty factors) are pre-selected multipliers or divisors applied to some endpoint parameter of toxicity data. They typically vary from 10 to 1000, are dependent on the data available and are usually used when few toxicity data are available (Selck *et al.* 2002).

In their first tier of risk assessment the European Union (EU) employs AFs in the form of Uniform Principles (UP) to set pesticide surface water threshold limits. They require that the concentration must be less than 0.01*acute L(E)C50 (Lethal or Effect Concentration for 50% of the test population) fish or Daphnia for insecticides and 0.1x EC50 algae for herbicides. They also specify for prolonged exposure the concentration should not exceed 0.1*NOEC (No Observable Effect Concentration) Daphnia (21days) and fish (28days).

AF methods have been criticised because they often do not have any scientific basis; they are deemed as using arbitrary values to predict an effect. However, they are much easier to use than the SSD. In addition, the AF approach is less time and data consuming.

1.2.1.2 Species Sensitivity Distributions

The SSD methods are based on the assumption that the variation in species sensitivity to a chemical is statistically distributed (Roelofs *et al.* 2003). Most SSD methods involve using single species toxicity data to generate cumulative distribution function curves, which are then used to extrapolate protective levels. However, a few methods that use ordered statistics (where the data are ranked before extrapolation) exist.

North American and European development of the SSD were independent and scientists from either location were not aware of the work being done in the other continent, until 1992 (Suter, 2002). The Netherlands has been using SSD for environmental risk assessment since 1989, while the European Community has employed its use to determine PNECs (Predicted No Effect Concentration) since 1996 (Duboudin *et al.* 2004).

The SSD was first developed and used by the USEPA (United States Environmental Protection Agency) in 1978 to derive water quality criteria (Suter, 2002). This method was based on LC50 values and used the Hazard Concentration for five percent (HC5) of the species. Today, the HC5 is the most widely accepted HC%, however the principal of

the 95% protection was disputed from the beginning (Forbes & Forbes, 1993; Hopkin, 1993; Smith & Cairns, 1993; Grist *et al.* 2002).

One of the major differences among SSD methods relate to the distribution used to model the toxicity data. Risk assessors are encouraged to use the best fitted distribution for their data samples on an individual toxicant basis (Wheeler *et al.* 2002). To date Europe and the US use the log-normal (Wagner & Lokke, 1991) or the log-logistic (Aldenberg & Slob, 1993) model whilst Australia and New Zealand use Burr III (Shao, 2000; Wheeler *et al.* 2002). The USEPA uses the log-triangular distribution.

The log-logistic and the log-normal models are still the most popular, but Grist *et al.* (2002) highlight that so far there has been no one predetermined statistical distribution that has been well-fitted for all data samples. The log-normal model is used often due to its simplicity and use in previous pesticide risk assessments. However, Wheeler *et al.* (2002) suggest that the log-logistic model generally produces a better goodness of fit for the toxicity data and has extended tails thereby making it more conservative; but its calculations are more complicated than the log-normal model.

Okkerman *et al.* (1993) claim to have validated the methods of Aldenberg and Slob (1993, log - logistic), Wagner and Løkke (1991, log – normal) and a modified version of the USEPA (1992) using organic compounds and results from multi-species tests (Grist *et al.* 2002). In addition, Selck *et al.* (2002) state that the Aldenberg & Slob method appears

to be more protective than that of Wagner & Løkke, a claim which supports Wheeler *et al.* (2002).

The SSD is not without its criticisms and its use in risk assessment is being debated especially as it relates to the species and type of toxicity data to be included, and the most appropriate end point of the SSD (Maltby *et al.* 2005; Schroer *et al.* 2004). First of all, many scientists point out that laboratory data do not reflect a wide range and random selection of the total environment, an assumption upon which SSD methods are based. In fact, the data available are mostly due to costs and manageability of the species.

Forbes & Calow (2001) criticise the use of values of species that are not found with the particular ecosystem. Even though some supporters of the SSD approach encourage the use of data that are of the same habitats, geographic regions and taxonomic groups, the situation remains that such data are often not available. However, studies done by Hose & Van den Brink (2004) and Maltby *et al.* (2005) support the claim that sensitivity of organisms is independent of their geographic origin. The more recent studies construct separate SSDs based on major taxonomic grouping, for example Brix *et al.* (2001) used fish and invertebrates, Duboudin *et al.* (2004) used vertebrates, invertebrates and algae and Maltby *et al.* (2005) used vertebrates, arthropods and non-arthropod invertebrates. Taxa selection for a distribution should be based on good biological and statistical grounds (Versteeg *et al.* 1999).

Since risk managers tend to be more interested in the effect of a toxicant in the long term, chronic values are often deemed more important than their acute counterparts. However, while acute data tend to be more readily available for most substances, chronic data are often severely lacking. In addition, the NOECs (No Observable Effect Concentrations) which are sought after have been heavily criticised as being statistically unsound and incapable of environmental protection (Scholz *et al.* 2001; Crane & Newman, 2000; Chapman *et al.* 1996).

NOECs are derived through hypothesis testing and as a result the power of the statistical test and level of significance chosen can affect the outcome. In addition, no confidence intervals are supplied with these values. Most importantly, failure to detect a statistically significant effect does not necessarily mean that there was not a biologically significant one. Chapman *et al.* (1996) concluded that even the software packages selected to determine the NOEC can produce different results for the same data. NOECs are also subject to experimental design and the levels of concentration chosen by the experimenter. Crane & Newman (2000) point out that some studies revealed that it takes a 20 – 25 percent change before the hypothesis testing indicates that there has been a significant level of effect.

A contentious issue relating to SSD construction is the number of data points needed to generate a proper model. OECD (1992) and the Australian water quality guidelines both stipulate the use of at least five data points to produce a SSD; while the European guidance document suggests eight (Hose & Van den Brink, 2004). Selck *et al.* (2002)

state that at least four values in order to make a prediction. Wheeler *et al.* (2002) posit that ten should be used and Newman *et al.* (2000) states 15 to 55, with a median of 30 datapoints are needed to get the optimal HC5. However, the amount of data points needed also relates to the statistical distribution that the SSD model will take, for it is found that if a bootstrap approach is used a minimum of 20 are needed.

However, despite the criticisms and concerns SSDs are increasingly being incorporated into risk assessments. Wheeler *et al.* (2002) propose that this is because they have greater statistical power than approaches that rely solely on AFs. To date SSDs have been used with a variety of chemicals, such as metals, surfactants, pesticides, organic substances and inorganic substances, which are potential threats to the environment (Maltby *et al.* 2005).

There have been arguments that state using a HC value does not take into account the effects on keystone species or other important species. The HC value which is based on structural endpoints is influenced by the debate between two contradicting theories on the relationship between ecosystem structure and function: 1) ecosystem structure will change before its function and 2) ecosystem function will change before its structure. If the former theory is true then vital species can be lost without a major change to ecosystems. Both structural endpoints and functional endpoints have been investigated, however there is still no consensus as to which is most appropriate for ecotoxicological studies (Selck *et al.* 2002).

1.2.1.3 Application Factors Vs Species Sensitivity Distribution

In an effort to evaluate which approach can better predict PNECs, Selck *et al.* (2002) used AFs and SSDs based on Aldenberg & Slob and Wagner & Løkke methods constructed with NOECs and EC50s (Effect Concentrations for 50% of the test population) or LC50s (Lethal Concentrations for 50% of the test population). When the PNECs generated were compared with field studies of Tributyltin (TBT) and alkylbenzene sulfonate (LAS), the results indicated that both AF and SSD appear to be protective - the lowest field effect concentration was higher than the PNECs (Selck *et al.* 2002).

Brock *et al.* (2004) also compared an AF method with a SSD method, assessing the effects of two photosynthesis-inhibiting herbicides-metribuzin and metamitron. Their results also reveal that both approaches produce protective estimates when compared with the NOEC results found within the enclosure experiments with these herbicides (Brock *et al.* 2004).

Forbes & Forbes (1993) did a comparison between an SSD against one of the AF methods that was being used- dividing the lowest fish, crustacean and alga NOEC by 10. Of the eight chemicals used, both methods produced the same value for two, while the SSD method produced lower values for five and a higher value for one chemical. These authors concluded that advocating a method based on a more conservative prediction is not justifiable.

1.2.1.4 Single Species Toxicity Data Vs Model Ecosystems Data

Since laboratory LC50, EC50 and NOEC do not account for the abiotic environment or interactions between species within the ecosystem, they are deemed as inadequate measures of effects on field populations and communities; and therefore thought to be unfit to produce results explaining an entire ecosystem (Forbes & Forbes, 1993; Newman *et al.* 2000; Pratt & Cairns, 1996; Ravera, 1989; Selck *et al.* 2002). Instead, the use of results from multi-species tests and model ecosystems (such as mesocosms, experimental ponds and ditches, artificial streams, and microcosms) is preferred.

Zeeman & Gilford (1993) suggest that the use of single species laboratory data to make predictions continue because of the general lack of more ecologically reliable data (Selck *et al.* 2002). However, ecotoxicological field studies are well established in risk assessment because of their ability to yield data on population and community effects. But, analysis of these data remains a challenge (ETC editorial, 1999), in addition for these studies to be representative of the natural environment they need to be of a certain size and complexity, which can be costly (Boxall *et al.* 2002).

Boxall *et al.* (2002) suggest that there are many limitations to using model ecosystems, especially as it relates to interpreting the results and extrapolating it to other situations (Brock *et al.* 2004). Some major reasons according to them include: 1) the NOECs depend on the test concentrations selected; 2) studies are usually performed at different times and starting conditions; 3) studies vary in duration; 4) differences in sensitivities across experiments; 5) measured end points are not always consistent or comparable; and

6) statistical concerns about the number replicates done and type of analysis used on the data.

Most of the risk assessments carried out on the effects of toxicants in the aquatic environment have been based on extrapolation methods that employed the use of single species laboratory data on *Daphnia*, fish and algae with survival, growth and reproduction being the usual endpoints (Selck *et al.* 2002). These are all structural endpoints and Forbes and Forbes (1993) emphasise that there are instances where community structure and function are not easily or consistently coupled. Therefore, in light of this information, using a protection level based on a structural change may be inadequate.

However, some researchers such as Pratt & Cairns (1996) and Van Leeuwen (1996) support the theory that ecosystem function is less sensitive than ecosystem structure to disturbance due to redundancy of ecological functions which can allow the death of the more sensitive species but yet maintain ecosystem functioning (Selck *et al.* 2002). This theory was also supported by the findings of Kälquist *et al.* (1994) who found that even though the species diversity had changed after pesticide exposure the primary production levels remained the same (Selck *et al.* 2002). However, Brock *et al.* (2000) concluded that functional endpoints are more sensitive than structural ones to photosynthesis inhibiting herbicides.

Single species extrapolation methods such as the SSD assume that the sensitivity of the organisms used for the laboratory studies is similar to that of the field (Wheeler *et al.* 2002). However, organisms used for laboratory studies appear to be more sensitive than those from mesocosm studies because of lack of random selection of species and differences in water quality and habitat and shelter availability (Hose & Van den Brink, 2004; Versteeg *et al.* 1999). Also standard toxicity tests are usually performed for the most sensitive life stages of a species, and as a result there can be overestimation of population effects (Maltby *et al.* 2005; Boxall *et al.* 2002).

Studies have shown that single species data can be extrapolated to make predictions similar to semi-field studies (Hose & Van den Brink, 2004; Boxall *et al.* 2002; ETC editorial, 1999). Hose & van den Brink (2002) and Schroer *et al.* (2004) showed that SSD using laboratory data can produce EC50 values that are similar to those attained through mesocosm (semi-field) experiments, even though field endpoints may differ (field - abundance vs. laboratory – immobility). Therefore SSDs can be used to predict safe environmental concentrations (Hose & Van den Brink, 2004).

1.2.2.1 Mesocosm Study Review: Mohlenberg et al. (2001)

Mohlenberg *et al.* (2001) modelled the lowest effect concentration (positive or negative) results obtained from model ecosystem studies, using Partial Least Squares (PLS) to describe their relationship with experimental characteristics (day number for 1st dosing, dosing interval, latitude, longitude, mesocosm size - volume and depth), single species toxicity (HC5 and OECD-lowest L(E)C50 standardised species x 0.1) and fate properties

(water solubility, log Kow and log Kd). The lowest effect concentrations were collected for macroinvertebrates (non-predatory, predatory, epibenthic fauna and sediment-living fauna); zooplankton (cladocera, copepoda and rotifera) and micro-algae.

The HC5 was taken at the 50% CI and determined using the Wagner and Løkke (1991, log – normal) method. Some of these HC5s were produced by pooling data according to taxonomic groups (algae, fish, insects, crustacea); however due to lack of toxicity data for some pesticides all data were pooled for SSD generation irrespective of taxonomic group.

The Mohlenberg *et al.* (2001) database included 91 experiments (both single and multiple application regimes) from 112 publications with 3,635 effect concentrations for 31 pesticides. Eight herbicides: 2,4 D, alachlor, atrazine, glufosinate-ammonium, glyphosate, hexazinone, linuron and triclopyr; 22 insecticides: aminocarb, azinphos-methyl, bifenthrin, carbaryl, carbofuran, chlorpyrifos, cyfluthrin, deltamethrin, diazinon, diflubenzuron, dimethoate, endosulfan, esfenvalerate, fenitrothion, fenvalerate, lambda-cyhalothrin, lindane, methoxychlor, mexacarbate, permethrin, tebufenozide, tralomethrin; and the fungicide propiconazole. However, the number used in each PLS model varied.

Their zooplankton model when restricted to single applications of insecticides had a predictability of 74%, while the predictability for herbicides and insecticide combined was 66%. The insecticide model was based on 11 experiments covering 11 insecticides: lindane, methoxychlor, chlorpyrifos, esfenvalerate, diflubenzuron, deltamethrin, diazinon, cyfluthrin, permethrin, bifenthrin, and lambda-cyhalothrin.

The factors that affected toxicity for cladocera and copepoda were day of application, latitude, longitude, log Kow, Volume, HC and LC50/10. The model indicates that toxicity decreases in cold climates (high latitude), but increases in longitude caused increased toxicity. During the summer (high Day number) and in systems with larger volumes more toxic effects are expected. In addition hydrophobic substances are more toxic. However log Kd or depth were not considered to have an impact on toxicity. Mohlenberg *et al.* (2001) also conclude that cladocera and chaoborus are more sensitive than copepoda and at any given concentration copepoda is expected to have 20% more reductions than cladocera.

The macroinvertebrates model can predict 63% of observed effects (multiple applications) found in nine experiments, with the best predictions being for systems with sediments and macrophytes. The PLS model indicates all four sub-groups of macroinvertebrates are equally sensitive to pesticide exposure. However, there appears to be a difference according to the habitat of the organisms. More effects are expected at high latitudes and low longitudes in addition to shorter interval between applications and low number of doses. Shallower systems are expected to produce increased toxicity and hydrophobic, adsorbable substances are more toxic. Low persistent pesticides had fewer effects than high ones with short term exposure.

The micro-algae model of this study is able to explain 72% of the variance seen in nine experiments carried out in the field. The model indicates that pesticides added over a

short period of time are more toxic to algae. In addition, algae are more sensitive to hydrophobic, adsorbable substances.

1.2.2.2 Mesocosm Study review of Brock et al. (2000)

The research undertaken by Brock *et al.* (2000) aimed to: 1) assess the ecosystem NOEC for individual pesticides, 2) to compare these threshold levels with water quality standards, and 3) to evaluate the ecological consequences of exceeding these standards. They collected ecological effects (on community metabolism, phytoplankton, periphyton, macrophytes, zooplankton, macro-crustacean and insects, molluscs, and fish and amphibian) from semi-field studies done using single, multiple and continuous applications in both running and stagnant water regimes. A total of fifty-six studies covering 20 herbicides and 62 studies on 21 insecticides were used.

The effects were classified according to their magnitude and duration using the following five categories – no effect, slight effect, pronounced short-term effect, pronounced effect in a short term study, pronounced long term effect. To enable comparison among pesticides a toxic unit was derived by dividing the nominal pesticide concentration by the geometric means of L(E)C50 values for the most sensitive standard test species. *Daphnia* or sometimes a standard test fish species values were used for insecticides and *Scenedesmus subspicatus*; *Selenastrum capricornutum* or *Chlorella vulgaris* values for herbicides.

The ecosystem NOEC was compared with the results from various methods used to set pesticide surface water thresholds such as:-

1) The Maximum Permissible Concentration (MPC) which uses at least four chronic toxicity points (NOEC) for aquatic organisms according to the Aldenberg & Slob (1993) method or a modified USEPA method (Crommentuijn *et al.* 1997) when there were fewer than four.
2) Uniform Principles (UP) which is used by the EU in their first tier of risk assessment and stipulates that surface water concentrations must be less than 0.01 * acute L(E)C50 fish of Daphnia and 0.1 * EC50 algae, and lower than 0.1 * NOEC Daphnia (21days) and fish (28days) prolonged exposure. Brock *et al.* (2000) used both the liberal and conservative interpretation of the UP. Both forms used the recommended multiplier for the species type, however the liberal form used the geometric mean L(E)C50 value while the conservative form used the most sensitive L(E)C50 value available.

Brock *et al.* (2000) concluded that values derived by the MPC and UP methods were sufficiently protective of aquatic ecosystems for all types of insecticide exposure regimes. These researchers consider the MPC values derived for herbicides by the modified EPA method as being protective, but nonetheless state some adjustments are needed for certain compounds. They propose that the UP criteria seem protective with photosynthesis and growth inhibiting herbicides, but not with auxin stimulators (underestimation factor as high as 100).

1.2.2.3 Review done by Brock et al. (2006)

This review examined the aquatic risk of pesticides, ecological protection goals and common aims in the EU legislation. The authors also make several proposals based on their findings with the aim to harmonise the system used in the EU. The scientific results used are largely based on the study briefly described above - Brock *et al.* (2000); Maltby *et al.* (2005) who tested the protectiveness of various HC5s against the ecosystem NOEC for 16 insecticides; Van den Brink *et al.* (2006) who examined the protectiveness of various HC5s against the ecosystem NOEC for nine herbicides; and current work on fungicides being carried out by Maltby.

The HC5 and HC50 values (at the median: 50% and lower limit: 95% confidence intervals) based on Aldenberg & Jaworska method (2000 – log-normal) using acute and chronic data were calculated in the Van den Brink *et al.* (2006) study. The SSDs were constructed for algae, macrophytes, invertebrates and vertebrates for three herbicides (atrazine, diquat, 2,4-D) and for the other herbicides (diuron, linuron, metamitron, metribuzin, pendimethalin, simazine) SSDs were generated for primary producers and invertebrates and/or vertebrates. These results were compared with semi-field studies. The research revealed that the lower limit HC5 value with acute data was able to protect the aquatic environment, while the acute data median HC5 was able to protect the ecosystems against short-term herbicide exposure.

Maltby *et al.* (2005) generated HC5 values (at the median: 50%, lower limit: 95% confidence intervals, and upper limit: 5%) for 16 insecticides: (azinphos – methyl,

carbofuron, chlorpyrifos, diazinon, fenithrothion, parathion – ethyl, parathion – methyl, cypermethrin, deltamethrin, fenvalerate, lambda – cyhalothrin, permethrin, lindane, methoxychlor, carbaryl, diflubenzuron). The SSDs were generated taxonomically (vertebrates, arthropods, non-arthropod invertebrates) and for different habitats (saltwater, freshwater, lentic, lotic) and geographic regions (palaeartic, neartic, temperate, tropical). The latter two groupings were not found to influence the HC5 results. Maltby *et al.* (2005) concluded that the lower limit HC5 value is able to protect aquatic ecosystems; while the median HC5 is generally protective of these freshwater environments.

Brock *et al.* (2006) relate that the results from Maltby's current work on fungicides (azoxystrobin, carbendazim, pentachlorophenol, triphenyltin acetate) indicate that the median HC5 is not protective of the environment. However, the lower limit HC5 is able to protect the ecosystem from two of the four fungicides (azoxystrobin, pentachlorophenol). The general conclusion from Brock *et al.* (2006) is that all median HC5 values can protect the environment against slight effects and the lower limit HC5 values can ensure against any effects.

1.3 Hypotheses

My research was aimed at testing the following hypotheses:-

1) Empirically based models using laboratory data such as toxicity, fate and physico-chemical properties of pesticides can be constructed to predict real world effects.

2 – The basic physico-chemical and fate properties of the pesticide, in addition to the structural properties of the system, help predict of the impacts seen.

3 – HC5 values are more predictive than LC50 values required for registration.

4 – Better predictions are obtained from modelling pesticides types (insecticides and herbicides) separately.

CHAPTER 2 - METHODS

2.1 Ecosystem studies

Pesticide impact studies in freshwater lentic and lotic mesocosms, ponds and streams were collected from the published literature, data evaluation records from unpublished studies submitted by registrants to the USEPA, and monitoring reports for Spruce Budworm and other forest pest spraying programmes.

Criteria for selection:-

1) The studies were performed using a realistic freshwater aquatic ecosystem of adequate size (more than 80L volume for lentic systems) and contained a range of species
2) The experimental design was clear or easily obtained if not included with results
3) Systems did not include possible traces of other pesticides
4) Effect information was available for a single application for which the pesticide water concentration was known or could be easily deduced
5) A pre-treatment or untreated control system was used
6) Statistical significance of the results were indicated, or studies had extremely sound experimental designs in addition to quantification of effects that can clearly be considered major (e.g. death of all organisms in the treated systems, compared to negligible change in the control systems)

Some studies contained more than one experiment, that is, more than one pesticide, concentration or system characteristic was used. Information extracted from the studies included system structure and location characteristics such as 1) type (pond, lake,

mesocosm or stream); 2) dimensions (length, diameter, width or depth); 3) size of enclosure; 4) volume of water within enclosure; 5) water regime (lentic or lotic) and 6) country. Water quality properties such as pH, temperature, dissolved oxygen, conductivity, total phosphorus and total nitrogen were noted when available. Species composition was also recorded, especially fish and macrophyte presence.

Endpoints in the two water regimes – lentic and lotic – were not easily comparable, consequently an attempt was made to model them separately. However, there were few lotic studies and therefore only entries from lentic systems were used. Also, for modelling purposes only two structural properties of the system were used – the volume and surface area to volume ratio which was calculated by using the dimensions or size of the system and volume within the enclosure.

While the number of replicates of control and treated systems was known for each experiment, the results reported were based on averages of these replicates. The trade name and formulation of the pesticide were recorded, along with the solvent into which it was dissolved. Application method (e.g. spray, spray drift, sub-surface release), regime (single or multiple), rate and date are included in the database.

Vital information such as peak pesticide concentration in the water column; taxonomic group and effect and type of control (pre-treatment or untreated) were recorded. And when available so were study duration; acclimatisation period of the system prior pesticide addition; sampling regime; type of effect (primary or secondary) and recovery.

2.2 Laboratory data

2.2.1 LC 50 and EC 50 laboratory single species toxicity data

Whiteside *et al.* (2007) compiled a database of single species toxicity values [L(E)C50], for both fresh and salt water species, based on the USEPA pesticide registration data, Agritox database, European Commission pesticide review reports , the Pesticide Manual (2003), and the USEPA ECOTOX database. They generated separate Species Sensitivity Distributions (SSDs) for crustacea, insecta, fish, algae and macrophytes from which a Hazard Concentration for the sensitive 5% of the population (HC5, median estimates) was obtained.

Both salt and fresh water species were grouped together (Maltby *et al.* 2005) and based on the statistical distribution of the data the SSDs were created in one of three ways:-

1) If the data available for a pesticide were (by visual examination) normally distributed and had at least 5 species values, the ETX2.0 programme was used. This software, which is based on a log-normal distribution, was developed by van Vlaardingen *et al.* (2004). Since this was the preferred method of HC5 estimation, outliers were removed from datasets in order to achieve normality; however in the event this did not happen the other methods were resorted to.
2) If the dataset was not normal or normality could not be achieved by removal of outliers, and there were more than ten values available the BurrliOZ programme was used. This software is based on Burr III distributions, which come from a family of very flexible distributions which makes good approximations of many commonly used distributions such as log-normal, log-logistic and Weibull.

3) If the dataset could not meet the requirements conditions for the other two programmes, in terms of size and distribution, the ETX 2.0 small sample method was used. The mean of the data was calculated and then an externally derived standard deviation was applied. This externally derived standard deviation was taken from those SSDs that were based on normally distributed data. It was done for the same major taxonomic groups (crustacea, insecta, fish, algae and macrophytes) and for similar classes of pesticides (insecticides, herbicides, fungicides and other pesticides).

The median estimate of the HC5 was generated (despite the risk of under-protection) so as to not bias the data because of lower sample sizes.

From the sources cited above, geometric means of LC50 data were calculated for *Daphnia spp*, *Scenedesmus subspicatus* and *Selenastrum capricornutum.*

2.2.2 Pesticide physico-chemical and fate properties

Octanol-water partition coefficient (Kow), organic carbon absorption coefficient (Koc), aerobic soil biotransformation half-life (ASB), aerobic aquatic biotransformation half-life (AAB) and water photolysis half-life (WPHL) were collected for those pesticides that had suitable model ecosystem studies. These data came from the database created by Whiteside *et al.* (2007), PMRA (Pest Management Regulatory Agency – Government of Canada), The Pesticide Manual (2003), European Commission Pesticide Review reports, EXTOXNET (EXtention TOXicology NETwork), USDA NRCS 2005 (United States

Department of Agriculture - National Resources Conservation Service), PAN (Pesticide Action Network), US EPA pesticide fate database and InChem. When more than one value was found for a property for the same pesticide, the geometric mean of those values were used.

Correlation analysis done using ASB and AAB values from 117 pesticides from the PMRA database revealed an expected strong relationship. The relationship equation was then used to fill in missing AAB values. When possible hydrolysis was matched according to the average pH of the system, alternately the half-life for a neutral pH was used.

Those fate properties which were considered as persistent were given a value which was more than the highest known half-life for the pesticides in the database. Tebuthiuron was assigned a value of 1520 days for aerobic soil biotransformation half-life; 1095 days was given as the water photolysis half-life of alachlor, carbendazim, hexazinone, lindane, metsulfuron methyl and simazine; and 14 pesticides were assigned a hydrolysis half-life of 1825 days.

2.3 Database construction

A database was constructed in Microsoft Excel® using the information extracted from the studies, laboratory data and fate characteristics of the pesticides. Standardisation was done prior modelling. First of all, entries for same variables were converted into common units.

Despite variation in power of the statistical tests and experimental designs used, responses to pesticide treatment were considered significant in accordance to the claims of individual study authors. A code was assigned to the results in order to indicate the taxonomic level they were reported at. Results were also coded binomially: 1 = effects (increase or decrease) and 0 = no significant effects.

Quantified effects of maximum impacts collected from tables, text or through estimation from graphs, were changed to percentages, which were then logged and arc-sine transformed in the case of decreases. It must be noted that the majority of statistically significant effects reported are at least a 70% change.

Since many studies did not include the magnitude of change for insignificant responses, an effect ratio of one (1) was assigned to cases with no significant effects. Effects were calculated using the following equations:-

1) decrease was observed

$$\text{effect ratio} = \frac{[quantity \quad in \quad control]}{[quantity \quad in \quad treatment]}$$

equation 1

2) increase was observed

$$\text{effect ratio} = \frac{[quantity \quad in \quad treatment]}{[quantity \quad in \quad control]}$$

equation 2

Using this system to quantity responses to pesticide treatment, effects are depicted by a value over one, and the larger that value is, the greater the magnitude of impact.

The laboratory data were standardised to produce Toxicity Units (TU), which entailed dividing the peak pesticide water column concentration by the species L(E)C50 geometric mean or HC5. (*See equation 3*) (Brock *et al.* 2000; Mineau 2002) These TU values along with the physico-chemicals properties and surface area to volume ratio were all log-transformed.

$$TU = \frac{[concentration\ of\ pesticide]}{[geometric\ mean\ L(E)C50 \ / \ HC5]}$$

equation3

The database was then carefully examined to determine the major taxonomic groups that were represented. It was found that crustacea, algae, and insecta were the groups with the most entries and there was enough data to model copepods and cladocera as distinct groups.

In order to avoid pseudo-replication, an overall experiment count ratio of effects was calculated within the three major sub-data groups (algae, insecta, crustacea) for effects recorded at the species, and family level in the case of insecta, since this group is extremely diverse and many studies reported at this taxonomic level. To build-up the

sample for insecta modelling, effects at the species levels were examined and included at the family level.

The number of affected species or families (both increases and decreases) was divided by the total number species or families recorded for that experiment (*See equation 4*).

count ratio of effect =

$$\frac{[number\ affected\ (species\ or\ families]}{[total\ number\ within\ system\ (species\ or\ families]}$$

equation 4

Increases were grouped with the decreases, because any statistically significant response was considered important and an indication of ecosystem change due to pesticide treatment. Overall, there were very few increases, with most being at the species level.

2.4 Modelling and Analysis

2.4.1 Sub-data sets

Prior to modelling data were pooled in the following groups:-

1) Taxonomic: genus to phylum
2) Pesticide type: all pesticides (AP), insecticides (IA), herbicides (HA).
3) Structural ecological endpoints: abundance, biomass, drift

In order to achieve a higher degree of realism the *a priori* decision was made to only derive a predictive model when there were at least six pesticides in the dataset.

2.4.2 Validation datasets

The sub-data sets used for modelling were first examined to see whether they were large enough to separate into training (2/3) and validation (1/3) sets. Generally if the data set contained over 30 entries, a validation set was collected. The entries were assigned numbers from one to three and all entries labelled as two were selected for the validation set. The selection of a validation set was not strictly random, the method outlined above was used to increase the type of pesticides available for modelling.

2.4.3 Empirical Modelling

STATISTICA® 6.0 was used to do all the modelling and statistical analysis. The original intent of this study was to generate models using both logistic and linear regressions. However, only linear regression models were generated, due to a number of reasons such as the nature of the data (inequitable distribution of effects to no effects, with effects accounting for over ¾ of the data in most samples), time constraints and exploratory analyses indicating that not much more would be gained from logistic modelling. In addition, it should be noted that only one effect entry per experiment was included (as dependent variables) for modelling, in order to eliminate pseudo-replication.

Prior to modelling the normality of independent continuous variables (fate properties, physico-chemical properties, TUs, structural properties of the system, quantified changes) was tested by visual examination of normal probability plots. It was found that these variables were all normal when log-transformed, and consequently the log-transformed values of these variables when used for subsequent analyses.

The correlations between these continuous independent variables were also tested. In a nutshell, the log-transformed geometric means values for both *Scenedesmus subspicatus* and *Selenastrum capricornutum* were highly correlated with each other (r = 0.93; p=0.000) and with HC5 values for algae (r = 0.80 (p=0.000) and r = 0.92 (p=0.000) respectively). Log-transformed *Daphnia* geometric L(E)C50 values were correlated with the log-transformed HC5crustacea (r= 0.95; p=0.000) and log-transformed HC5insecta (r= 0.83; p=0.000).

Table 2.1 contains the correlations between the different fate parameters. Log Koc is only correlated with log Kow (r = 0.38; p=0.009); while log Kow is also correlated with log WPHL (r = -0.31; p=0.050). Log ASB is correlated with log WPHL and log AAB; and log HHL is correlated with both log ASB and log AAB (r = 0.26; p=0.000 and r = 0.50; p=0.000 respectively).

In order to effectively test the hypotheses it was decided that the relevant effects and all explanatory variables would be subjected to the Akaike's Information Criterion (AIC) for the selection of the best approximating models (Burnham and Anderson, 2002). The AIC is based on maximum likelihood estimation and it penalises for the number of parameters within a model, thereby facilitating parsimony. AIC works on a relative scale and the model with the smallest AIC value is considered the best.

As a rule of thumb AIC correction estimates (AICc) should be used preferentially to AIC values from samples sizes that produce a quotient of less than 40 when divided by the total number of estimated parameters (*Df* (degrees of freedom) + 2) (Burnham and Anderson, 2002). Consequently, the AICc, which is based on the AIC, sample size (n) and total number of estimated parameters (K) was calculated for each model (*See equation 5*).

$$\mathrm{AICc} = \mathrm{AIC} + \frac{2K(K+1)}{n-K-1}$$

equation 5

There were a few instances that pivot sweep errors occurred which sometimes lead to under-reporting of the D*f*; this was corrected before any further calculations or analyses were done. It appears that these errors occur in small dataset with many correlated variables. Models with correlated variables or without a TU were not considered to be acceptable for predicting effects and were therefore omitted from further analyses.

In order to get the best approximate model the AICc difference was calculated by subtracting the AICc values from the lowest AICc value (the posited best model). The rough rule of thumb states that those models that had a difference of less than or equal to two can also be considered as the best approximating models. Those models with a difference between two and four have less proof that they are probably the best, while there is no evidence to support models with a difference of ten or greater as plausible.

Akaike's weights ratio were also calculated since it is credited as a better means of deciding whether the proposed best model truly is the best model from the list of candidate models. First Akaike's weights (w_i) calculated for each model based on the AICc difference (AICc) and the total Δ AICc for all models (*See equation 6*).

$$w_i = \frac{\exp\left(-\frac{1}{2}\Delta i\right)}{\sum_{r=1}^{R} \exp\left(-\frac{1}{2}\Delta i\right)}$$

equation 6

Then the Akaike's weight ratio for each model is attained by simply dividing the highest weight (proposed best model) by the Akaike's weight of that model.

Those models that had an AICc difference of less than or equal to two, and models based solely on the toxicity units were created using linear regression in order to investigate their predictive strengths. Consequently, the adjusted coefficient of determination (R^2) was noted, since it takes into account the number of variables and whether improvements they produce are more than would be expected by chance. In addition the statistical significance status (at the 0.05 level) of each predictor variable in the model was collected from the regression output data sheet.

Model validation was done by predicting impacts using the model equations of the best approximating models or any of particular relevance to analysis (mostly those that used only a TU explanatory variable) and the data from these validation sets. These predicted

values were then compared with the actual observed values by means of correlation analysis.

The first hypothesis states that empirically based models using laboratory toxicity data, fate properties and physico-chemical properties of pesticides can be constructed to predict real world effects. In order to ascertain whether this hypothesis can be accepted the statistical significance of the model was checked by looking at the p-values found within the output sheets from the AIC and linear regression.

The other hypotheses within this study are comparative in nature. Some were tested by examination of the AICc model selection, AICc difference and Akaike's weight ratio. Evaluation of models based on linear regressions was done through examination of the R^2s along with the statistical contribution of each model parameter.

Table for Chapter 2

Table 2. 1 - Correlations for 41 pesticides.

	log Kow	log Koc	LASB	LAAB	L WPHL	L HHL	L Volume	L TSA/V R
log Kow	1.0000	.3757	-.126	.055	-.3050	.024	.055	.049
	p= ---	*p=.009	p=.408	p=.720	*p=.050	p=.691	p=.464	p=.513
		N = 47	N = 45	N = 45	N = 42	N = 287	N = 233	N = 179
log Koc	.3757	1.0000	0.12	.2700	-.2717	.046	-.092	-.037
	*p=.009	p= ---	p=.418	p=.073	p=.082	p=.438	p=.220	p=.626
	N = 47		N = 45	N = 45	N = 42	N = 287	N = 233	N = 179
LASB	-.126	0.12	1.0000	.6376	.3822	.2575	-.255	.1950
	p=.408	p=.418	p= ---	*p=.000	*p=.014	*p=.000	*p=.000	*p=.010
	N = 45	N = 45		N = 45	N = 41	N = 278	N = 219	N = 174
LAAB	.055	.2700	.6376	1.0000	.2046	.5005	-.1244	-.0132
	p=.720	p=.073	*p=.000	p= ---	p=.199	*p=0.00	p=.066	p=.863
	N = 45	N = 45	N = 45		N = 41	N = 278	N = 219	N = 174
L WPHL	-.3050	-.2717	.3822	.2046	1.0000	-.0687	-.1137	.1086
	*p=.050	p=.082	*p=.014	p=.199	p= ---	p=.277	p=.147	p=.166
	N = 42	N = 42	N = 41	N = 41		N = 252	N = 164	N = 164
L HHL	.024	.046	.2575	.5005	-.0687	1.0000	.0463	-.0476
	p=.691	p=.438	*p=.000	*p=0.00	p=.277	p= ---	p=.486	p=.532
	N = 287	N = 287	N = 278	N = 278	N = 252		N = 228	N = 174
L Volume	.055	-.092	-.255	-.1244	-.1137	.0463	1.0000	-.8775
	p=.464	p=.220	*p=.000	p=.066	p=.147	p=.486	p= ---	*p=0.00
	N = 233	N = 233	N = 219	N = 219	N = 164	N = 228		N = 179
L TSA/V R	.049	-.037	.0195	-.0132	.1086	-.0476	-.8775	1.0000
	p=.513	p=.626	*p=.010	p=.863	p=.166	p=.532	*p=0.00	p= ---
	N = 179	N = 179	N = 174	N = 174	N = 164	N = 174	N = 179	

Key

LAAB – log-transformed aerobic aquatic biotransformation

LASB – log-transformed aerobic soil biotransformation

L HHL - log-transformed hydrolysis half-life

LTSA/V R – log-transformed total surface area to volume ratio

L Volume - log-transformed volume

L WPHL – log-transformed water photolysis half-life

log Koc – log-transformed organic carbon absorption coefficient

Log Kow - log-transformed octanol –water partition coefficient

* indicates statistical significance

CHAPTER 3 - CRUSTACEA

3.1 Modelling Crustacea Abundance Responses

The data used for these models reflect crustacea abundance responses to pesticide applications in lentic systems. Data were separated and modelled according to the following categories:-

1) crustacea species response
2) cladocera response
3) copepoda response

The explanatory variables entered into the AIC analyses included log-transformed toxic units based on the geometric means of *Daphnia* species (L TU Daphnia) and hazard concentration for 5% of crustacean species (L TU HC5 - C), this was done to test the hypothesis that hazard concentrations are better predictors of ecosystem effects than single species toxicity data. Log-transformed structural properties of system (volume – L volume and surface area to volume ratio - L TSA/V R) and all log-transformed fate and physico-chemical properties of the pesticide (octanol-water partition coefficient – log Kow, organic carbon absorption coefficient – log Koc, aerobic soil biotransformation - L ASB, aerobic aquatic biotransformation - L AAB, water photolysis half-life - L WPHL, hydrolysis half-life - LHHL) were initially entered into the AIC.

The dataset allowed modelling with two different pesticide groupings: 1) all pesticides combined (AP) and insecticides alone (IA). Modelling data for herbicides alone (HA)

was not possibly due to under-representation. Validation sets obtained were used to test the validity (by means of correlation analyses) of the best approximate and other models of interest, such as those based solely on the Toxic Units (TUs).

The magnitude of impact is assessed at 0.01 * TU Daphnia to enable comparison with the EU's Uniform Principles that use a multiplier factor of 0.01 on Daphnia L(E)C50 to derive a No Effect Concentration (NEC) for pesticides in surface water (Van den Brink *et al.* 2003; Brock *et al.* 2001). The impact is also assessed for 0.1 * TU HC5-C for best models that were based on this parameter and / or L TU HC5-C models. Risk managers usually use NOEC values for SSD extrapolations, however this research uses HC5 values based on geometric means for L(E)C50s. A safety factor of ten is deemed appropriate to extrapolate chronic data based on acute values when there is more than one test done (Chapman *et al.* 1997). In addition, Van den Brink *et al.* (2003) used a safety factor of ten in their calculation of NECs using HC5 values based on EC50 data, so their principle was adopted.

3.2 Modelling Crustacea Species Responses

These data report on 14 families in eight orders (*see figure 3.1*). About 65% of the species belong to the order cladocera, which was represented by seven families: bosminidae, chydoridae, daphniidae, holopediidae, ilyocryptidae, macrothricidae and sididae. Approximately 25% of the species came from the three families of the sub-class copepoda: cyclopidae, diaptomidae and *eudiaptomus graciloides* (a calanoida species).

The remaining ten percent were from the families - hyalellidae, asellidae, candonidae and moinidae.

Seventy-eight entries were available for modelling. They are drawn from 30 studies covering 21 pesticides – one fungicide (carbendazim); five herbicides (atrazine, glufosinate-ammonium, hexazinone, metribuzin, metsulfuron methyl) and 15 insecticides (carbaryl, carbofuran, chlorpyrifos, cyfluthrin, cypermethrin, diflubenzuron, esfenvalerate, fenitrothion, lindane, methyl parathion, permethrin, phorate, pyridaben, tebufenozide, temephos).

The dependent variable is a count ratio of effect, derived by dividing the number of statistically significantly affected crustacea species (increases and decreases in abundance) by the total number crustacea species recorded for that experiment (*see equation 4 in 2.3*). Four experiments within the data used for modelling had at least one species that statistically significantly increased when compared to the concurrent control; two were from permethrin studies and the other two from tebufenozide.

The validation set consists of one fungicide (carbendazim); five herbicides (glufosinate-ammonium, hexazinone, linuron, metribuzin, metsulfuron methyl); and 14 insecticides (azinphos-methyl, carbofuran, chlorpyrifos, cypermethrin, diflubenzuron, esfenvalerate, fenitrothion, lindane, methyl parathion, permethrin, phorate, pyridaben, tebufenozide, temephos).

3.2.1 Model based on All Pesticide Data

Of 96 possible model combinations consisting of a TU and uncorrelated variables, 14 are considered as the best approximating models based on AIC difference of less than two (*See table 3.1*). All of these models are statistically significant, can be statistically validated using the independent dataset and account for 46 to 50 percent of the variance in crustacea overall species responses.

The AIC scores revealed that models based solely on the TUs were statistically significant, however there is not enough evidence supporting them as being the best to explain the effects seen. The L TU Daphnia model has an adjusted R^2 of 0.45 (*see equation 7*) and the L TU HC5-C model has an adjusted R^2 of 0.45 (*see equation 8*).

L Daphnia TU

$$\textit{Count Ratio of Effect} = [0.7129 + (0.141 * \text{L TU Daphnia})]$$

Equation 7

L TU HC5-C

$$\textit{Count Ratio of Effect} = [0.4751 + (0.142 * \text{L TU HC5-C})]$$

equation 8

Eleven of the 13 best approximating models contain the Daphnia TU and the other two are based HC5-C TU. More than half of these 13 models have L HHL as a predictor variable and many of the others contain LAAB. An increase in either of these variables is predicted to reduce the effects seen on crustacean species abundance.

Eight of the models, proposed by AIC to be the possible best, have L Volume as a predictor variable; while an additional model has the negatively correlated L TSA/V R. However, it must be noted that, with the exception of one model, these variables were not statistically significant contributors to their respective models. Systems of smaller volumes and larger surface area to volume ratio are expected to show more effects. L WPHL, log Kow and log Koc are not considered to be statistically significant contributors to their respective models below p=0.05.

The results indicate that Daphnia TU is better at predicting effects of pesticides on crustacean species than HC5-C TU and HHL is the most important fate parameter for model prediction using this dataset. The L TU Daphnia & L HHL (adjusted $R^2 = 0.48$, *see equation 9, see figure 3.2*) model has both predictor variables as being statistically significant factors below p=0.05; and it is the most parsimonious among the best approximating 14 models. The results seem to suggest that this model is the best for the data.

L TU Daphnia & L HHL

Count Ratio of Effect = [0.9075 + (0.1250 * L TU Daphnia) + (-0.0883 * L HHL)]

equation 9

3.2.2 Models based on All Pesticides Data from Fish-Free Systems

To check whether fish within a system influences the results and therefore the predictive ability of the models, a separate AIC table was generated using the previously outlined

criteria with data from fish-free systems only. This data sample contains 58 entries from 19 studies covering 16 pesticides – one fungicide (carbendazim), four herbicides (glufosinate-ammonium, hexazinone, metribuzin, metsulfuron methyl) and 11 insecticides (carbaryl, carbofuran, chlorpyrifos, cypermethrin, diflubenzuron, fenthion, lindane, permethrin, phorate, tebufenozide, temephos). The validation set consists of carbendazim; four herbicides (glufosinate-ammonium, hexazinone, metribuzin, metsulfuron methyl) and eight insecticides (carbofuran, chlorpyrifos, cypermethrin, lindane, permethrin, phorate, tebufenozide, temephos).

Of the 95 possible models with a TU and uncorrelated variables, five are indicated by the AICc difference being best approximating models (*see table 3.2*). All five models are statistically significant, can be statistically validated using the independent dataset and account for 55 – 57% of the variance seen in the species effects, suggesting that, indeed, predictability of effects is higher when without fish as a confounding factor. The L Daphnia TU (adjusted $R^2 = 0.55$, *see equation 10, see figure 3.3*) model was among the five; however the model based on HC5 – C TU alone (adjusted $R^2 = 0.51$, *see equation 11*) showed less evidence of being the best for this data.

L TU Daphnia

$$\textit{Count Ratio of Effect} = [0.7788 + (0.1579 * \text{L TU Daphnia})]$$

equation 10

L TU HC5-C

$$\textit{Count Ratio of Effect} = [0.5091 + (0.11504 * \text{L TU HC5-C})]$$

equation 11

The L TU Daphnia model is a strong competitor for best approximating model based on the principle of parsimony and minute model improvements in predictive power received through the addition of other variables. In fact, the only statistically significant variable below p=0.05 level for these best approximating models is L TU Daphnia. L HHL, though not statistically significant, is present in 3/5 of the most probable models. However, the improvement it brings in comparison to the L TU Daphnia model is minimal, the difference in adjusted R^2 ranges from 0.01 to 0.02. As hydrolysis half-life decreases the effects seen is expected to increase.

The results indicate that Daphnia TU is better to use when predicting the effects of pesticides from lentic fish-free systems and HHL may be an important fate parameter for modelling. The L TU Daphnia & HHL (adjusted R^2 = 0.57, *see equation 12, see figure 3.4*) can vie as the best model for these data because it contains both variables that appear to be important for prediction, in addition to being the best model as selected by the AIC scores.

L TU Daphnia & HHL

Count Ratio of Effect = [0.9328 + (0.1406 * L TU Daphnia) + (-0.0756 * L HHL)]

equation 12

3.2.3 Models based on Insecticides Data

When the insecticide data were modelled alone, of the 96 possible models without correlated variables and a TU, only two are considered by AICc difference to be the best

approximating models for the data (*see table 3.3*). Both models are statistically significant, can be validated using an independent dataset, could account for 48% of the variance of overall species system effects and are based on Daphnia TU. This predictive strength is comparable to the models generated with all pesticide data, but less than the best approximating models based on all pesticide data but came from fish-free systems.

Models based on either Daphnia or HC5 – C TUs alone are not considered to have enough evidence supporting them as the best approximating models for these data (adjusted R^2s = 0.22; *see equations 13 and 14*).

L TU Daphnia

Count Ratio of Effect = [0.7065 + (0.1100 * L TU Daphnia)]

equation 13

L TU HC5-C

Count Ratio of Effect = [0.3986 + (0.1639 * L TU HC5-C)]

equation 14

The next best six models were included in the analyses to enable examination of some possible trends. Firstly, all of these models contain L HHL as a statistically significant contributing factor and as HHL increases, less crustacea species are predicted to be affected. Secondly, three-quarters have either Kow or its positively correlated Koc counterpart. Increases in either of these two coefficients are predicted to induce more effects. Thirdly, half of these models also include a structural property of the system,

though it is not a statistically significant contributor and did not improve model prediction by much, if at all.

The results indicate that Daphnia TU better predicts the proportion of crustacea species affected by insecticide exposure than HC5-C TU. In addition, it appears that HHL and Kow enable the generation of stronger predictive models. L TU Daphnia, log Kow & L HHL (adjusted $R^2 = 0.48$, *see equation 15, see figure 3.5*) is very likely the best model for predicting effects of insecticides on crustacea species in lentic systems. This model is supported by 1) the principle of parsimony, 2) no variation between the coefficients of determination of the two best approximate models, and 3) its selection by AIC to be the best.

L TU Daphnia, log Kow & L HHL

$$\textit{Count Ratio of Effect} = [0.7022 + (0.0998 * \text{L TU Daphnia}) + (0.0750 * \log \text{Kow}) + (-0.1704 * \text{L HHL})]$$

equation 15

3.2.4 Models based on Insecticides Data from Fish-Free Systems

Modelling only insecticide data was done using entries that come solely from fish free systems. The AICc difference indicated that of the 96 models possible that have a TU and no correlating variables, four compete as being the best for these data (*see table 3.4*). All of these models are statistically significant, could explain 64 – 68% of the variance on the species effect and are based on Daphnia TU. The predictive strength of these models is much better than their counterparts based on data from both fish-present and fish-free

systems combined. Three of the four models can be statistically validated with an independent dataset.

L TU Daphnia (adjusted R^2s = 0.37; *see equation 16*) or L TU HC5 – C (adjusted R^2s = 0.29; *see equation 17*) models are not considered to have enough evidence to support that they are the best for these data.

L TU Daphnia

Count Ratio of Effect = [0.7651 + (0.1287 * L TU Daphnia)]

equation 16

L TU HC5-C

Count Ratio of Effect = [0.3734 + (0.1932 * L TU HC5-C)]

equation 17

Hydrolysis half-life is included in all four models and it is a statistically significant contributor in each. Three of the models also contain WPHL as a predictor variable, while one had Kow and three included Koc. The two physico-chemical properties are not statistically significant contributors to their respective models, however WPHL is statistically significant in one of the three best approximating models in which it is included. Shorter hydrolysis and water photolysis half-lives are expected to increase the proportion of crustacea species affected. While high Koc or Kow values are expected to facilitate increased effects.

The results indicate Daphnia TU better predicts the proportion of crustacea species affected by insecticide exposure in fish-free systems than HC5-C TU. In addition, the fate parameters – HHL and WPHL – appear to be important for modelling. The L TU Daphnia, L WPHL & L HHL model (adjusted $R^2 = 0.65$; *see equation 18*; *see figure 3.6*) can be considered the best for this data since it is parsimonious and can be validated using an independent data set, in addition to being the only best approximate model with all its variables as statistically significant contributors.

L TU Daphnia, L WPHL & L HHL

Count Ratio of Effect = [1.0436 + (0.1035 * L TU Daphnia) + (-0.0580 * L WPHL) + (-0.1301 * L HHL)]

equation 15

3.2.5 Summary of Crustacea Species Modelling Results

The best crustacea species count ratio of effect models are able to account for between 48 to 65 percent of the variance observed in the proportion of species affected by pesticide exposure. The model with the strongest predictive ability is from insecticide data that came from fish-free systems.

Overall Daphnia TU is the better predictor of crustacea species results and best and TU alone models from fish-free systems have stronger predictive abilities compared to their counterparts derived from data that came from combined entries on fish present and fish-free experiments. Fish-free data produced better best approximating models for

insecticides than all pesticides together; however the best approximating model from fish-present and fish-free systems data had the same predictive strength for insecticides only and all pesticides together.

Hydrolysis half-life is the single most important fate parameter for modelling proportion of crustacea species affected. Kow or water photolysis half-life was included to better explain responses observed in systems treated with insecticides.

Given that the most predictive models came from the fish-free insecticide data, new models using all available data (to maximise sample size) were generated using the variable of the best models, in order to estimate the proportion of crustacea species that will be affected at 0.01 * TU Daphnia. These new models included five additional insecticides (bendiocarb, carbaryl, cyfluthrin, deltamethrin, fenthion) for modelling, thereby producing a model based on 16 insecticides.

Even though the best approximating models for insecticide models based on fish-free data seem to indicate L WPHL is a statistically significant contributor to model prediction; modelling with the much larger data set (N=49 vs. N = 24, and 11 insecticides vs. 16) revealed that this fate parameter was not statistically significant. Instead, the best model proved to be L TU Daphnia, log Kow and L HHL (adjusted R^2 = 0.55; *see equation 16, see figure 3.7*), which had all of its variables considered as statistically significant contributors.

L TU Daphnia, log Kow & L HHL

Count Ratio of Effect = [0.7390 + (0.1165 * L TU Daphnia) + (0.0631 * log Kow) + (-0.1685 * L HHL)]

equation 16

Low and high values or both low or high, or average values were paired for model input. The log Kow values used are 1.52, 6.6 and 4.06; and the L HHL values used are -0.17, 3.26 and 1.55. The only conditions under which the 0.01 * TU Daphnia threshold can be protective is hydrophilic (or intermediate hydrophobic) insecticides that are extremely resistant to hydrolysis (5% of species are predictive to be affected). However, if an insecticide contains the reverse characteristics (hydrophobic with a short HHL) then all crustacea species are predicted to be affected. The model also predicts an insecticide of intermediate hydrophobicity and a HHL of 16 hours can cause 63% of the crustacea species to be affected at 0.01 * TU Daphnia, while a highly hydrophobic insecticide that is resistant to hydrolysis can lead to 37% of the species being affected. An insecticide that is hydrophobic with a HHL of 35.5 days is predicted to affect 50.25% of the crustacea species within a system.

*These results clearly show that a threshold level of 0.01 * TU Daphnia would not be protective enough of crustacea species in the aquatic environment against most insecticides.*

3.3 Modelling Cladocera Abundance Responses

The abundance responses used for these analyses were those that were reported by the individual study authors at the order level (cladocera). The dependent variable was a log-transformed abundance change ratio (LAR) calculated by dividing the control by the post-treatment numbers to reflect decreases or assigning a fix value of one to the no effects.

The data set consists of 56 entries from 21 studies reporting on 20 pesticides – the fungicide (carbendazim); four herbicides (atrazine, glufosinate-ammonium, hexazinone, metsulfuron-methyl) and 15 insecticides (azinphos-methyl, carbaryl, chlorpyrifos, cypermethrin, deltamethrin, diflubenzuron, esfenvalerate, fenvalerate, lambda-cyhalothrin, lindane, methoxychlor, permethrin, pyridaben, tebufenozide, temephos). Data were modelled from all pesticides combined (AP) and insecticides alone (IA).

The validation set is based on the herbicides hexazinone and metsulfuron, along with the insecticides azinphos-methyl, chlorpyrifos, cypermethrin, deltamethrin, diflubenzuron, esfenvalerate, fenvalerate, lambda-cyhalothrin, lindane, methoxychlor, permethrin, pyridaben, tebufenozide and temephos; and the fungicide carbendazim.

3.3.1 Models based on All Pesticides Data

Of the 95 possible models with a TU and uncorrelated variables, ten have an AICc difference of less than two, thereby having enough evidence to support the claim of being the best approximating models (*see table 3.5*). All ten models are statistically significant,

contain HC-5 TU, and were statistically validated using the independent dataset. The models account for 31 – 34% of the variance seen in the cladocera effects.

The AIC scores show that there is less evidence to support L TU Daphnia (adjusted R^2 = 0.19, *see equation 17*) or L TU HC5-C (adjusted R^2 = 0.25, *see equation 18*) being considered as a best approximating model; however these models are statistically significant.

L TU Daphnia

$$LAR = [1.3888 + (0.3277 * \text{L TU Daphnia})]$$

equation 17

L TU HC5-C

$$LAR = [0.4751 + (0.142 * \text{L TU HC5-C})]$$

equation 18

Four of the ten best approximate models contain WPHL and it is statistically significant in three. Kow is a statistically significant factor in all of the three models that it was included in; however Koc is not considered a statistically significant factor in any of its respective models. The models indicate that as WPHL increases so will the effect seen on cladocera; while fewer effects will be seen as Kow increases.

Structural property of the system - volume and TSA/V R - , AAB, and HHL, though present in many of the models, were not considered to be statistically significant contributors to model prediction.

The results show that HC5-C TU is better than Daphnia TU and Kow is an important physico-chemical property for predicting effects on cladocera. WPHL and AAB appear in many of the best approximate models however their contribution is minimal. The only model that has all of its variables considered as statistically significant contributors is L TU HC5-C and log Kow. So given this factor and the small range in predictive power of the models, this model can be considered the best for the data set. The adjusted R^2 for this model is 0.33 and the actual model equation is as follows (*See figure 3.8*):

$$LAR = [1.1711 + (0.5310 * \text{L TU HC5-C}) + (-0.1406 * \log \text{Kow})]$$

equation 19

3.3.2 Models based on All Pesticides Data from Fish-Free Systems

Data from fish-free systems were modelled in order to investigate whether having fish within the system influences the results and therefore the predictive ability of the models. The AIC analysis was done using the previously outlined criteria, except this time only with data from fish-free systems. This data sample contains 43 entries from 14 studies covering 13 pesticides – carbendazim, three herbicides (glufosinate-ammonium, hexazinone, metsulfuron methyl) and nine insecticides (carbaryl, chlorpyrifos,

cypermethrin, diflubenzuron, esfenvalerate, fenvalerate, lambda-cyhalothrin, methoxychlor, permethrin).

The validation set is made up of two herbicides (hexazinone, metsulfuron methyl) and ten insecticides (chlorpyrifos, cypermethrin, deltamethrin, esfenvalerate, fenvalerate, lambda-cyhalothrin, lindane, methoxychlor, permethrin, temephos).

Of the 96 possible models with a TU and uncorrelated variables, five are indicated by the AICc difference as being the best approximating models (*see table 3.6*). All five models are statistically significant and statistically validated using the independent dataset. They are able to explain 48 to 51% of the variance seen in the changes of cladocera, clearly higher than the performance obtained with all studies combined.

Even though the L TU HC5-C model (adjusted $R^2 = 0.41$, *see equation 20*) and L TU Daphnia (adjusted $R^2 = 0.32$, *see equation 21*) models are statistically significant and can be validated with an independent dataset, they were not considered as being among the best approximating models by the AICc scores.

L TU HC5-C

$$LAR = [0.4751 + (0.142 * \text{L TU HC5-C})]$$

equation 20

L TU Daphnia

$$LAR = [1.5537 + (0.3810 * \text{L TU Daphnia})]$$

equation 21

All five models are based on TU HC5-C and four have Kow, which is a statistically significant contributing factor for three of the four models. Two of the five models include AAB, but it is only statistically significant for one. As Kow or AAB increase, less cladocera effects are expected. The best approximating model that includes both Kow and AAB as predictor variables, indicate that neither of them is a statistically significant contributing factor. While volume and its statistically negatively correlated counterpart TSA/V R are considered as insignificant contributors to their respective models.

The results indicate that the HC5-C TU is better than the Daphnia TU for modelling cladocera responses to pesticide treatments. In addition, hydrophobicity is an important fate variable; hydrophilic pesticides are expected to reduce cladocera populations. L TU HC5-C & log Kow (adjusted $R^2 = 0.51$, *see equation 22, see figure 3.9*) model, which has both variables as statistically significant contributors, is considered to be the best by AICs scores for this dataset (all pesticides from systems without fish).

L TU HC5-C & log Kow

$$LAR = [1.1662 + (0.6063 * \text{L TU HC5-C}) + (-0.1356 * \log \text{Kow})]$$

equation 22

3.3.3 Models based on Insecticides Data

Modelling insecticide data separately produced 96 models with a TU and no correlated variables, five of which are considered by AICc difference as having enough evidence to

be the best approximating model for the data (*see table 3.7*). These models explain between 25 to 27 percent of the variance seen in cladocera effects, which is less predictive than models based on grouping all pesticide data together. Even though these models were statistically significant, none could be validated using the independent dataset - the predicted results failed to show any correlations with the observed study impacts. This may have occurred partially due to small sample size.

The L TU Daphnia model (adjusted $R^2 = 0.09$) provides a really poor fit for cladocera abundance response to insecticide exposure, while the L TU HC5-C model (adjusted $R^2 = 0.15$, *see equation 23*) though statistically significant did not have enough evidence to support it being considered as one of the best approximating models.

L TU HC5-C

$$LAR = [0.4751 + (0.142 * \text{L TU HC5-C})]$$

equation 23

All five of these models are based on HC5-C TU and had WPHL; the latter is a statistically significant contributor in four of the five models. As photolysis half-life increases, the effects on cladocera seen are expected to increase. However, volume, TSA/V R, Koc and HHL while all being included in at least one of the best approximating models were considered to be insignificant contributors.

The results indicate that the HC5-C TU is better than the Daphnia TU and WPHL is an important fate variable for predicting reductions in cladocera abundance caused by

insecticides. The only model that has all of its variables considered as statistically significant contributors is L TU HC5-C & L WPHL (adjusted $R^2 = 0.27$, *see equation 24, see figure 3.10*), which was selected by the AICc as the most likely best model. There is no strong evidence against this model being considered the best for this data set.

L TU HC5-C & L WPHL

$$LAR = [-0.1494 + (0.6322 * \text{L TU HC5-C}) + (0.4103 * \text{L WPHL})]$$

equation 24

3.3.4 Models based on Insecticides Data from Fish-Free Systems

Modelling insecticide data was done using entries that came only from fish free systems. The AICc difference indicates that of the 96 models possible that have a TU and no correlating variables, eight can be considered as the best for these data (*see table 3.8*). All of these models are statistically significant and they can explain 42 – 49% of the variance in cladocera abundance response. This is twice as better as the models generated based on insecticide data from both fish-free and fish-present systems.

Four models were statistically validated with an independent dataset, regardless of the number of the validation sample: only data from that contained entries that had all of its fate, physico-chemical, respective TUs and system structural properties, and a data set that was only modified according to the parameters present in the model.

Half of the eight best approximating models are based on the HC5-C TU and the other half Daphnia TU. The model that used HC5-C TU as the sole predictor was among these eight models, and was selected by the AICc difference as the most likely model; however this model could not be validated with an independent data set.

L TU Daphnia model (adjusted $R^2 = 0.25$, *see equation 25*) while statistically significant, was not considered as having enough evidence to be among the best approximating models. However, the L TU HC5-C (adjusted $R^2 = 0.44$, *see equation 26, see figure 3.11*) model based on the principle of parsimony can be considered as one of the best models for predicting cladocera responses to insecticide exposure.

L TU Daphnia

$$LAR = [1.4904 + (0.42 * \text{L TU Daphnia})]$$

equation 25

L TU HC5-C

$$LAR = [0.1512 + (0.7471 * \text{L TU HC5-C})]$$

equation 26

WPHL is present in five models and Kow in four. Koc, TSA/V R and volume were present in at least one of the eight best approximating models, however none is considered as statistically significant contributors. According to the regression equations, as WPHL increases, fewer reductions should be seen in cladocera abundance. This prediction is not only counter-intuitive, but also the opposite of what is expected when WPHL when the model was generated based on insecticides only but from both fish-present and fish-free systems.

The results do not clearly indicate which of the two TUs – Daphnia or HC5-C – is better, however WPHL seems to be an important fate parameter in predicting effects on cladocera abundance. As previously mentioned, the influence of WPHL (like that of other loss rates in crustacea species analyses) seems counter-intuitive; the shorter half-lives contribute to reduced cladocera abundance. In addition to having both of its predictor variables as statistically significant contributors, L TU Daphnia & L WPHL (adjusted R^2 = 0.44, *see equation 27, se figure 3.12*) was the only model to be statistically validated with the both independent data sets; and can be considered as one of the best models for predicting cladocera abundance response to insecticide exposure.

L TU Daphnia & L WPHL

$$LAR = [2.1802 + (0.5739 * \text{L TU Daphnia}) + (-0.6884 * \text{L WPHL})]$$

equation 27

3.3.5 Summary of Cladocera Modelling Results

Best models for cladocera data can account for 27 to 51 percent of variance caused by pesticide exposure and the model with the greatest predictive power is based on all pesticides from fish-free systems. The least predictive best model is based on insecticide data that came from both fish-present and fish-free systems, and the better models came from fish-free systems. Generally, models were more predictive when all pesticide data on cladocera were pooled together. Overall HC5-C TU is the better TU predictor variable for responses of cladocera abundance to pesticide exposure.

In order to estimate the impacts a concentration of 0.1 * TU HC5-C or 0.01 TU Daphnia would have on cladocera populations, the best fish-free models were generated with all available data from fish-free systems. Both insecticides alone and all pesticides models were done because cladocera is sensitive to the former group (Brook *et al.* 2000, Mohlenberg *et al.* 2001), but it was the latter group that had the strongest predictive model.

Upon modelling the best model based on all pesticides it was found that the Kow which was statistically significant with the more constrained dataset (N = 28), was no longer a statistically significant contributor, and the model predictive strength plummeted to an adjusted R^2 of 0.35. The L TU HC5-C & L WPHL (adjusted R^2 = 0.55, *see equation 28, see figure 3.13*) model based on fish-free insecticide data gave a higher predictive power than its Daphnia counterpart, and the L WPHL which was not considered a statistically significant contributor to the model prediction under the constrained dataset (N = 20), was now considered as statistically significant in the expanded dataset (N= 48). Consequently, this model was selected to estimate the expected impacts of insecticides on cladocera abundance at 0.1 * TU HC5-C using the lowest (-1.9031), highest (3.0394) and average (1.0680) values for L WPHL based on the database.

L TU HC5-C & L WPHL

$$LAR = [0.7474 + (0.5507 * \text{L TU HC5-C}) + (-0.4219 * \text{L WPHL})]$$

equation 28

The results show that insecticides that have a water photolysis half life of more than 12 days will not decrease cladocera abundance at concentrations that are 0.1 * TU HC5-C. However those pesticides that undergo rapid water photolysis degradation (e.g. 7 hours) can cause a 74% reduction in abundance. If a threshold of 1 * TU HC5-C was used to protect cladocera, it would prove to be inadequate since about 50% loss is expected with an insecticide with a water photolysis half-life of 12 days. But, insecticides that are resistant to water photolysis break down are not predicted to have an impact.

*These results indicate that a threshold level of 1 * TU HC5-C would not be protective but 0.1 * TU HC5-C would be protective of the aquatic environment against insecticides that are not resistant to water photolysis.*

3.4 Modelling Copepoda Abundance Responses

The abundance responses used for these analyses were those reported by the individual study authors at the sub-class level (copepoda). The dependent variable was a log-transformed abundance change ratio (LAR) which was calculated by dividing the control numbers by the post-treatment numbers to reflect decreases and a fix value of one was assigned to the no effects.

The 66 entries available for modelling were collected from 22 studies and reported on 20 pesticides – the fungicide (carbendazim), four herbicides (atrazine, glufosinate-ammonium, hexazinone, metsulfuron-methyl) and 15 insecticides (carbaryl, chlorpyrifos, cypermethrin, deltamethrin, diflubenzuron, esfenvalerate, fenthion, fenvalerate, lambda-

cyhalothrin, lindane, methoxychlor, methyl-parathion, pyridaben, tebufenozide, temephos). The validation set consists of entries from the herbicides metsulfuron-methyl, hexazinone and metsulfuron; the insecticides chlorpyrifos, cypermethrin, deltamethrin, diflubenzuron, lambda-cyhalothrin, lindane, methoxychlor, methyl parathion, pyridaben, tebufenozide and temephos; and the fungicide carbendazim.

3.4.1 Models based on All Pesticides Data

Of the 96 possible models with a TU and uncorrelated variables, three can be considered as the best approximating models based on AICc difference of less than two (*see table 3.9*). All three models are statistically significant and can be statistically validated using the independent dataset. The models are able to explain 36 to 38% of the variance seen in the changes of copepoda abundance.

The first two less approximating model will be included in the analysis because they provide further evidence to support trends seen in the best approximating models. Neither the L TU HC5-C (adjusted $R^2 = 0.20$; *see equation 29*) nor L TU Daphnia (adjusted $R^2 = 0.14$, *see equation 30*) model could best predict the responses observed in copepoda upon exposure to pesticides.

L TU HC5-C

$$LAR = [0.4751 + (0.142 * \text{L TU HC5-C})]$$

equation 29

L TU Daphnia

$$LAR = [0.9290 + (0.19797 * \text{L TU Daphnia})]$$

equation 30

All the models in consideration – best approximating and first two less approximating – have HC5-C as the predictor TU; and include Kow as a statistically significant contributing factor. More hydrophilic pesticides are predicted to have fewer effects on copepoda populations.

Four of the five models in consideration include a structural property; three contain volume and one TSA/V R. The former variable is a statistically significant contributor to its respective models, however the latter is not. Increased volumes or decreased TSA/V R are expected to contribute to fewer effects. Neither HHL nor AAB is a statistically significant contributor.

The results suggest that HC5-C is the better TU predictor and that Kow is an important fate parameter when predicting the effects of pesticides on copepoda abundance. The volume of the system is also essential for such predictive purposes. L Volume, L TU HC5-C and log Kow (adjusted $R^2 = 0.38$, *see equation 31, see equation 3.14*) was the only best approximating model with all of its predictor variables being considered statistically significant contributors below p=0.05; and since there is no strong evidence to suggest that there is a better model for the data, it can be considered as the best.

L Volume, L TU HC5-C and log Kow

$$LAR = [1.2398 + (-0.2370 * \text{L Volume}) + (0.5310 * \text{L TU HC5-C}) + (-0.1406 * \log \text{Kow})]$$

equation 31

3.4.2 Models based on All Pesticides Data from Fish-Free Systems

To check whether fish within the system influenced the results and therefore the predictive ability of the models, a separate AIC table was generated with data from fish-free systems. This data sample contains 50 entries from 15 studies covering 15 pesticides – the fungicide (carbendazim); three herbicides (glufosinate-ammonium, hexazinone, metsulfuron methyl) and 11 insecticides (carbaryl, chlorpyrifos, cypermethrin, deltamethrin, esfenvalerate, fenthion, fenvalerate, lambda-cyhalothrin, lindane, methoxychlor, temephos).

The validation set consists of three herbicides (glufosinate-ammonium, hexazinone, metsulfuron methyl); eight insecticides (chlorpyrifos, cypermethrin, deltamethrin, esfenvalerate, lambda-cyhalothrin, lindane, methoxychlor, temephos); and the fungicide carbendazim.

Ninety-six models with a TU and uncorrelated variables were possible, six of which have an AICc difference of less than two (*see table 3.10*). All six models are statistically significant and were statistically validated using the independent dataset. These best

approximating models are able to explain 42 to 53% of the variance seen in the changes in copepoda abundance. Overall, this is slightly better predictive strengths than the models for all pesticides based on data from both fish-present and fish-free systems.

The L TU HC5-C (adjusted $R^2 = 0.25$, *see equation 32*) and L TU Daphnia TU (adjusted $R^2 = 0.31$, *see equation 33*) models were not considered as being among the best approximating models by the AICc scores.

L TU HC5-C

$$LAR = [0.4751 + (0.142 * \text{L TU HC5-C})]$$

equation 32

L TU Daphnia TU -

$$LAR = [1.1472 + (0.2519 * \text{L TU Daphnia})]$$

equation 33

All five best approximating models include TU HC5-C and Kow as statistically significant contributing factors. AAB, ASB, volume and TSA/V R though included in at least one of the best approximating models are not statistically significant contributing factors to their respective models. Greater copepoda reductions are expected with more hydrophilic pesticides.

The results indicate that the HC5-C TU is better than the Daphnia TU and Kow is an important fate variable for predicting copepoda responses to pesticide treatments. The L TU HC5-C & log Kow model (adjusted R^2 = 0.43, *see equation 34, see figure 3.15*), which was selected by the AIC scores as being the best model for these data, is the only best approximating model with all of its variables as statistically significant contributors. Given this and the close predictive abilities of the other models, this model can be considered at the best model for the data.

L TU HC5-C & log Kow

$$LAR = [1.0675 + (0.4541 * \text{L TU HC5-C}) + (-0.1425 * \log \text{Kow})]$$

equation 34

3.4.3 Models based on Insecticides Data

Modelling insecticide data separately produced 96 models without correlated variables and a TU; five are considered by AICc difference as having enough evidence to be the best approximating model for the data (*see table 3.11*). All five models are statistically significant and were statistically validated using the independent dataset. These best approximate models explain between 36 to 42 percent of the variance seen in copepoda responses, this level of predictive strength is similar to that of models based all pesticides from both fish-free and fish-present systems.

Though statistically significant, the L TU HC5-C (adjusted $R^2 = 0.26$, *see equation 35*) or the L TU Daphnia (adjusted $R^2 = 0.13$, *see equation 36*) model is not among the best approximating models for predicting copepoda abundance responses to insecticide exposure.

L TU HC5-C

$$LAR = [0.4751 + (0.142 * \text{L TU HC5-C})]$$

equation 35

L TU Daphnia

$$LAR = [0.8649 + (0.2337 * \text{L TU Daphnia})]$$

equation 36

All five models are based on the HC5-C TU and have a structural system property as a predictive variable. Three models include volume and the other two have TSA/V R; both parameters are statistically significant contributors to their respective models. More abundance reductions are expected when TSA/V R increases or volume decreases.

WPHL is included in four of the best approximating models; however it is only statistically significant in two models. It should be noted that these four models all have an adjusted coefficient of determination of 0.42 and the sole model without WPHL has a six percent points decrease in model predictability. More copepoda reductions are expected with increased WPHL. Two models contain Koc, however it is not a statistically significant contributor to either of the model.

The results show that the HC5-C is a better predictor than Daphnia TU, and WPHL is an important fate parameter for modelling. The structural property of the system appears to influence insecticide toxicity. In light of these results the L TSA/V R, TU L HC5-C & L WPHL (adjusted $R^2 = 0.42$, *see equation 37, see figure 3.16*) and L Volume L HC5-C & L WPHL (adjusted $R^2 = 0.42$, *see equation 38, see figure 3.17*) models can vie to be the best for the dataset.

L TSA/V R, TU L HC5-C & L WPHL

$$LAR = [-0.5340 + (0.9487 * \text{L TSA/V R}) + (0.3805 * \text{L TU HC5-C}) + (0.1976 * \text{L WPHL})]$$

equation 37

L Volume, L HC5-C & L WPHL

$$LAR = [0.2214 + (-0.2937 * \text{L Volume}) + (0.4141 * \text{L TU HC5-C}) + (0.1894 * \text{L WPHL})]$$

equation 38

3.4.4 Models based on Insecticides Data from Fish-Free Systems

Like, with all pesticide data, modelling only insecticide data was also done using entries that come from fish free systems. The AICc difference indicated that of the 96 models possible that have a TU and no correlating variables, four contend as being the best for this data (*see table 3.12*). All of these models were statistically significant, however only three could be validated with an independent dataset. These four best approximating models can explain 62 – 64% of the variance in copepoda responses to insecticide

exposure, which is clearly better than the models based on insecticide data from both fish-free and fish-present systems and all pesticides grouped together.

The L TU HC5-C (adjusted $R^2 = 0.40$, *see equation 39*) and L TU Daphnia TU (adjusted $R^2 = 0.24$, *see equation 40*) models are not considered to be among the best approximating models by the AICc scores.

L TU HC5-C

$$LAR = [0.4751 + (0.142 * \text{L TU HC5-C})]$$

equation 39

L TU Daphnia TU

$$LAR = [1.1007 + (0.2649 * \text{L TU Daphnia})]$$

equation 40

All four models are based on HC5-C TU and include AAB as a statistically significant contributing factor. More reductions in copepoda abundance are expected with longer AAB half-lives. WPHL, volume and TSA/V R are considered statistically insignificant contributors to their respective models.

The results from these analyses reveal that HC5-C TU is a better predictor for effects on copepoda abundance. It also appears that AAB is an important fate parameter when predicting the effects that insecticides from fish free systems would have on copepoda

abundance. The evidence seems to support the L TU HC5-C & L AAB (adjusted R^2 = 0.63, *see equation 41, see figure 3.18*) model being the best approximate for this data.

L TU HC5-C & L AAB

$$LAR = [-2.2353 + (0.6638 * \text{L TU HC5-C}) + (1.3257 * \text{L AAB})]$$

equation 41

3.4.5 Summary of Copepoda Modelling Results

The best models for copepoda data can account for 38 to 63 percent of variance observed in the experiments. Similar to crustacea species, the best model with the highest predictive power originated from insecticides from fish-free systems. The least predictive model is based on all pesticides from fish-present and fish-free systems. In general, the better models came from fish-free systems and models were more predictive when data came strictly from insecticide treatments.

The HC5-C TU is the better predictor TU variable for responses of copepoda abundance to pesticide exposure. Best models based on all pesticides included Kow, and when these data came from combined both fish-present and fish-free entries, systems volume proved to be an important variable. For the best insecticide models based on data that combined entries from systems both with and without fish, water photolysis coupled with surface area to volume ratio or volume, are best. But for insecticide models based on fish-free systems aquatic aerobic biotransformation is the only other variable required for the best model.

To estimate the likely impacts of 0.1 * TU HC5-C on copepod abundance, the best model was selected – L TU HC5-C & L AAB from fish-free insecticide data and remodelled using all available fish-free insecticide data, partially because of small sample size. The new L TU HC5-C & L AAB (adjusted $R^2 = 0.62$; *see equation 42, see figure 3.19*) model had two additional insecticides (azinphos-methyl; methyl-parathion) and twice as many entries (N = 44). The lowest, highest and average values for L AAB (0.56, 2.31, 1.43 respectively) based on the database were placed into the model equation.

L TU HC5-C & L AAB

$$LAR = [-1.2931 + (0.5788 * \text{L TU HC5-C}) + (0.8244 * \text{L AAB})]$$

equation 42

The results show that insecticides at 0.1 * TU HC-5 C concentration with an aerobic aquatic biotransformation half-life of less than 27 days will not decrease copepoda abundance. However, insecticides that are resistant to aerobic aquatic biotransformation are predicted to cause a 19% reduction. The newer L TU HC5-C & L AAB (*equation* 42) model also predicts that at the HC5-C TU concentration copepods are not likely to incur any impacts if exposed to an insecticide with an aerobic aquatic biotransformation half-life of less than 27 days. However, a 79% decrease is expected if the insecticide is resistant to aerobic aquatic biotransformation.

*These results indicate that a threshold level of 0.1 * TU HC5-C or 1 * TU HC5-C would be protective of the aquatic environment against insecticides that are not resistant to anaerobic aquatic biotransformation.*

3.5 Discussion

The results show that crustacea laboratory single species toxicity data as geometric means of Daphnia species (Daphnia) or in the form of a Hazard Concentration for five percent of crustacea species (HC5-C) are able to produce statistically significant models capable of predicting field effects of pesticide exposure on crustacea abundance within lentic systems. The best models are based on the toxic units derived from these single species laboratory toxicity data (TU Daphnia & TU HC5-C) alone and/or in conjunction with other predictor variables such as fate, physico-chemical and system properties. All the proposed best models for crustacea species, cladocera and copepoda effects were validated using an independent dataset. Crustacea species produced the most predictive models, followed by copepoda. However, cladocera models were less predictive and showed inconsistency in terms of the influence of fate parameters on the toxic effects seen. This was probably due to small sample size.

3.5.1 Pesticide Grouping

It is expected that insecticides data should yield better models for crustacea, since these organisms are sensitive to the specific modes of action of these pesticides. In fact, Brock *et al.* (2000a) found that insects and crustaceans are the most sensitive groups in insecticide treatments. However, the cladocera and to a lesser extent crustacea species models were generally more predictive with data from all pesticides while copepoda models were better when the came from insecticide only data, even though insecticides account for about 75% of the pesticides in all three crustacean taxonomic groupings (crustacea species, cladocera, copepoda) and the actual pesticide composition is similar

among these sub-data sets. However, it must be noted that the best models based on larger sample sizes were all from insecticide only data from fish-free systems. However, the data from fish-free and fish-present systems combined produced models of similar predictability, with the best models based on insecticide or all pesticides grouped together having less than five percent difference in predictive power.

Competition and other biological factors such as feeding habits may explain why copepoda data produce better models when restricted to insecticides. While some members of these two groups generally feed at the same level, the majority of cladocera are unselective filter-feeders, while copepoda tend to actively select their food with a preference for larger particles (Becker *et al.* 2004, Brandl, 2005). Consequently, it is possible that they would respond differently to the different types of pesticides, since herbicides are not directly toxic to crustaceans.

The models in the current study are based on single species laboratory data done using standardised tests which do not reflect natural aquatic ecosystem conditions, extrapolations to selective feeders may be more accurate than to filter feeders that probably ingest more pesticides from feeding than their copepoda relatives. This is in part supported by the fact that the best copepoda models are substantially more predictive than those of cladocera. However, it does not explain why the best copepod models had about the same predictive strength as the best crustacean species models, in light of the fact that the majority of species used to derive that model belong to the order cladocera.

But it must be noted that the crustacea species responses were quantified using a species count ratio of effect, while the copepoda used an abundance effect ratio.

3.5.2 Presence of Fish

The most predictive best models come from fish-free insecticide systems while the least predictive are based on data that grouped fish-present and fish-free systems. It appears that the presence of fish causes secondary effects that greatly distort the effects seen, and consequently explaining the variance observed becomes more difficult. In fact, the coefficients of determination for the best models are better by 10 to 20% in those models derived from data without fish. These results seem to support the recommendation of the workshop on Community Level Aquatic System Studies – Interpretation Criteria (CLASSIC) held during May –June, 1999 in Germany - fish should not be included in a micro- or meso- cosm study if effects on invertebrates are important endpoints.

An interesting finding in our study is that the best copepod models required the use of a structural property of the system (volume or surface area to volume ratio) if the data grouped entries from fish-present and fish-free systems; however if the data came only from fish-free systems no structural property was needed. The results also indicate that more effects are expected in smaller system or those with larger surface area to volume ratio (which is usually associated with smaller systems). An examination of the experiments used for modelling show that fish are generally only present in the larger systems. This tendency was also found by Belanger (1997) who analysed data from over 150 model stream ecosystems (Brock *et al.* 2006). However, it seems as though the fish

may be causing secondary effects which are more pronounced in the smaller systems. It is also quite possible that the smaller systems have more fish biomass per area than the larger ones, and this therefore enhances secondary effects caused by fish.

3.5.3 HC5c vs Daphnia TU

It was hypothesised that the HC5 would have been the better predictor since it is made up of various crustacea species, which should in turn reflect the range of toxicity sensitivities present in the real world. In essence, the HC5 is thought to help produce a laboratory based value that was more reflective of an ecosystem. While the results from the cladocera and copepoda show that HC5 for crustacea is the better toxicity predictor, the best models for effects at the species level (i.e. proportion of species affected) indicate that the Daphnia toxicity unit is better suited for this type of data. However, the crustacea species models based solely on the toxic units as predictor variables have approximately the same predictive strength. This scenario is likely due to the strong correlation between these two toxicity units.

It is no surprise that the hazard concentration toxicity unit based on crustacea is better for copepoda since it is less taxonomically related to Daphnia; while cladocera models' preference for this predictor variable may be caused by hazard concentration values being extrapolated from species sensitivity distributions which were comprised of many cladocerans. However, if this hold true it does not explain why crustacea species models preferred the hazard concentration toxicity unit based on crustacea species, given that 65% of the species used to get the count ratio of effect are from the order cladocera.

It must be noted that the dataset used for modelling crustacea species effects included responses of *Daphnia* species; so this may possibly explain why the crustacea species best models preferred *Daphnia* based toxicity unit. However, the preference of toxicity unit predictor variable may be better explained by the fact that the dependent variable for crustacea species differed from that used for predicting cladocera or copepoda abundance (both preferred HC5-C). Scatterplots of the HC5 for crustacea toxicity units versus effect ratio (*see appendix 9*) and Daphnia toxicity unit versus species count ratio of effect ratio (*see appendix 10*), reveal that the former is much more scattered since it covers a larger range of toxicity units than the latter. While visual inspection of the distribution of HC5 for crustacea and Daphnia toxicity units against abundance ratio of response, show no major differences.

3.5.4 Fate, physico-chemical and Structural Properties of the System

Addition of fate, physico-chemical or structural system property variables improved model prediction for all crustacea groups, with the sole exception of models based on insecticides from fish-free systems for cladocera (note that the sample size was quite small). The degree of improvement by addition of such variables varied tremendously from merely a few percent better to twice better.

Hydrolysis half-life is the single most important fate parameter for crustacea species regardless of the dataset used (insecticides or all pesticides and their fish-free counterparts). The models indicate that more effects are expected from those chemicals

that have lower hydrolysis half-lives; which is somewhat counter-intuitive since it is expected that pesticides that are more persistent will lead to higher toxicity. It is possible that pesticides degrade into compounds that are more toxic than the parent material and some pesticides (like organothiophosphates, which were well-represented in the dataset used for modelling) need to be transformed to be toxic. But unfortunately the former hypothesis has generally been under-researched and there are studies reporting that several organophosphorus pesticides degrade into compounds that are more, less or similar to the parent in terms of its toxicity (Pehkonen & Zhang, 2002). So in order to test whether direct or metabolite-mediated toxicity may have contributed to the results indicated in the models, modelling was re-done using the variables of the best models but instead by grouping the insecticide data according to whether they needed to be transformed or were directly toxic. The models for both groups resulted in insecticides with shorter hydrolysis half-lives leading to more effects, thereby not supporting the hypothesis that that metabolite toxicity played a role in the impacts seen.

About half the data used to generate the best crustacea species model came from organophosphorus (OPs) insecticides, however results obtained from models generated using the same variables but without the OPs showed that hydrolysis was still considered a statistically significant contributor and rapid hydrolysis was predicted to affect more crustacea species.

Water photolysis half-life is included in the best cladocera models for data based on 1) all pesticides from systems with and without fish, 2) insecticides from systems with and

without fish, and 3) insecticides from fish free-systems. The first two sets of data produced models that indicate longer water photolysis half-lives would give rise to more toxic effects, however the last set of data (which produced the best model) show that shorter water photolysis half-lives would reduce cladocera abundance. Approximately 38% of the data used to generate the best cladocera model were from pyrethroids, which had the shortest water photolysis half-lives in the entire dataset. However, models generated using the same variables and non-pyrethroids still resulted in photolysis half-life being considered statistically significant and rapid photolysis leading to higher reductions in cladocera abundance.

Water photolysis half-life is also included in the best copepoda insecticide models from systems with and without fish, while Kow is included in the best models based on all pesticides. More copepoda reductions were predicted with longer water photolysis half-lives and hydrophilic compounds. In addition, structural property of the system was included in the best models based on data from both fish-present and fish-free systems and it was indicated that effects were more in smaller systems. Since these structural parameters were coupled with hydrophilic pesticides and insecticides that are resistant to water photolysis degradation, it therefore it appears as though the smaller systems can result in fewer refugia for these water-dwelling organisms, which can exacerbate effect of persistent pesticides.

However, the models based insecticide data from fish-free systems, which is the dataset that produced the best copepoda models, have aerobic aquatic biotransformation half-life

as the best explanatory fate variable. More effects are predicted if the degradation time is longer, which is logical because the insecticide would spend a longer time within the system. However, the selection of aerobic aquatic biotransformation half-life as the best fate variable is of some interest because unlike the other fate parameters it can be considered the closest laboratory equivalent to a field half-life test given (it is done using both water and sediment).

Hydrophobic insecticides contributed to more effects for crustacea species; however the best approximating models for cladocera and copepoda indicated that hydrophilic pesticides caused more effects. Our crustacea species (made up of 65% cladocera and 25% copepoda species) results are similar to Mohlenberg *et al.* (2001) who found that hydrophobic insecticides increase the toxicity for cladocerans and copepods. Unfortunately, Kow (or Koc) when present was not statistically significant in the best approximating all pesticides crustacea species models and insecticide models for cladocera or copepoda. Even though Mohlenberg *et al.* (2001) used few insecticides for modelling (less than 11), with the exception of diazinon and bifenthrin, the rest of their insecticides are included in the dataset of our study; consequently similar effects on cladocera and copepoda might be expected.

In essence, these results reiterate the fact that the physico-chemical and fate properties of the pesticide, along with actual system characteristics and application method of the pesticide can greatly influence the toxic effects seen. Hydrolysis is affected by temperature and pH; photolysis can be accelerated or inhibited depending on depth and

availability of sunlight; aerobic aquatic biotransformation is influenced by the physico-chemical properties of the system; and structural properties such as volume influences bioavailability of the pesticide. Consequently, using a tool that does not take into account these factors can severely underestimate the effects of worse case scenarios and result in poor predictability of effects overall.

3.5.5 Conservativeness of Hazard Quotients

Using the best models to draw conclusions about protective threshold concentrations revealed more effects are bound to occur at the standard Daphnia level (0.01 * Daphnia) than the L TU HC5-C threshold concentration with a safety factor of 10 (0.1 * HC5-C). This is hardly surprising since with the exception of a few pesticides (temephos, metribuzin and azinphos-methyl) all others used for modelling have 0.1 * HC5-C values that are between 1.28 (chlorpyrifos) and 246 (lindane) times more sensitive than the 0.01 * Daphnia values.

Our study shows about 50% of crustacea species can be affected by insecticide exposure at concentrations of 0.01 * Daphnia TU. These results directly contradict Brock *et al.* (2000) who found that this threshold level is protective of insecticide impacts on the aquatic environment. The disparity may partially be caused by the method used to determine an effect. Our research did not discriminate between slight effects and transient effects versus clear effects; any statistical significant change attributable to the pesticide treatment regardless of duration was considered to be an effect, especially since the study reports show that most effects are only considered as being statistically significant when

there is a major change (usually more than 70%). In addition, some of the studies used in our research are done at lower concentrations, and they showed effects. Brock *et al.* (2000) mentioned that one of the problems that they had in deriving a true NOEC was that studies were generally done at higher concentrations and some of the lowest concentrations they had showed clear effects (11 insecticides of 21).

It is also extremely important to note that the models do not contain entries at extremely low toxic units such as 0.01 * Daphnia TU or 0.1 * HC5-C TU. So even though the predicted impacts at these concentrations are estimated from fairly strong models (adjusted $R^2 \geq 0.55$) there is an unknown amount of error associated with the extrapolation of impacts beyond the range of available data.

Within their report Brock *et al.* (2000) conclude based on limited data that the crustacean groups of cladocera and copepoda are not affected at 0.01 – 0.1 * TU Daphnia; there are many more studies showing clear negative effects at 0.1 – 1 * TU Daphnia following a single application of acetylcholinesterase-inhibiting insecticides increases dramatically. Eighty-three percent of these studies show effects for cladocera and 30% for copepoda. Even though a similar approach was used to calculate the toxicity units, examination of Brock *et al.* (2006) publication shows that the HC5 values varies between the two studies (theirs and ours). So this may in part explain the variation in results obtained.

The best models for cladocera and copepoda generally support that a threshold value at 0.1 * HC5-C would be protective, with the major exception being insecticides that

degrade rapidly by water photolysis (cladocera). In addition, insecticides that are extremely resistant to aerobic aquatic biotransformation can possibly cause minor reductions in copepods (a maximum of 19%) according to extrapolations from the best fitting copepoda model. However, on average a threshold level of 1 * median HC5-C TU is predicted as being incapable of offering enough protection to cladocera, but this level can protect copepods that are not exposed to insecticides which are extremely resistant to aerobic aquatic biotransformation. This therefore does not confirm Maltby *et al.* (2005) claims that the lower limit (95% CI) value of HC5 is able to protect aquatic ecosystems; while the median (50% CI) HC5 is generally protective of these freshwater environments.

Tables and Figures for Chapter 3

Table 3. 1 - Model selection by AIC for Count Ratio of Effect for crustacea species based on all pesticides using both Daphnia and HC5c TUs, fate and structural properties of the system.

Var 1	Var 2	Var 3	Var 4	Var 5	Df	Δ AICc	w_i ratio	Adjusted R^2	L ratio Chi^2	p	r
* L TU Daphnia	L HHL				2	0.00	1.00	0.48	36.45076	0.000000	.6886
											p=.000
L Volume	L TU Daphnia	L WPHL	LAAB		4	0.03	1.01	0.50	40.56890	0.000000	.6916
											p=.000
L Volume	L TU Daphnia	L HHL			3	0.28	1.15	0.49	38.24190	0.000000	.7010
											p=.000
L Volume	L TU Daphnia	LAAB			3	1.01	1.65	0.48	37.51622	0.000000	.6956
											p=.000
L TSA/V R	L TU Daphnia	L HHL			3	1.17	1.79	0.48	37.35644	0.000000	.7044
											p=.000
L Volume	L TU Daphnia				2	1.19	1.81	0.47	35.26438	0.000000	.6523
											p=.001
L Volume	L TU Daphnia	L WPHL	L HHL		4	1.57	2.19	0.49	39.02737	0.000000	.6903
											p=.000
L TU HC5 - C	LAAB				2	1.57	2.19	0.47	34.88331	0.000000	.7097
											p=.000
L Volume	L TU Daphnia	log Kow	L HHL		4	1.61	2.24	0.49	38.98617	0.000000	.7093
											p=.000
L Volume	L TU Daphnia	log Koc	L WPHL	LAAB	5	1.62	2.24	0.50	41.05880	0.000000	.6960
											p=.000
L TU Daphnia	log Kow	L HHL			3	1.76	2.41	0.48	36.76292	0.000000	.6919
											p=.000
L TU Daphnia	L WPHL	L HHL			3	1.94	2.64	0.47	36.58503	0.000000	.6841
											p=.000
L TU HC5 – C	L HHL				2	1.95	2.66	0.46	34.49847	0.000000	.7133
											p=.000
L TU Daphnia					1	2.56	3.60	0.45	31.82382	0.000000	.6088
											p=.002
L TU HC5 - C					1	2.01	2.73	0.45	32.37372	0.000000	.6839
											p=.000

Key

Var – variable
Df – degree of freedom
ΔAICc - AICc difference
w_i ratio- Akaike's weight ratio
r – correlation coefficient

LAAB – log-transformed aerobic aquatic biotransformation

LHHL – log-transformed hydrolysis half-life

LTSA/V R – log-transformed total surface area to volume ratio

LTU Daphnia – log-transformed toxic unit ba geometric mean for Daphnia species

L TU HC5 – C – log-transformed toxic unit ba hazard concentration for 5% of crustacea species

L Volume - log-transformed volume

L WPHL – log-transformed water photolysis half-life

log Koc – log-transformed organic carbon absorption coefficient

log kow – log-transformed octanol-water coefficient

N= 52 for training set, N = 24 for validation set.

* proposed best model for dataset

Table 3. 2 – Model selection by AIC for Count Ratio of Effect for crustacea species based on all pesticides from systems without fish using both Daphnia and HC5c TU, fate and structural properties of the system.

Var 1	Var 2	Var 3	Df	Δ AICc	w_i ratio	Adjusted R^2	L ratio Chi^2	P	r
* L TU Daphnia	L HHL		2	0.00	1.00	0.57	34.38206	0.000000	.6776 p=.000
* L TU Daphnia			1	0.78	1.48	0.55	31.49877	0.000000	.6088 p=.002
L TU Daphnia	LAAB		2	1.28	1.90	0.56	33.10294	0.000000	.6387 p=.001
L TU Daphnia	log Kow	L HHL	3	1.41	2.02	0.57	35.08227	0.000000	.6832 p=.000
L Volume	L TU Daphnia	L HHL	3	1.96	2.67	0.56	34.52676	0.000000	.6831 p=.000
L TU HC5 - C			1	3.91	7.07	0.51	28.36877	0.000000	.6839 p=.000

N = 38 for training set, N = 20 for validation set * proposed best model/s for dataset

Key

Var – variable **Df** – degree of freedom **ΔAICc** - AICc difference **w_i ratio**- Akaike's weight ratio **r** – correlation coefficient

LAAB – log-transformed aerobic aquatic biotransformation **L Volume** - log-transformed volume

LHHL – log-transformed hydrolysis half-life **log kow** - log-transformed octanol-water coefficient

LTU Daphnia – log-transformed toxic unit based on geometric mean for Daphnia species

L TU HC5 – C – log-transformed toxic unit based on hazard concentration for 5% of crustacea species

Table 3. 3 – Model selection by AIC for Count Ratio of Effect for crustacea species based insecticides using both Daphnia and HC5c TUs, fate and structural properties of the system.

Var 1	Var 2	Var 3	Var 4	Df	Δ AICc	w_i ratio	Adjusted R^2	L ratio Chi^2	p	r
* L TU Daphnia	log Kow	L HHL		3	0.00	1.00	0.48	27.39866	0.000005	.6760
										p=.003
L Volume	L TU Daphnia	log Kow	L HHL	4	0.92	1.58	0.48	28.59695	0.000009	.6659
										p=.004
L TSA/V R	L TU Daphnia	log Kow	L HHL	4	2.09	2.84	0.46	27.42790	0.000016	.6773
										p=.003
L Volume	L TU Daphnia	L HHL		3	3.93	7.15	0.42	23.46526	0.000032	.6347
										p=.006
L TU Daphnia	L HHL			2	4.02	7.45	0.40	21.27202	0.000024	.6409
										p=.006
L TU Daphnia	log Koc	L HHL		3	4.19	8.12	0.42	23.21122	0.000036	.6206
										p=.008
L TU HC5 - C	log Kow	L HHL		3	4.45	9.25	0.41	22.95010	0.000041	.6496
										p=.005
L Volume	L TU Daphnia	log Koc	L HHL	4	4.59	9.90	0.43	24.93014	0.000052	.6132
										p=.009
L TU Daphnia				1	13.07	688.16	0.22	10.11766	0.001469	.4214
										p=.092
L TU HC5 - C				1	13.17	724.01	0.22	10.01611	0.001552	.5050
										p=.039

N = 37 for training set, N = 17 for validation set.
* proposed best model for dataset

Key

Var – variable
Df – degree of freedom
ΔAICc - AICc difference
w_i ratio- Akaike's weight ratio
r – correlation coefficient

LAAB – log-transformed aerobic aquatic biotransformation

LHHL – log-transformed hydrolysis half-life

LTSA/V R – log-transformed total surface area to volume ratio

LTU Daphnia – log-transformed toxic unit based on geometric mean for Daphnia species

L TU HC5 – C – log-transformed toxic unit ba hazard concentration for 5% of crustacea species

L Volume - log-transformed volume

L WPHL – log-transformed water photolysis half-life

log Koc – log-transformed organic carbon absorption coefficient

log kow – log-transformed octanol-water coefficient

Table 3. 4 – Model selection by AIC for Count Ratio of Effect for crustacea species from insecticides from systems without fish using both Daphnia and HC5c TU, fate and structural properties of the system.

Var 1	Var 2	Var 3	Var 4	Var 5	Df	Δ AICc	w_i ratio	Adjusted R^2	L ratio Chi^2	p	r
L TU Daphnia	log Koc	L WPHL	L HHL		4	0.00	1.00	0.68	31.76399	0.000002	.6130 p=.009
L Volume	L TU Daphnia	log Koc	L WPHL	L HHL	5	1.49	2.11	0.67	32.52792	0.000005	.3949 p=.117
* L TU Daphnia	L WPHL	L HHL			3	1.08	1.71	0.65	28.45890	0.000003	.6424 p=.005
L TU Daphnia	log Kow	L HHL			3	1.65	2.29	0.64	27.88075	0.000004	.6715 p=.003
L TU Daphnia	log Koc	L HHL			3	2.01	2.73	0.63	27.52537	0.000005	.6075 p=.010
L TU Daphnia					1	12.92	12.92	0.37	12.22593	0.000471	.4214 p=.092
L TU HC5 - C					1	15.92	15.92	0.29	9.22304	0.002390	.5050 p=.039

N =24 for training set, N = 13 for validation set

* proposed best model for dataset

Key

Var – variable
Df – degree of freedom
ΔAICc - AICc difference
w_i ratio- Akaike's weight ratio
r – correlation coefficient

LHHL – log-transformed hydrolysis half-life

LTU Daphnia – log-transformed toxic unit based on geometric mean for Daphnia species

L TU HC5 – C – log-transformed toxic unit based on hazard concentration for 5% of crustacea species

L Volume - log-transformed volume

L WPHL – log-transformed water photolysis half-life

log Koc – log-transformed organic carbon absorption coefficient

log kow – log-transformed octanol-water coefficient

Table 3. 5 - Model selection by AIC for Log Abundance Ratio Change for cladocera for all pesticides using both Daphnia and HC5c TUs, fate and structural properties of the system.

Var 1	Var 2	Var 3	Var 4	Df	Δ AICc	Akaike weight ratio	Adjusted R^2	L ratio Chi^2	p	r	N	r (N=20)
L TU HC5 - C	L WPHL	LAAB		3	0.00	1.00	0.34	18.43268	0.000358	.4164 p=.038	25	.4721 p=.036
* L TU HC5 - C	log Kow			2	0.03	1.02	0.33	16.28399	0.000291	.4513 p=.024	25	.4936 p=.027
L Volume	L TU HC5 - C	log Kow		3	1.06	1.70	0.32	17.37002	0.000593	.4398 p=.028	25	.5151 p=.020
L TU HC5 - C	log Koc			2	1.05	1.69	0.31	15.26603	0.000484	.4780 p=.024	22	.5360 p=.015
L Volume	L TU HC5 - C	L WPHL	LAAB	4	1.43	2.05	0.34	19.12012	0.000744	.4674 p=.028	22	.5031 p=.024
L TU HC5 - C	log Koc	L WPHL	LAAB	4	1.54	2.16	0.33	19.01030	0.000782	.5123 p=.021	20	.5123 p=.021
L TSA/V R	L TU HC5 - C	log Kow		3	1.45	2.07	0.32	16.98058	0.000713	.4179 p=.038	25	.4812 p=.032
L TSA/V R	L TU HC5 - C	L WPHL	LAAB	4	1.75	2.40	0.33	18.80044	0.000860	.4860 p=.030	20	.4860 p=.030
L TU HC5 - C	log Koc	LAAB		3	1.79	2.45	0.31	16.63843	0.000839	.4271 p=.033	25	.4970 p=.026
L TU HC5 - C	log Kow	L HHL		3	1.98	2.69	0.31	16.45327	0.000915	.4534 p=.023	25	.4897 p=.028
L TU HC5 - C				1	2.7344	3.92	0.19	11.47753	0.000704	.4734 p=.017	25	.5094 p=.022
L TU Daphnia				1	5.5959	16.40	0.25	8.61600	0.003332	.4761 p=.016	25	.5300 p=.016

N = 36

* highlight – proposed best model for dataset

Key

Var – variable
Df – degree of freedom
ΔAICc - AICc difference
w_i ratio- Akaike's weight ratio
r – correlation coefficient

LAAB – log-transformed aerobic aquatic biotransformation

LHHL – log-transformed hydrolysis half-life

LTSA/V R – log-transformed total surface area to volume ratio

LTU Daphnia – log-transformed toxic unit based on geometric mean for Daphnia species

L TU HC5 – C – log-transformed toxic unit based on hazard concentration for 5% of crustacea species

L Volume - log-transformed volume

L WPHL – log-transformed water photolysis half-life

log Koc – log-transformed organic carbon absorption coefficient

log kow – log-transformed octanol-water coefficient

Table 3. 6 – Model selection by AIC for Log Abundance Ratio Change for cladocera based on all pesticides from systems without fish using both Daphnia and HC5c TU, fate and structural properties of the system.

Var 1	Var 2	Var 3	Df	Δ AICc	Akaike weight ratio	Adjusted R^2	L ratio Chi^2	p	r	N	r (N=15)
* L TU HC5 - C	log Kow		2	0.00	1.00	0.51	22.12591	0.000016	.5614	18	.5882
									p=.015		p=.021
L TSA/V R	L TU HC5 - C	log Kow	3	1.07	1.71	0.51	23.21549	0.000036	.6103	15	.6103
									p=.016		p=.016
L Volume	L TU HC5 - C	log Kow	3	1.28	1.90	0.50	23.00625	0.000040	.5532	17	.6202
									p=.021		p=.014
L TU HC5 - C	LAAB		2	1.53	2.15	0.48	20.59499	0.000034	.6048	18	.6578
									p=.008		p=.008
L TU HC5 - C	log Kow	LAAB	3	1.99	2.70	0.49	22.29839	0.000057	.5844	18	.6110
									p=.011		p=.016
L TU HC5 - C			1	4.05	7.57	0.41	15.92731	0.000066	.6121	18	.6257
									p=.007		p=.013
L TU Daphnia			1	8.08	56.93	0.32	11.89307	0.000563	.6519	18	.6418
									p=.003		p=.010

N = 28

Key

Var – variable
Df – degree of freedom
ΔAICc - AICc difference
w_i ratio- Akaike's weight ratio
r – correlation coefficient

LAAB – log-transformed aerobic aquatic biotransformation

LTSA/V R – log-transformed total surface area to volume ratio

LTU Daphnia – log-transformed toxic unit based on geometric mean for Daphnia species

L TU HC5 – C – log-transformed toxic unit based on hazard concentration for 5% of crustacea species

L Volume - log-transformed volume

log kow – log-transformed octanol-water coefficient

* proposed best model for dataset

Table 3. 7 – Model selection by AIC for Log Abundance Ratio Change for insecticides using both Daphnia and HC5c TUs, fate and structural properties of the system.

Var 1	Var 2	Var 3	Df	Δ AICc	Akaike weight ratio	Adjusted R^2	L ratio Chi^2	p	r	N	r (N=15)
* L TU HC5 - C	L WPHL		2	0.00	1.00	0.27	10.78328	0.004555	.3392	22	.4444
									p=.123		p=.097
L Volume	L TU HC5 - C	L WPHL	3	1.41	2.03	0.26	11.54342	0.009123	.3119	19	.4412
									p=.194		p=.100
L TSA/V R	L TU HC5 - C	L WPHL	3	1.57	2.19	0.26	11.38793	0.009803	.4903	16	.4595
									p=.054		p=.085
L TU HC5 - C	log Koc	L WPHL	3	1.89	2.58	0.25	11.06079	0.011402	.3259	22	.4612
									p=.139		p=.084
L TU HC5 - C	L WPHL	L HHL	3	1.99	2.70	0.25	10.96921	0.011894	.3435	22	.4442
									p=.118		p=.097
L TU HC5 - C			1	3.34	5.30	0.15	5.29163	0.021428	.4189	22	.4771
									p=.052		p=.072
L TU Daphnia			1	4.91	11.67	0.09	3.71455	0.053940	.4001	22	.4350
									p=.065		p=.105

N = 27

* proposed best model for dataset

Key

Var – variable
Df – degree of freedom
ΔAICc - AICc difference
w_i ratio- Akaike's weight ratio
r – correlation coefficient

LHHL – log-transformed hydrolysis half-life

LTSA/V R – log-transformed total surface area to volume ratio

LTU Daphnia – log-transformed toxic unit based on geometric mean for Daphnia species

L TU HC5 – C – log-transformed toxic unit based on hazard concentration for 5% of crustacea species

L Volume - log-transformed volume

L WPHL – log-transformed water photolysis half-life

log Koc – log-transformed organic carbon absorption coefficient

Table 3. 8 – Model selection by AIC for Log Abundance Ratio Change for cladocera based on insecticides from systems without fish using both Daphnia and HC5c TU, fate and structural properties of the system.

Var 1	Var 2	Var 3	Var 4	Df	ΔAICc	Akaike weight ratio	Adjusted R^2	L ratio Chi^2	p	r	N	r (N=12)
* L TU HC5 - C				1	0.00	1.00	0.44	12.79	0.000349	.4443 p=.074	17	.4230 p=.171
L TU HC5 - C	L WPHL			2	0.68	1.41	0.45	14.35	0.000767	.5454 p=.024	17	.5769 p=.050
L TSA/V R	L TU Daphnia	log Koc	L WPHL	4	1.28	1.89	0.49	18.35	0.001056	.4817 p=.081	14	.6704 p=.017
* L TU Daphnia	L WPHL			2	1.03	1.67	0.44	14.00	0.000913	.5365 p=.026	17	.6517 p=.022
L TU Daphnia	log Koc	L WPH L		3	1.56	2.18	0.46	15.75	0.001279	.5250 p=.030	17	.6162 p=.033
L TSA/V R	L TU HC5 - C			2	1.51	2.13	0.43	13.52	0.001160	.4596 p=.133	12	.4596 p=.133
L TSA/V R	L TU Daphnia	L WPH L		3	1.98	2.69	0.45	15.32	0.001561	.5443 p=.044	14	.7054 p=.010
L Volume	L TU HC5 - C			2	1.88	2.56	0.42	13.15	0.001394	.3646 p=.165	16	.4590 p=.133
L TU Daphnia				1	5.84	18.55	0.25	6.95	0.008399	.5096 p=.037	17	.4496 p=.143

N = 20

* proposed best model for dataset

Key

Var – variable
Df – degree of freedom
ΔAICc - AICc difference
w_i ratio- Akaike's weight ratio
r – correlation coefficient

LTSA/V R – log-transformed total surface area to volume ratio

LTU Daphnia – log-transformed toxic unit based on geometric mean for Daphnia species

L TU HC5 – C – log-transformed toxic unit based on hazard concentration for 5% of crustacea species

L WPHL – log-transformed water photolysis half-life

log Koc – log-transformed organic carbon absorption coefficient

Table 3. 9 – Model selection by AIC for Log Abundance Ratio Change for copepoda based on all pesticides using both Daphnia and HC5c TU, fate and structural properties of the system.

Var 1	Var 2	Var 3	Var 4	Df	Δ AICc	Akaike weight ratio	Adjusted R^2	L ratio Chi^2	p	r
* L Volume	L TU HC5 - C	log Kow		3	0.00	1.00	0.38	23.55057	0.000031	.6970 p=.000
L TSA/VR	L TU HC5 - C	log Kow		3	1.25	1.87	0.36	22.30475	0.000056	.7158 p=.000
L Volume	L TU HC5 - C	log Kow	L HHL	4	1.97	2.68	0.36	23.67782	0.000093	.6897 p=.000
L Volume	L TU HC5 - C	log Kow	LAAB	4	2.01	2.74	0.36	23.63552	0.000094	.7109 p=.000
L TU HC5 - C	log Kow			2	2.96	4.39	0.32	18.50257	0.000096	.6660 p=.001
L TU HC5 - C				1	8.66	76.15	0.20	10.71191	0.001064	.6250 p=.001
L TU Daphnia				1	11.71	349.03	0.14	7.66702	0.005624	.4787 p=.021

N = 43

* proposed best model for dataset

Key

Var – variable
Df – degree of freedom
ΔAICc - AICc difference
r – correlation coefficient

LAAB – log-transformed aerobic aquatic biotransformation

L HHL - log-transformed hydrolysis half-life

LTU Daphnia – log-transformed toxic unit based on geometric mean for Daphnia species

L TU HC5 – C – log-transformed toxic unit based on hazard concentration for 5% crustacea species

L TSA/VR - log-transformed total surface area to volume ratio

L Volume - log-transformed volume

Log Kow - log-transformed octanol-water coefficient

Table 3. 10– Model selection by AIC for Log Abundance Ratio Change for copepoda from all pesticides from systems that contained no fish using both Daphnia and HC5c TU, fate and structural properties of the system.

Var 1	Var 2	Var 3	Var 4	Df	Δ AICc	Akaike weight ratio	Adjusted R^2	L ratio Chi^2	p	r
* L TU HC5 - C	log Kow			2	0.00	1.00	0.43	21.63431	0.000020	.7321
										p=.002
L Volume	L TU HC5 - C	log Kow		3	0.73	1.44	0.43	23.02625	0.000040	.7505
										p=.001
L TU HC5 - C	log Kow	LASB		3	0.84	1.52	0.43	22.91700	0.000042	.7387
										p=.002
L TSA/V R	L TU HC5 - C	log Kow		3	1.21	1.83	0.42	22.54512	0.000050	.7455
										p=.001
L TU HC5 - C	log Kow	LAAB		3	1.56	2.18	0.42	22.19471	0.000059	.7504
										p=.001
L Volume	L TU HC5 - C	log Kow	LAAB	4	1.81	2.47	0.43	24.07290	0.000077	.7769
										p=.001
L TU Daphnia				1	8.59	73.27	0.25	10.93521	0.000944	.5713
										p=.026
L TU HC5 - C				1	5.44	15.14	0.31	14.08845	0.000174	.7011
										p=.004

N = 35

Key

Var – variable
Df – degree of freedom
ΔAICc - AICc difference
r – correlation coefficient

LAAB – log-transformed aerobic aquatic biotransformation

LASB – log-transformed aerobic soil biotransformation

LTU Daphnia – log-transformed toxic unit based on geometric mean f Daphnia species

L TU HC5 – C – log-transformed toxic unit based on hazard concentration for 5% of crustacea species

L TSA/VR - log-transformed total surface area to volume ratio

L Volume - log-transformed volume

Log Kow - log-transformed octanol-water coefficient

* proposed best model for dataset

Table 3. 11 – Model selection by AIC for Log Abundance Ratio Change for copepoda based on insecticides using both Daphnia and HC5c TU, fate and structural properties of the system.

Var 1	Var 2	Var 3	Var 4	Df	Δ AICc	Akaike weight ratio	Adjusted R^2	L ratio Chi^2	p	r
* L TSA/VR	L TU HC5 - C	L WPHL		3	0.00	1.00	0.42	19.63412	0.000202	.6706 p=.004
* L Volume	L TU HC5 - C	L WPHL		3	0.08	1.04	0.42	19.55796	0.000210	.6230 p=.010
L TSA/VR	L TU HC5 - C	log Koc	L WPHL	4	0.87	1.54	0.42	20.92309	0.000328	.6843 p=.003
L Volume	L TU HC5 - C	log Koc	L WPHL	4	0.88	1.55	0.42	20.91494	0.000329	.6464 p=.007
L Volume	L TU HC5 - C			2	1.91	2.59	0.36	15.58067	0.000414	.6020 p=.014
L TU HC5 - C				1	5.32	14.29	0.26	10.03262	0.001538	.6188 p=.011
L TU Daphnia				1	10.26	168.76	0.13	5.09413	0.024007	.2587 p=.333

N = 30

Key

Var – variable
Df – degree of freedom
ΔAICc - AICc difference
r – correlation coefficient

LTU Daphnia – log-transformed toxic unit based on geometric mean for Daphnia species

L TU HC5 – C – log-transformed toxic unit based on hazard concentration for 5% of crustacea species

L TSA/VR - log-transformed total surface area to volume ratio

L Volume - log-transformed volume

L WPHL – log-transformed water photolysis half-life

log Koc - log-transformed octanol-carbon partition coefficient

* proposed best model for dataset

Table 3. 12 – Model selection by AIC for Log Abundance Ratio Change for all insecticides from systems that contained no fish using both Daphnia and HC5c TU, fate and structural properties of the system.

Var 1	Var 2	Var 3	Df	Δ AICc	Akaike weight ratio	Adjusted R^2	L ratio Chi^2	p	r	N
* L TU HC5 - C	LAAB		2	0.00	1.00	0.63	24.36561	0.000005	.7153 p=.009	12
L TSA/VR	L TU HC5 - C	LAAB	3	0.93	1.59	0.64	25.67073	0.000011	.6232 p=.073	9
L Volume	L TU HC5 - C	LAAB	3	1.37	1.98	0.63	25.23568	0.000014	.6588 p=.027	11
L TU HC5 - C	L WPHL	LAAB	3	1.93	2.63	0.62	24.66845	0.000018	.7191 p=.008	12
L TU Daphnia			1	15.17	1966.07	0.24	6.98846	0.008204	-.0720 p=.824	12
L TU HC5 - C			1	9.99	147.90	0.40	12.16298	0.000487	.5338 p=.074	12

N = 22

* proposed best model for dataset

Key

Var – variable
Df – degree of freedom
ΔAICc - AICc difference
r – correlation coefficient

LAAB – log-transformed aerobic aquatic biotransformation

LTU Daphnia – log-transformed toxic unit based on geometric mean for I species

L TU HC5 – C – log-transformed toxic unit based on hazard concentration for 5% of crustacea species

L TSA/VR - log-transformed total surface area to volume ratio

L Volume - log-transformed volume

L WPHL – log-transformed water photolysis half-life

Figure 3. 1– Taxonomic tree of crustaceans in dataset used for modelling responses at the species level.

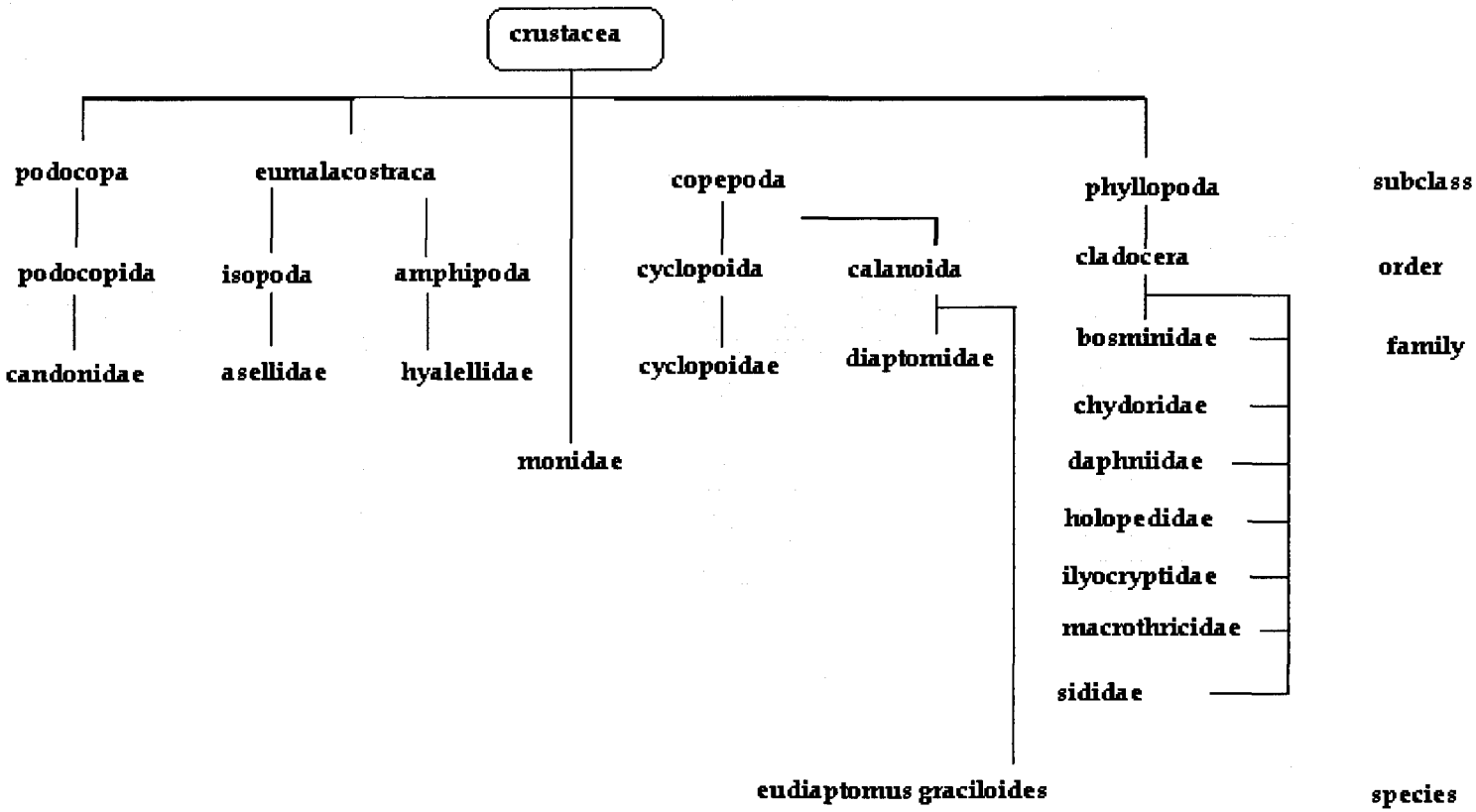
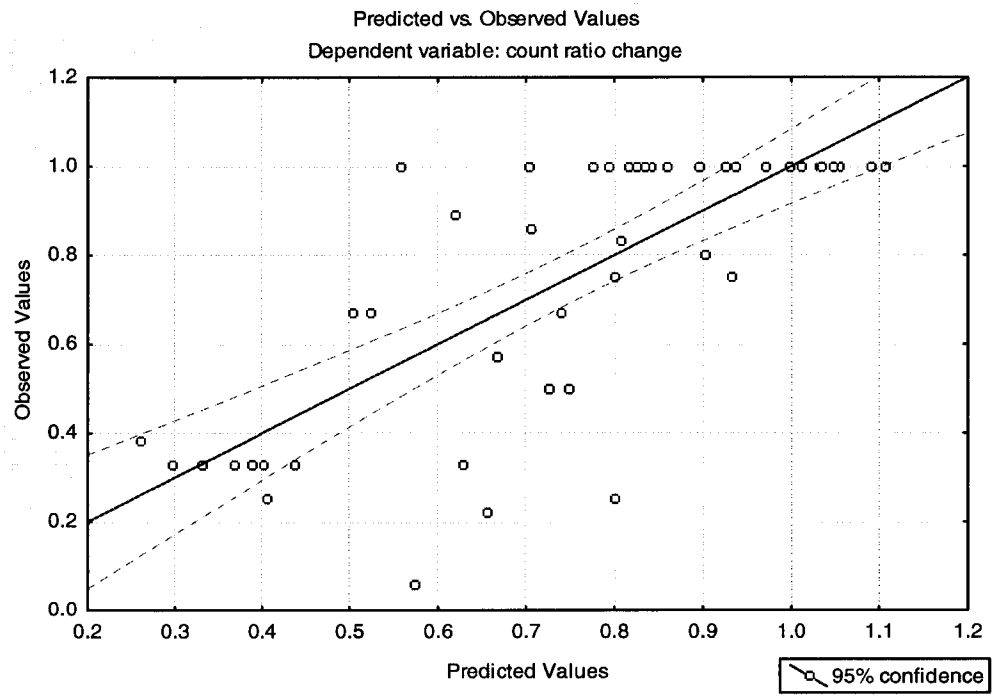


Figure 3. 2– Best model for crustacea species data – L Daphnia TU, log Kow & L HHL.



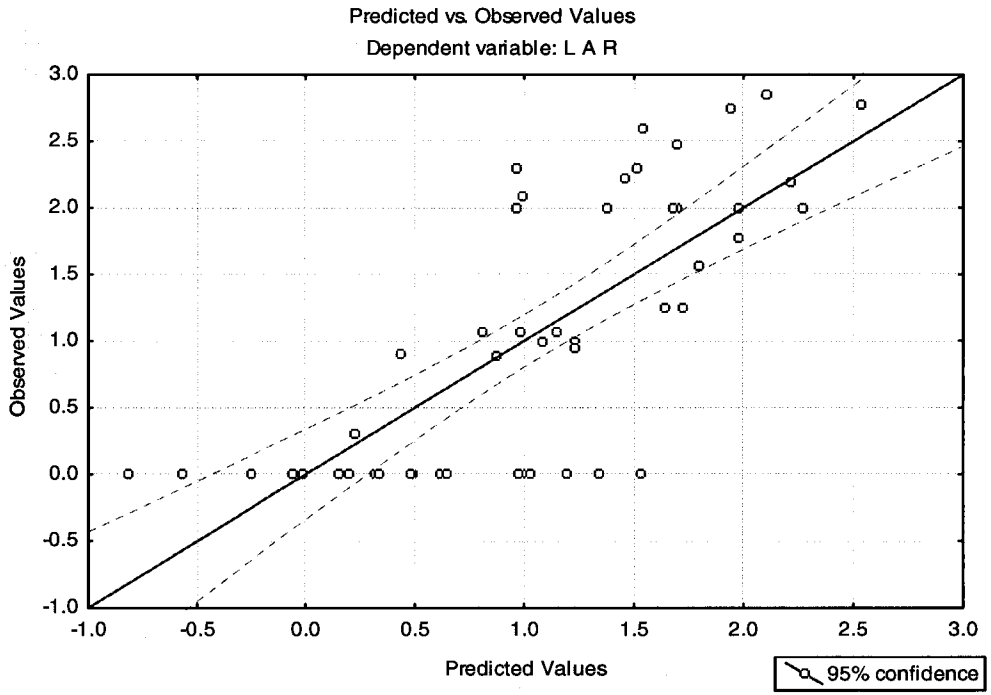
Key

LHHL – log-transformed hydrolysis half-life

LTU Daphnia – log-transformed toxic unit based on geometric mean for Daphnia species

log Kow - log-transformed octanol-water coefficient

Figure 3. 3– Best model for cladocera – L TU HC5-C * L WPHL

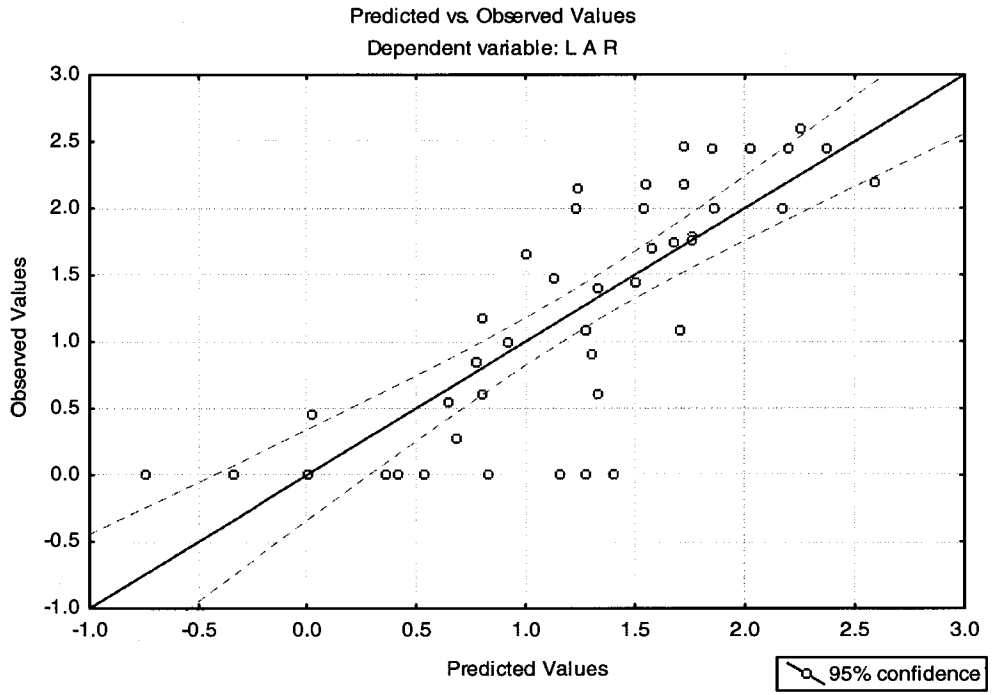


Key

L TU HC5 – C – log-transformed toxic unit based on hazard concentration for 5% of crustacea species

L WPHL – log-transformed water photolysis half-life

Figure 3. 4 - Best copepoda model – L TU HC5-C & L AAB.



Key

L TU HC5 – C – log-transformed toxic unit based on hazard concentration for 5% of crustacea Species

LAAB – log-transformed aerobic aquatic biotransformation

CHAPTER 4 – INSECTA

4.1 Modelling Insecta Abundance Responses

The data used for these models are based on insecta abundance responses to pesticide application in lentic systems. However, due to few entries on herbicides, carbendazim or rotenone modelling was done only with insecticide data. Many studies reported responses at the family level as well as the species level; consequently the dependent variable - count ratio of effect - was derived at both taxonomic levels. The count ratio was calculated by dividing the number of statistically affected insecta species/families by the total number insecta species/families recorded for that experiment (*see equation 4 in 2.3*). All effects are abundance decreases.

Three log-transformed toxic units (TUs) were used as explanatory variables: 1) geometric means of Daphnia species (L TU Daphnia), 2) hazard concentrations for 5% of the crustacean species (L TU HC5 - C), and 3) hazard concentrations for 5% of the insecta species (L TU HC5 - I). This was done to test the hypothesis that states the HC5s better predict the effects seen. The inclusion of the L TU HC5 – C was partially based on its strong statistical correlation with both L TU HC5 – I and L TU Daphnia. Another reason for inclusion is that registrants are not obligated to give laboratory single species data for insecta in order to assess aquatic impacts of pesticides.

Log-transformed structural properties of system (volume – L volume and surface area to volume ratio - L TSA/V R) and all log-transformed fate and physico-chemical properties

of the pesticide (octanol-water partition coefficient – log Kow, organic carbon absorption coefficient – log Koc, aerobic soil biotransformation - L ASB, aerobic aquatic biotransformation - L AAB, water photolysis half-life - L WPHL, hydrolysis half-life - LHHL) were initially entered into the model.

Inclusion of all these explanatory variables in the AIC reduced the number of entries available for modelling and created sub-data sets of 35 entries for species and 42 for family. Given these small sample sizes, it was decided that modelling would be done based on all available data as well as on a more restricted training set: 2/3 of the data used for modelling, and the remaining 1/3 used to validate the best models for species data. However, since there were 69 entries for family, it was decided that all the data with all independent parameters known would be used for AIC analyses and the best approximating models would be validated using the remaining data.

4.2 Modelling Insecta Species Abundance Responses

The 35 available entries came from 13 studies that reported on nine insecticides – carbaryl, carbofuran, chlorpyrifos, deltamethrin, diflubenzuron, lindane, methyl parathion, permethrin and phorate. Eleven families within six orders were represented; 61% of the data came from the order diptera and its three families - ceratapogonidae, chaoboridae and chironomidae, approximately 19% are from the ephemeroptera families of baetidae and caenidae and the remaining species were from the families of dytiscidae, gerridae, hydrometridae, leptoceridae, limnephilidae and sialidae (*see diagram 4.1*).

The AIC based on the training set data indicate that five models can be considered to the best approximating based on an AICc difference of less than two (*see table 4.1*), while the AIC scores for all available data considered ten models as the best approximating models (*see table 4.2*). All these models are statistically significant and the training set models are able to explain 29 to 34% of the variance observed in the insecta species response ratios, but those based on all available data are only able to explain 13 to 20 percent. In addition, all the training set models failed the validation test using an independent data set – the predicted impacts were not statistically correlated to the observed study impacts.

Even though these models were poor predictors, after modelling all insecta species collected (48 entries) using the variable of the best approximating models the following trends were observed/confirmed:

- HC5-C and HC5-I TUs were the only statistically significant contributors.
- HC5-C is the best TU predictor variable for modelling the proportion of insect species that will be affected when exposed to insecticides
- The best model which was L TU HC5-C because it had a consistent, though poor, adjusted R^2 of 0.19 (based on 23, 35 or 48 entries) and it was the only model that could be statistically validated.

Given the poor strength of the models no extrapolations pertaining to expected effects at the standard threshold level were done and the following discussions will not include details of these results.

4.3 Modelling Insecta Family Abundance Responses

The 42 values used for AIC analysis came from 14 known families within eight orders; given data gaps (in species taxonomic classification) and derived values the total number of families reflected is unknown. About 62% of the data came from diptera families, with 15% belonging to chaoboridae and 23% to chironomidae; the remaining 24% represented ceratapogonidae, culicidae and tipulidae. Approximately 17% of the data came from ephemeroptera families of caenidae and baetidae. The remaining data represented the families of coenagrionidae, dytiscidae, hydrometridae, leptoceridae, limnephilidae and sialidae (*See diagram 4.1*).

The entries for AIC analysis came from 17 studies based on ten insecticides: carbaryl, carbofuran, chlorpyrifos, cypermethrin, deltamethrin, diflubenzuron, lindane, methyl parathion, permethrin and phorate, while the validation set consisted of 25 entries from eight studies covering nine: insecticides bendiocarb, carbofuran, deltamethrin, diflubenzuron, esfenvalerate, fenitrothion, methyl parathion, permethrin and trichlorphon.

Of the 93 model combinations possible with a TU and no correlating variables, 19 can be considered as the best approximating models according to the AICc difference (*see table 4.3*). All of the 19 candidate models were statistically significant and the adjusted R^2s range from 0.20 to 0.27. Since, many structural system properties were missing and most of the adjusted R^2s models are within a small range, only the most parsimonious models that did not include a structural property were validated using the independent data. All

these models (six) were statistically validated; the predicted impacts are statistically correlated with those reported in the experiments.

HC5-C and HC5-I are statistically significant contributors in all the models; the former is found in 16 models while the latter only contribute to three. The L TU HC5-I (adjusted $R^2 = 0.18$, *see equation 43*), even though statistically significant, does not have enough evidence to support it being considered as one of the best approximate models. But the L TU HC5-C (adjusted $R^2 = 0.20$, *see equation 44)* model is among the 19 best approximating models, is the only model with all of its predictor variables being considered as statistically significant contributors below p=0.05, and has been statistically validated. The comparatively strong predictive power of just the HC5-C TU may explain why so many models qualify as being the best approximating model.

L TU HC5-C

$$\textit{Count Ratio of Effect} = [0.4429 + (0.1717 * \text{L TU HC5-C})]$$

equation 43

L TU HC5-I

$$\textit{Count Ratio of Effect} = [0.6505 + (0.1396 * \text{L TU HC5-C})]$$

equation 44

With the exception of the TU predictors, the only other variable that was statistically significant below p=0.05 is L AAB, which is a statistically significant contributor in two of its seven models. Two models have L ASB and another four models contain L HHL,

while seven models include L TSA/V R and another had L Volume. Log Kow is found in seven models, two others have L WPHL and one has log Koc.

Using the results based on all 19 models does not greatly assist in deciphering the better models; however when the selection is restricted to only those models with an Akaike weight ratio of less than two, a new trend is seen. First of all, five of the nine models contain L AAB as a predictor and two others its correlated counterparts L ASB and L HHL. Only a third of these models have log Kow as a contributing factor and one other contains L WPHL. Another third of these models include L TSA/V R.

The results seem to indicate that the L TU HC5-C & L AAB (adjusted R^2 = 0.25, *see equation 45, see figure 4.2*), which was also statistically validated, or L TU HC-C (adjusted R^2 = 0.20, *see equation 43*) models are the best for predicting responses of insecta families to insecticide exposure.

L TU HC5-C & L AAB

Count Ratio of Effect = [0.2002 + (0.1950 * L TU HC5-C) + (0.1374 * L AAB)]

Equation 45

4.3.1 Summary of Insecta Family Modelling Results

The results from the best models indicate that HC5-C is the best TU predictor variable, and L AAB is an important fate parameter for modelling the proportion of insect families affected by insecticide exposure. However, it should be noted that the predictive power

for insecta is not as good as for crustacea with adjusted R^2 values within the range of 0.20 to 0.27. Nonetheless, the proposed best models for predicting impacts of insecticides on insect family (L TU HC5-C & LAAB, L TU HC5-C) and L TU HC5-I were generated using all data collected. Since L AAB was statistically significant and both the L TU HC5-C and L TU HC-I models had the lower adjusted R^2s (0.24 and 0.18 respectively), the impact predicted at 0.1 * TU was calculated based on the L TU HC5-C & L AAB (adjusted $R^2 = 0.29$, *see equation 46, see figure 4.3*) model.

L TU HC5-C & L AAB

Count Ratio of Effect = [0.1410 + (0.2104 * L TU HC5-C) + (0.1321 * L AAB)]

equation 46

The lowest (-0.34), highest (2.31) and average (0.985) values for L AAB found in the database were placed into the model equation to determine the level of effect a 0.1 * TU HC5-C would have on insecta families. On average, an estimated six percent of insect families are predicted to be affected at insecticide concentrations at 0.1 * TU HC5-C. However, insecticides that degrade rapidly by means of aquatic aerobic biotransformation are not predicted to affect abundance, but those that are resistant to this breakdown are predicted to cause up to 24% of families being affected. A protective threshold concentration at 1 * TU HC5-C will reduce at least 10% of insect families with an average reduction of about 27%. Therefore such a threshold is not protective of aquatic insects.

4.4 Discussion

The results indicate that laboratory toxicity data alone and/or with other variables can create statistically significant models capable of predicting field effects; and generally inclusion of fate, physico-chemical and system structure properties to the models improved predictions, however in most cases they (these additional variables) were not statistically significant. Aerobic aquatic biotransformation half-life, which is the closest laboratory fate parameter to a field half-life, was the best and only statistically significant predictor variable other than the toxicity units.

Unfortunately, the validation of all the best approximating models with an independent dataset proved to be problematic, due to missing toxicity units, fate properties and most popularly system structural properties. But, all the models that were tested for validation passed; the impacts they predicted are statistically correlated to the impacts seen in insecticide experiments.

However, the adjusted R^2s are low, ranging from 0.20 to 0.27. While the fact that the count ratio of effect (the dependent variable) did not take into account when the maximum effect was detected or its duration and some were made up from results reported at the species level, may be on reason for poor model predictability; it is more likely the predictor variables initially entered into the AIC are not the best to predict impacts of pesticides on insects. Therefore when coupled with the diversity of insects and complexities of dynamic ecosystem relationships and conditions (predator-prey, generation time, life-cycle stage, system physico-chemical properties, etc) the models

were unable to predict the likely effects of insecticide exposure on insect families. This is partly supported by the preference of a crustacea based toxicity unit to an insecta based one.

The hazard concentration for crustacea toxicity unit is not only included in the majority of best approximating models, but is also found in the more predictive models, i.e. those models with higher adjusted coefficients of determination. It should be noted that the coefficients of determination for those models based on HC5-I or HC5-C TUs alone, are not very different, varying by two (42 entries) to six (>60 entries) percent. Nonetheless, the results seems to indicate laboratory insect toxicity data is rather lacking to the extent that a hazard concentration value based on insecta less reflects the effects of insecticides on insects than a hazard concentration value based on crustacea. This poor characterisation of insect toxicity data may explain why the best models have such low predictive powers.

Our models support the hypothesis that TUs based on HC5s (HC5-C and HC5-I) are better predictors than those based on the geometric means of the most common required species for registration (*Daphnia*). However, it is worthy to note that the Daphnia toxicity unit was unable to statistically explain the variance observed in insecta responses to insecticide exposure, especially since risk assessors widely use some laboratory based *Daphnia* value to determine the effect an insecticide would have on the aquatic environment. This is done simply because *Daphnia* is thought to be the most sensitive or amongst the most sensitive to insecticides. Given that the Daphnia toxicity units can not

predict insecticide effects on aquatic insects, it is left to wonder if they can therefore protect them.

Our best model based on hazard concentration for crustacea toxicity units and aerobic aquatic half-lives (L TU HC5-C & L AAB; adjusted $R^2 = 0.29$, *see equation 46*) predicts that an average of 6% of families can be affected at 0.1 times a median HC5-C based on acute toxicity data and at least 10% will be affected at the HC5-C concentration. With the exception of a few insecticides (temephos, azinphos-methyl) all others in our database show that 0.1 * HC5-C values that are between 1.28 (chlorpyrifos) and 246 (lindane) times more conservative than the 0.01 * Daphnia values. So our results contradicts Brock *et al.* (2000a) and Brock *et al.* (2006) who concluded that their 0.01 * Daphnia and median HC5 values (our study also used median HC5 values) can protect the environment against insecticide induced effects. While differences exist among the toxicity values, classification of effects and methods of determining protectiveness of the threshold concentration used by Brock *et al.* (2000a), Brock *et al.* (2006) and our study may account for the different conclusions, it still appears very unlikely that a threshold concentration at 0.01 * Daphnia can protect aquatic insect.

Tables and Figures for Chapter 4

Figure 4. 1 - Taxonomic tree of insects in dataset used for modelling responses at the species level.

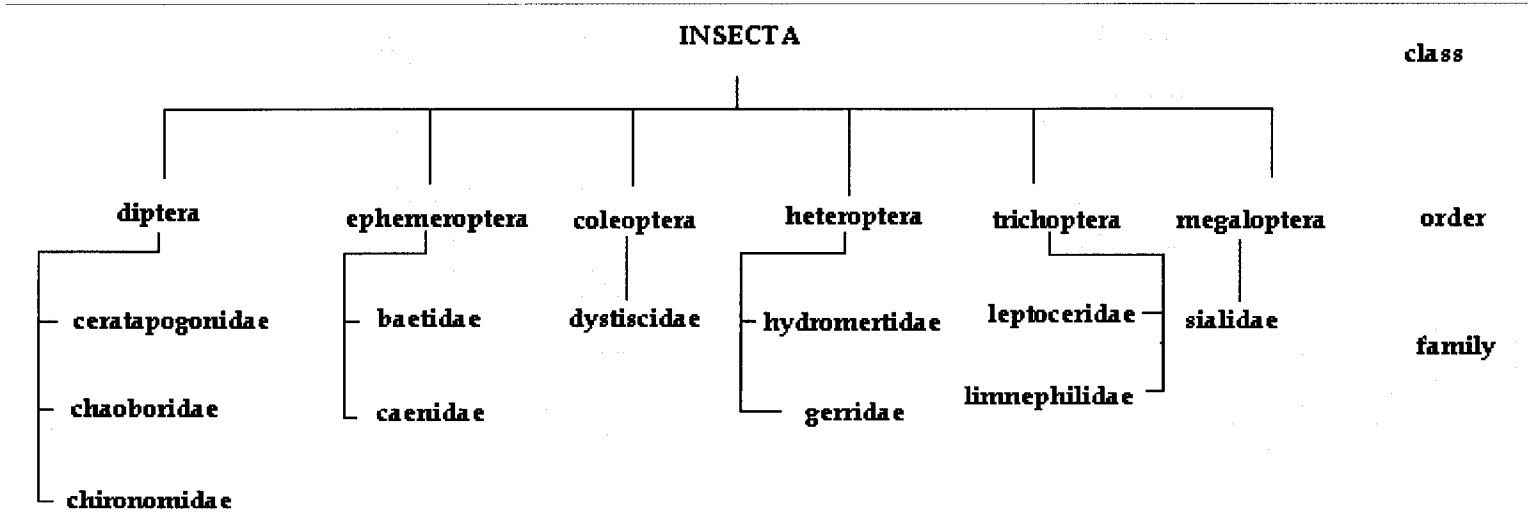


Table 4. 1 – Model selection by AIC for Count Ratio of Effects for insecta species validation-training set data.

Var 1	Var 2	Var 3	Df	Δ AICc	Akaike weight ratio	Adjusted R^2	L ratio Chi^2	p	r	N	r (N = 12)
*L TU HC5 - C	L HHL		2	0.00	1.00	0.34	11.85175	0.002669	.3719 p=.156	16	.2976 p=.347
L TSA/V R	L TU HC5 - C	L HHL	3	1.44	2.05	0.33	12.63474	0.005497	.3433 p=.275	12	.3433 p=.275
L TU HC5 - C	L WPHL	L HHL	3	1.49	2.10	0.33	12.58550	0.005624	.3549 p=.177	16	.2421 p=.448
L TU HC5 - C	LAAB		2	1.86	2.53	0.29	9.99321	0.006761	.4726 p=.064	16	.2416 p=.449
L TU HC5 - C	log Kow	L HHL	3	1.99	2.70	0.32	12.08255	0.007106	.3764 p=.151	16	.2957 p=.351
											.4126
L TU HC5 - C			1	3.79	6.65	0.19	5.86557	0.015440	.5574 p=.025	16	p=.183 .4111
L TU HC5 - I			1	5.14	13.07	0.14	4.51453	0.033608	.4193 p=.136	14	p=.184 .2976

N = 23

* proposed best model for dataset

Key

Var – variable
Df – degree of freedom
ΔAICc - AICc difference

r – validation correlation coefficient

LAAB – log-transformed aerobic aquatic biotransformation

LHHL – log-transformed hydrolysis half-life

L TU HC5 – C – log-transformed toxic unit based on hazard concentration for 5% of crustacea species

LTU HC5 - I – log-transformed toxic unit based on hazard concentration for 5% of insecta species

L TSA/V R - log-transformed total surface area to volume ratio

Table 4. 2 – Model selection by AIC for Count Ratio of Effects for insect species using all available data.

Var 1	Var 2	Var 3	Df	Δ AICc	Akaike weight ratio	Adjusted R^2	L ratio Chi^2	p
* L TU HC5 - C	L HHL		2	0.00	1.00	0.20	10.06081	0.006536
L TU HC5 - C			1	0.62	1.36	0.16	7.33062	0.006779
L TU HC5 - C	log Koc		2	1.12	1.75	0.18	8.94336	0.011428
L TSA/V R	L TU HC5 - C	L HHL	3	1.26	1.88	0.20	10.91470	0.012196
L Volume	L TU HC5 - C	log Koc	3	1.59	2.22	0.19	10.58683	0.014183
L TU HC5 - C	LAAB		2	1.75	2.40	0.16	8.31306	0.015662
L TU HC5 - C	log Koc	L HHL	3	1.86	2.54	0.18	10.31898	0.016041
L Volume	L TU HC5 - C	L HHL	3	1.86	2.54	0.18	10.31878	0.016042
L TU HC5 - I			1	1.87	1.00	0.13	6.07979	0.013674
L Volume	L TU HC5 - C		2	1.91	2.60	0.16	8.15316	0.016965
L TU Daphnia			1	5.45	2.55	0.04	2.49993	0.113851

N = 35

Key

Var – variable
Df – degree of freedom
ΔAICc - AICc difference
r – validation correlation coefficient

LAAB – log-transformed aerobic aquatic biotransformation

LHHL – log-transformed hydrolysis half-life

LTU Daphnia – log-transformed toxic unit based on geometric mean for Daphnia species

L TSA/V R – log-transformed total surface area to volume ratio

L TU HC5 – C – log-transformed toxic unit based on hazard concentration for 5% of crustacea species

L TU HC5 – I – log-transformed toxic unit based on hazard concentration for 5% of insecta species

L Volume - log-transformed volume

L WPHL – log-transformed water photolysis half-life

log Koc – log-transformed organic carbon absorption coefficient

* highlight – proposed best model for dataset

Table 4. 3 – Model selection by AIC for Count Ratio of Effect from insecta family using all data with all dependent variables.

Var 1	Var 2	Var 3	Var 4	Df	Δ AIC c	Akaike weight ratio	Adjusted R^2	L ratio Chi^2	p	r	N
* L TU HC5C	L AAB			2	0.00	1.00	0.25	14.27225	0.000796	.6455 p=.002	21
L TSA/V R	L TU HC5C	L AAB		3	0.59	1.34	0.26	15.77622	0.001260		
L TU HC5C	L WPHL	L AAB		3	0.62	1.36	0.26	15.74399	0.001280		
L TU HC5C	Log kow	L AAB		3	0.72	1.43	0.26	15.64612	0.001340		
L TU HC5C	L HHL			2	0.77	1.47	0.24	13.49771	0.001172	.4795 p=.028	21
L TSA/V R	L TU HC5C	L WPHL	L AAB	4	1.09	1.73	0.27	17.37253	0.001636		
L TU HC5C	LOG KOW			2	1.12	1.75	0.23	13.15705	0.001390	.5181 p=.014	22
L TSA/V R	L TU HC5 I			2	1.24	1.86	0.23	13.02921	0.001482		
L TU HC5C	Log kow	LASB		3	1.36	1.97	0.25	15.00413	0.001813		
L TU HC5C	LASB			2	1.45	2.06	0.23	12.82506	0.001641	.7640 p=.000	21
L TU HC5 I	L HHL			2	1.48	2.09	0.22	12.79302	0.001667	.5608 p=.012	19
L Vol	L TU HC5C	log Kow		3	1.55	2.17	0.24	14.81637	0.001980		
L TU HC5C	Log kow	L HHL		3	1.66	2.29	0.24	14.70608	0.002086		
L TSA/V R	L TU HC5C	Log kow	L AAB	4	1.78	2.43	0.26	16.68499	0.002225		
L TSA/V R	L TU HC5C			2	1.78	2.72	0.22	12.49296	0.001937		
L TSA/V R	L TU HC5 I	L HHL		3	1.89	2.43	0.24	14.47160	0.002329		
L TSA/V R	L TU HC5C	Log kow		3	1.94	2.58	0.23	14.42767	0.002377		
L TU HC5C	LOG KOC	L AAB		3	1.95	2.63	0.23	14.41609	0.002390		
L TU HC5C				1	1.85	2.65	0.20	10.33423	0.001306	.7030 p=.000	22
L TU HC5 I				1	2.63	3.73	0.18	9.54992	0.002000	.6550 p=.002	20

N = 42

* proposed best model for dataset

Key

Var – variable
Df – degree of freedom
ΔAICc - AICc difference
r – validation correlation coefficient

LAAB – log-transformed aerobic aquatic biotransformation

LASB - log-transformed aerobic soil biotransformation

LHHL – log-transformed hydrolysis half-life

L TSA/V R – log-transformed total surface area to volume ratio

L TU HC5 – C – log-transformed toxic unit b hazard concentration for 5% of crustacea species

L TU HC5 – I – log-transformed toxic unit based on hazard concentration for 5% of insecta species

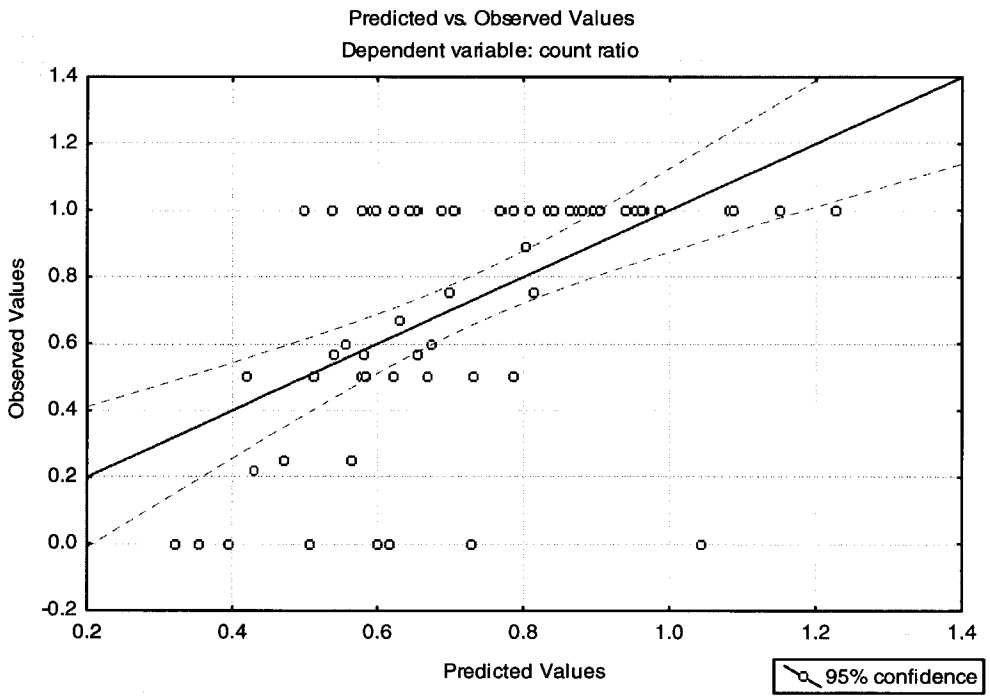
L Vol - log-transformed volume

L WPHL – log-transformed water photolysis half-life

log Koc – log-transformed organic carbon absorption coefficient

log kow - log-transformed octanol – water coefficient

Figure 4. 2- Best insecta family model (based on 65 entries) – L HC5-C & L AAB



Key

L TU HC5 – C – log-transformed toxic unit based on hazard concentration for 5% of crustacea species

LAAB – log-transformed aerobic aquatic biotransformation

CHAPTER 5 - ALGAE

5.1 Modelling Algal Species Abundance Responses

Algae are important to the aquatic community and changes in their function or structure can impact the entire ecosystem. They are the main primary producers in most freshwater ecosystems, and are food for many aquatic invertebrates such as cladocera and copepoda. Given their trophic level and function, they are sensitive to both changes in biological and physico-chemical properties of a system. For example, a reduction in the predatory organism or increase in phosphorus can both lead to an algal bloom. So a change in their abundance can be a quick indication of changes within an aquatic ecosystem.

Data used for generating models for this aspect of the research came from lentic systems and represented effects (both increases and decreases) based on species abundance. Eight experiments had at least one algae species that increased metribuzin (2), atrazine (1), fenthion (2), and cypermethrin (4).

A count ratio of effect, which represented the number of algal species affected (both increases and decreases) divided by the total number of algal species found in that experiment, was the dependent variable (*see equation 4 in 2.3*).

Given the lack of single species toxicity data it was decided that TUs based on these values would not be entered into the AIC. The three log-transformed toxic units (TUs) entered into the AIC analysis were: 1) hazard concentrations for 5% of the algal species (L TU HC5 - A); 2) geometric means of *Daphnia* species (L TU Daphnia); and 3) hazard

concentrations for 5% of the crustacean species (L TU HC5 - C). This was done to test the hypothesis that states the HC5s better predict the effects seen. Even though the crustacea toxicity data were not correlated with HC5-A, they were included to see how well they predict in comparison to these more scientifically based TUs. In addition, the EU's Uniform Principles use a multiplier factor based on the most sensitive standard test species for a pesticide to derive a No Effect Concentration (NEC) for pesticides in surface water (Van den Brink *et al.* 2003; Brock *et al.* 2001). A multiplier of 0.01 is used on Daphnia L(E)C50 or a more sensitive standard fish is used for insecticides; and 0.1 is used for EC50 algae for herbicides.

The magnitude of impact was assessed at 0.01 * TU Daphnia or 0.1 * TU HC5-C or 0.1 * TU HC5-A for the best models. The latter two levels are selected because risk assessors usually using NOEC values for SSD extrapolations, but our research uses HC5 values based on geometric means for L(E)C50s. Therefore a safety factor of ten, similar to Van den Brink *et al.* (2003), was used.

Both structural properties of system log-transformed (volume – L volume and surface area to volume ratio - L TSA/V R) and all log-transformed fate and physico-chemical properties of the pesticide (octanol-water partition coefficient – log Kow, organic carbon absorption coefficient – log Koc, aerobic soil biotransformation - L ASB, aerobic aquatic biotransformation - L AAB, water photolysis half-life - L WPHL, hydrolysis half-life - LHHL) were included. This facilitates testing the hypothesis stating these properties help to predict the effects observed.

5.2 Modelling Algal Species Abundance Responses to All Pesticides

The 41 entries (30 from herbicides) available for modelling, i.e. those having all fate, physico-chemical properties and system structural properties, came from 12 studies; and covered three insecticides (cypermethrin, fenthion, lambda-cyhalothrin) and seven herbicides (alachlor, atrazine, dichlobenil, hexazinone, metamitron, metribuzin, metsulfuron methyl). A validation sample was collected from the available algae data; it consisted of four insecticides (fenthion, lambda-cyhalothrin, permethrin, esfenvalerate) and six herbicides (alachlor, atrazine, hexazinone, metamitron, metribuzin, metsulfuron methyl).

The species that make up the data used for modelling came from nine phyla, however only 5% of the species belonged to dinophyta, euglenophyta, haptophyta, myzozoa or pyrophyta. Chlorophyta species contributed to about 33% of the data, with the class chlorophyceae containing 31% of the total data and conjugophyceae and eophyceae making up the remaining two percent. The cryptophyta families of pyrenomonadaceae and cryptomonadaceae added nine percent of the species data; while the cyanobacteria contributed 19%, with the class chroobacteria being the major contributor (12% of total species data). Thirty – four percent of the species belonged to the phylum ochrophyta of which the class bacillariophyceae was the main contributor (17% of total species data). The ochrophyta class Coscinodiscophyceae added to about eight percent of the total data, and its other classes - chrysophyceae, fragilariophyceae and synrophyceae contained approximately eight percent of the species used for modelling.

The good representation of herbicide data allowed for separate AIC analyses, which thereby facilitated testing the hypothesis that better model predictability is achieved when data is grouped according to its pesticide type. Given the small sample size, it was decided that a validation- training set method would be used in addition to modelling all available data.

Unfortunately computational violations occurred when all the data was entered into the software for AIC analysis. The programme encountered a few pivot errors, which led to an under reporting of degrees of freedom values. This was rectified before further analyses were done.

5.2.1 Models based on Training Set Data

Seventy-one model combinations were possible with a TU and uncorrelated variables. Seven of these models can be considered to the best according as the AICc difference of less than or equal to two (*see table 5.1*). All of these models were statistically significant below p= 0.05 and the adjusted R^2s are within a range of 0.31 to 0.37.

The validation of the seven models varied according to the size of the validation sample used. Validation done using entries with all its fate, physico-chemical, respective TUs and system structural properties, resulted in none of the models being statistically validated below p=0.05. However, when the dataset was only modified according to the parameters present in the model, three models could be validated. All three of the

validated models had either log Koc or log Kow as an explanatory variable, but the four that did not contain these variables could not be validated.

Five of the seven models have HC5-C as its predicting TU variable, and the other two use Daphnia spp. Of the 999 models generated by the AIC the L TU HC5-A model was not included and when the model was generated it was found that it is not statistically significant. However, the model based on the HC5-C TU (adjusted $R^2 = 0.12$, *equation 47*) is statistically significant, but the one based on the Daphnia TU is not.

L TU HC5-C

$$\textit{Count Ratio of Effect} = [0.1233 + (0.1692 * \text{L TU HC5-A})]$$

equation 47

All seven best approximating models contain a structural property of the system; three have L Volume and the other four L TSA/V R. These structural properties are statistically significant contributors in all of the models. More effects are expected in the systems that had smaller volumes and those with greater surface area to volume ratio. L AAB is considered a statistically significant contributing factor in all of the seven models. An increased level of effects is expected with increased aerobic aquatic biotransformation half-lives. Two models include log Kow and another has log Koc, however none of these properties were considered as statistically significant contributors to the models at p-values below 0.05.

These results indicate that LAAB, physical properties of the system and either the Daphnia or HC5-C TU are important for modelling proportion of algal species affected

by pesticide exposure. Given the close predictive range of these best approximating models, the statistical significance of the previously mentioned variables and statistical validation, proposing a single best model for this data is difficult. So using an Akaike weight ratio of less than two, the two best models are 1) L TSA/VR , L TU Daphnia & LAAB (adjusted R^2 = 0.33, *see figure 5.1*) and 2) L TSA/VR , L TUHC5-C & LAAB (adjusted R^2 = 0.32, *see figure 5.2*). The equations for these two models are:-

L TSA/VR, L TU Daphnia & LAAB

Count Ratio of Effect = [-0.318 + (0.3466 * L TSA/VR) + (0.1153 * L TU Daphnia) + (0.4519 * L AAB)]

equation 48

L TSA/VR, L TU HC5-C & LAAB

Count Ratio of Effect = [-0.4174 + (0.3240 * L TSA/VR) + (0.0913 * L TU HC5-C) + (0.4087 * L AAB)]

equation 49

5.2.2 Models based on All Available Pesticide Data

Forty-four model combinations were possible with a TU and uncorrelated variables. Only two these models can be considered to the best according as the AICc difference of less than two (*see table 5.2*). Both models are statistically significant below p=0.05 and their adjusted R^2s are 0.33 and 0.36. However, models based only on TUs predictors are not

included among the 999 models generated by the AIC. Upon generation it was found that these models were statistically insignificant.

Inclusion of the four models that have an AICc difference of less than three enables the examination of some possible trends. All six models contain a structural property of the system; the best five have L TSA/V R and the sixth include L Volume. These structural properties are statistically significant contributors in all of the models. More effects are predicted in the systems that have smaller volumes and those with greater surface area to volume ratio.

Aerobic aquatic biotransformation half-life is considered a statistically significant contributing factor in all of the seven models, and increased levels of effects are expected with increased L AAB values. Three models include log Kow, two others contain log Koc and the best two models (L TSA/V R, L TU HC5-C, log Kow & L AAB and L TSA/V R, L TU HC5-C, log Koc & L AAB) have one of these properties and in both cases they are considered as statistically significant contributors to the models at p-values below 0.05. However, they are only considered as a statistically significant contributor in one of the other three models. Increased Kow or Koc are expected to produce less observed algal effects.

Both best approximating models according to the AICc difference of less than two have all of its variables as statistically significant contributing factors. Even though the predictive ability of these two models is relatively close (3% difference), the Akaike

weight ratio indicates that the most likely model is more than twice better than the next probable model. Consequently, the most likely model - L TSA/VR, L TUHC5-C, log Kow & LAAB (adjusted $R^2 = 0.36$, *see equation 50, see figure 5.3*) can be considered as the best.

L TSA/VR, L TUHC5-C, log Kow & LAAB

$$\textit{Count Ratio of Effect} = [-0.3638 + (0.5026 * \text{L TSA/VR}) + (0.2137 * \text{L TUHC5-C}) + (-0.1227 * \log \text{Kow}) + (0.5466 * \text{L AAB})]$$

Equation 50

5.3 Modelling Algal Abundance Response to Herbicides

Most pesticides have very specific modes of actions that enable them to kill their intended target and in many cases organisms that have similar physiology as the intended target species. Consequently, algae are much more sensitive towards herbicides than any other groups of pesticides. In fact, many of the insecticide studies reviewed for this research showed marked effects on invertebrates but none was seen with algae (Stephenson *et al.* 1989; Kristin & Kaushik, 1987; Wendt-Rasch *et al.* 2003; Samsoe-Peterson *et al.* 2001; Hanazato & Kasai, 1995; Flidnew & Klein, 1996; etc.). Therefore, there is much to gain from predicting algal effects based only on herbicides.

Given the small sample size of the herbicide dataset; 30 entries on seven herbicides (alachlor, atrazine, dichlobenil, hexazinone, metamitron, metribuzin, metsulfuron-methyl), data from a validation-training set method in addition to grouping all available

entries were used to model algal effects. The validation sample consisted of alachlor, atrazine, hexazinone, metamitron, metribuzin and metsulfuron-methyl.

5.3.1 Models based on Training Set Data

One hundred and sixteen model combinations were possible with a TU and uncorrelated variables, ten can be considered to the best according as the AICc difference of less than or equal to two *(see table 5.3)*. All ten models were statistically significant below p=0.05, however only four of the ten best approximate models can be validated using the data from the independent dataset. The adjusted R^2s range from 0.18 to 0.32.

All ten models have HC5-A as its predicting TU variable, in addition the L TU HC5-A (adjusted R^2 = 0.18, *see equation 51*) model is included among these ten. The models based only on HC5-C and Daphnia TUs are included among the 999 models generated by the AIC. However, these models were not of statistical significance and had similar adjusted R^2s.

L TU HC5-A

$$\textit{Count Ratio of Effect} = [0.1233 + (0.1692 * \text{L TU HC5-A})]$$

equation 51

Nine of the ten best approximating models contain a structural property of the system; five have L volume and the other four L TSA/V R. These structural properties are statistically significant contributors in five of the models. More effects are expected in the

systems that have smaller volumes and those with greater surface area to volume ratio. L AAB, L WPHL and log Koc are all statistically significant contributors to the best approximating models.

It seems as though HC5-A TU is the best for modelling effects of herbicides on algal species, and the structural properties of the system and L AAB are also important. The L TSA/V R, L TU HC5-A & L AAB (adjusted $R^2 = 0.32$, *see equation* 52, *see figure 5.4*) and L Volume, L TU HC5-A & L AAB (adjusted $R^2 = 0.31$, *see equation 53, see figure 5.5*) models are probably the best for the dataset.

L TSA/V R, L TU HC5-A & L AAB

Count Ratio of Effect = [-0.5734 + (0.3384 * L TSA/VR) + (0.1906 * L TUHC5-A) + + (0.2793 * L AAB)]

equation 52

L Volume, L TU HC5-A & L AAB

Count Ratio of Effect = [-0.2546 + (-0.1261 * L Volume) + (0.2013 * L TUHC5-A) + + (0.2522 * L AAB)]

equation 53

5.3.2 Model based on All Available Herbicide Data

Sixty-five model combinations were possible with a TU and uncorrelated variables, eight of which can be considered to the best according as the AICc difference of less than two (*see table 5.4*). All of these models are statistically significant below p=0.05. The adjusted R^2s fall within the range of 0.38 to 0.41.

All eight models have HC5-A as its predicting TU variable, and the model with HC5-A (adjusted $R^2 = 0.26$, *see equation 54)* as the sole predictor was included among the 999 models generated by the AIC. However, the L HC5-A model did not have enough evidence supporting it is one of the best approximating models for this dataset. The models based on HC5-C and Daphnia TUs were not among the AIC model output and when modelled they were found to be statistically insignificant.

L TU HC5-A model

$$\textit{Count Ratio of Effect} = [0.1031 + (0.1930 * \text{L TU HC5-A})]$$

equation 54

The eight best approximating models all contain a structural property of the system; four have L Volume and the other four L TSA/V R. These structural properties are statistically significant contributors in six of the models. More effects are expected in the systems that have smaller volumes and those with greater surface area to volume ratio.

L AAB is found in all of these best approximating models; it is considered statistically insignificant in four models, and more effects are expected with increased L AAB. Two

models included L WPHL as an explanatory variable, two contained log Kow and one has log Koc. However, none of these properties can be considered as statistically significant contributors to the models.

It must be noted that all the models can be grouped into four sets of pairs with the only differing variable being which structural property it contained. The results seem to indicate that HC5-A TU is the best for modelling effects of herbicides on algal species; and structural properties of the system along with L AAB are also vital for improving model predictions.

The only best approximating models with all of its predictor variables as statistically significant contributing factors is L TSA/V R, L TU HC5-A & L AAB (adjusted R^2 = 0.39, *see equation 55, see figure 5.6*) and there is no evidence to not accept this model as the best for this dataset.

L TSA/V R, L TU HC5-A & L AAB

$$\textit{Count Ratio of Effect} = [-0.6618 + (0.3036 * \text{L TSA/VR}) + (0.2105 * \text{L TUHC5-A}) + (0.3268 * \text{L AAB})]$$

equation 55

5.4 Summary of Algal Species Modelling Results

The best models for algal species abundance count ratio of effect can account for 31 to 39% of the variance seen and the best models based on all pesticides and herbicides alone

based models have similar predictive powers for the best training set models; the adjusted coefficients of determination ranged from 0.31 to 0.33. While the best model based on all available herbicide data (see equation 55) is slightly better than the two best models based on all available pesticide data (*see equations 52 and 53*). In addition, the former model is more parsimonious than the latter ones.

Similar predictor variables are used for best models based on all pesticides and herbicides only, with the main difference being the predictor toxicity unit variable. Models that are based on all pesticides have HC5-C or Daphnia as the best predictor, while models based on herbicides alone have HC5-A. However, best models from both types of modelling show aquatic aerobic half-life and surface area to volume ratio improves model predictions.

Given the relatively small sample size and poor representation of insecticides, impacts at the threshold concentration was calculated using the most predictive model - L TSA/V R, L TU HC5-A & L AAB (adjusted R^2 = 0.39, *see equation 55*), which is based only on herbicide data. It must be noted that this model was not statistically validated under p=0.05, however the correlation analysis show significance at p=0.09. Since the entire database only has 32 count ratio entries on algal abundance response to herbicide treatments, and model is based on 30 entries, predicted impacts were calculated at 0.1 * TU HC-A using equation 55.

Low and high values or both low or high, or average values were paired for model input. The L AAB values used are 0.38, 2.28 and 1.33 and the L TSA/VR values used are -1.09, 2.10, and 0.51. The L TSA/VR, L TU HC5-A & LAAB model predicted that no impacts to algae should be expected at or below 0.1 * TU HC5-A under the following conditions:-

1) herbicides resistant to AAB found in systems with low surface area to volume ratio (e.g. 1.2 : 1, which is characteristic of larger bodies of water).

2) herbicides with very short half-lives (<2.4days) found in systems with high surface area to volume ratios (e.g. a ratio of 26)

3) herbicides with very short half-lives (<2.4days) found in systems with low surface area to volume ratios (e.g. a ratio of 26)

4) herbicides with an AAB of 21 days found in a system that has a surface area to volume ratio of about 6 : 1.

However, 0.1 * TU HC5-A threshold would allow 30% of algae species to be affected by herbicides that are persistent found in systems with high surface area to volume ratios (e.g. a ratio of 26, most likely characteristic of a small system). While, a threshold value of 1 * TU HC5-A would not be able to protect algae from herbicides that resistant to AAB, leading to a 22% change in large systems and a 51% change in small systems.

5.5 Discussion

The results indicate that laboratory toxicity data alone and/or with other variables can create statistically significant models capable of predicting field effects. Unfortunately most of these models, including the best model, failed to be statistically validated with an

independent dataset at $p \leq 0.05$, in addition statistical validation varied with differing size of validation set. This alludes to small sample size having played a huge role in the results obtained. However, it must be noted that the correlation between the impacts predicted by the best model equation (L TU HC5-A & L AAB, *see equation 55*) is statistically significant at p=0.09.

The relative poor predictive power of the best models (adjusted R^2s = 0.31 to 0.39) may also be attributed to small sample size, since the sub-datasets used for modelling contained a minimum of 27 entries and a maximum of 41. The fact that all of the best models had three predictor variables may be caused by poor explanatory power of the variables used for modelling and/or biological factors such as competition, secondary effects, predation, generation time of the species and variation in species sensitivity.

As mentioned previously data used for modelling contained statistically significant increases and decreases for both insecticides and herbicides (fenthion, cypermethrin, metribuzin, atrazine). This provides evidence that biological factors as well as toxicity played a major role in determining algal response, and it appears as though accounting for these species interactions can not be accomplished through the use of laboratory single species toxicity data. The possibility exists that the effects caused by the insecticides are largely secondary, probably stemming from reduction in algae feeders such as crustacea.

Another factor that may have contributed to an overall poor fit was the method used to obtain the overall species impacts – the count ratio. The count ratio does not take into account the when the maximum effect was detected or its duration, and is dependent on the number of species the authors choose to report on. Of course, the variation in experimental design and tests used to ascertain impacts, may have affected how well modelling can be done. It is interesting to note that half of the count ratio entries are no effects which were even observed at high concentrations. It is quite possible that the fast generational times algae may have hidden any pesticide impacts.

Even though the three to four insecticides (cypermethrin, fenthion, lambda-cyhalothrin and/or permethrin) in the dataset only represented about a quarter of the entries used for modelling, models based on all pesticide data had a distinct preference for Daphnia or HC5-C toxicity predictor variable. While, models based only on herbicide data preferentially included the HC5-A variable. It must be noted that HC5-A and Daphnia or HC5-C toxicity values were not shown to be correlated (*see table 2.1*).

The best fate predictor variable - aerobic aquatic biotransformation half-life - is predicted to cause more algal species effects if the degradation time is longer, which is as expected because the pesticide would spend a longer time within the system. Unlike the other fate parameters, aerobic aquatic biotransformation half-life can be considered the laboratory equivalent to a field half-life test given it is done using both water and sediment. Also compared to the other fate parameters used (hydrolysis, photolysis, aerobic soil biotransformation), the aerobic aquatic biotransformation half-life values were more

varied compared to having nearly many herbicides being resistant to degradation through those processes. However, the number of pesticides used for modelling is quite small (at most seven herbicides, four insecticides), and this may have played a major role in the selection of the most predictive fate parameter.

Our models predict that 0.1 * TU HC5 – A should comfortably protect algal species against herbicides that have an aerobic aquatic biotransformation half-life of 21days or less, but probably not against those herbicides that are resistant to aerobic aquatic biotransformation and found in systems with high surface area to volume ratio (which is characteristic of smaller bodies of water). While the concentration at HC5 for algae would not protect against persistent herbicides, regardless of how large the system is. Our results contradict those of Brock *et al.* (2006) who claim that a threshold concentration at the median HC5 based on acute data or the lower-limit HC5 based on chronic data would be protective of algae. Like our study their conclusion is based on very few herbicides (7) but given the possibility of small sample bias the percent affected at 0.1 * TU HC5-A or 1 * TU HC5-A should be accepted with caution, since it appears that small sample size may have had an impact on model predictability and validity. However, the best model - L TU HC5-A & L AAB - did have an adjusted R^2 = 0.39 (*see equation55)* and was statistically validated at p=0.09.

*A threshold herbicide concentration at 0.1 * TU HC5-A should not impact algae species abundance under normal conditions and even in the worse case scenario.*

Tables and Figure for Chapter 5

Table 5. 1 – Model selection by AIC for System Effect Change Ratio for all pesticides a validation-training set method.

Var 1	Var 2	Var 3	Var 4	Df	Δ AICc	Akaike weight ratio	Adjusted R^2	L ratio Chi^2	p	r	N	r (N=12)
L TSA/V R	L TU HC5 - C	log Kow	LAAB	4	0.00	1.00	0.37	17.03449	0.001903	.5966 p=.024	14	.5714 p=.052
* L TSA/V R	L TU Daphnia	LAAB		3	0.70	1.42	0.33	14.14525	0.002714	.5243 p=.054	14	.4992 p=.098
* L TSA/V R	L TU HC5 - C	LAAB		3	1.10	1.74	0.32	13.74489	0.003274	.4113 p=.144	14	.3725 p=.233
L TSA/V R	L TU HC5 - C	log Koc	LAAB	4	1.21	1.83	0.34	15.82711	0.003260	.5941 p=.025	14	.5546 p=.061
L Volume	L TU Daphnia	LAAB		3	1.42	2.04	0.31	13.42203	0.003807	.5085 p=.053	15	.4905 p=.105
L Volume	L TU HC5 - C	LAAB		3	1.66	2.29	0.31	13.18608	0.004251	.3908 p=.150	15	.3630 p=.246
L Volume	L TU HC5 - C	log Kow	LAAB	4	1.90	2.59	0.33	15.13261	0.004434	.5355 p=.040	15	.5282 p=.078
L TU HC5 - C				1	5.84	18.54	0.13	4.67968	0.030521	.1372 p=.626	15	.1491 p=.644
L TU Daphnia				1	6.83	30.39	0.09	3.69166	0.054685	.2129 p=.446	15	.2279 p=.476

N = 27

* proposed best model for dataset

Key

Var – variable **Df** – degree of freedom **ΔAICc** - AICc difference **r** – validation correlation coefficient
LAAB – log-transformed aerobic aquatic biotransformation **L Volume** - log-transformed volume
LTSA/V R – log-transformed total surface area to volume ratio **log Koc** – log-transformed organic carbon absorption coefficient
LTU Daphnia – log-transformed toxic unit based on geometric means for Daphnia species **log kow** – log-transformed octanol-water coefficient
L TU HC5 – C – log-transformed toxic unit based on hazard concentration for 5% of crustacea species

Table 5. 2 – Model selection by AIC for System Effect Change Ratio for all pesticides using all data.

Var 1	Var 2	Var 3	Var 4	Df	Δ AICc	Akaike weight ratio	Adjusted R^2	L ratio Chi^2	p
L TSA/V R	L TU HC5 - C	log Kow	LAAB	4	0.00	1.00	0.36	22.46830	0.000162
L TSA/V R	L TU HC5 - C	log Koc	LAAB	4	1.78	2.43	0.33	20.68885	0.000365
L TSA/V R	L TU Daphnia	log Kow	LAAB	4	2.14	2.92	0.32	20.32395	0.000431
* L TSA/V R	L TU Daphnia	LAAB		3	2.32	3.20	0.30	18.04251	0.000431
L TSA/V R	L TU Daphnia	log Koc	LAAB	4	2.70	3.86	0.31	19.76732	0.000555
L Volume	L TU HC5 - A	log Kow	LAAB	4	2.93	4.33	0.31	19.53839	0.000616

N = 41

* proposed best model for dataset

Key

Var – variable
Df – degree of freedom

ΔAICc - AICc difference

LAAB – log-transformed aerobic aquatic biotransformation

L Volume - log-transformed volume

LTSA/V R – log-transformed total surface area to volume ratio

log Koc – log-transformed organic carbon absorption coefficient

LTU Daphnia – log-transformed toxic unit based on geometric means for Daphnia species

log kow – log-transformed octanol-water coefficient

L TU HC5 – A – log-transformed toxic unit based on hazard concentration for 5% of algal species

L TU HC5 – C – log-transformed toxic unit based on hazard concentration for 5% of crustacea species

Table 5. 3 – Model selection by AIC for System Effect Change Ratio for all herbicides using a validation-training set method.

Var 1	Var 2	Var 3	Var 4	Df	Δ AICc	Akaike weight ratio	Adjusted R^2	L ratio Chi^2	p	r
* L TSA/V R	L TU HC5 - A	LAAB		3	0.00	1.00	0.32	11.36622	0.009902	.5907
										p=.094
* L Volume	L TU HC5 - A	LAAB		3	0.03	1.01	0.31	11.34055	0.010020	.7205
										p=.029
L Volume	L TU HC5 - A			2	0.19	1.10	0.27	8.91707	0.011579	.6080
										p=.082
L TSA/V R	L TU HC5 - A			2	0.60	1.35	0.26	8.51409	0.014164	.6014
										p=.087
L TSA/V R	L TU HC5 - A	L WPHL	LAAB	4	0.63	1.37	0.33	13.02633	0.011148	.7229
										p=.028
L Volume	L TU HC5 - A	L WPHL	LAAB	4	0.89	1.56	0.32	12.76370	0.012490	.7183
										p=.029
L Volume	L TU HC5 - A	L WPHL		3	1.27	1.88	0.27	10.09903	0.017743	.6240
										p=.072
L TSA/V R	L TU HC5 - A	L WPHL		3	1.60	2.22	0.26	9.76843	0.020641	.6231
										p=.073
L TU HC5 - A				1	1.73	2.37	0.18	5.15861	0.023131	.6588
										p=.054
L Volume	L TU HC5 - A	log Koc	LAAB	4	1.82	2.48	0.29	11.83978	0.018583	.7512
										p=.020
L TU Daphnia				1	4.90	11.59	0.04	1.98793	0.158557	.6720
										p=.047
L TU HC5 - C				1	4.94	11.84	0.04	1.94575	0.163046	.6257
										p=.071

N = 21

* proposed best model for dataset

Key

Var – variable
Df – degree of freedom
ΔAICc - AICc difference
r – validation correlation coefficient

LAAB – log-transformed aerobic aquatic biotransformation

LTSA/V R – log-transformed total surface area to volume ratio

LTU Daphnia – log-transformed toxic unit based on geometric mean for Daphnia species

L TU HC5 – A – log-transformed toxic unit based on hazard concentration for 5% of algal species

L TU HC5 – C – log-transformed toxic unit based on hazard concentration for 5% of crustacea species

L Volume - log-transformed volume

L WPHL – log-transformed water photolysis half-life

log Koc – log-transformed organic carbon absorption coefficient

Table 5. 4 – Model selection by AIC for System Effect Change Ratio for herbicides using all data.

Var 1	Var 2	Var 3	Var 4	Df	Δ AICc	Akaike weight ratio	Adjusted R^2	L ratio Chi^2	p
L TSA/V R	L TU HC5 - A	L WPHL	LAAB	4	0.00	1.00	0.41	20.27134	0.000441
* L TSA/V R	L TU HC5 - A	LAAB		3	0.00	1.00	0.39	18.11648	0.000416
L Volume	L TU HC5 - A	LAAB		3	0.12	1.06	0.39	17.99689	0.000440
L Volume	L TU HC5 - A	L WPHL	LAAB	4	0.52	1.29	0.40	19.75438	0.000558
L Volume	L TU HC5 - A	log Koc	LAAB	4	0.93	1.59	0.39	19.34362	0.000673
L TSA/V R	L TU HC5 - A	log Koc	LAAB	4	1.33	1.94	0.38	18.94870	0.000804
L Volume	L TU HC5 - A	log Kow	LAAB	4	1.37	1.97	0.38	18.91057	0.000818
L TSA/V R	L TU HC5 - A	log Kow	LAAB	4	1.70	2.34	0.38	18.57349	0.000953
L TU HC5 - A				1	3.80	6.67	0.26	10.03538	0.001536

N = 30

* proposed best model for dataset

Key

Var – variable
Df – degree of freedom
ΔAICc - AICc difference

LAAB – log-transformed aerobic aquatic biotransformation

LTSA/V R – log-transformed total surface area to volume ratio

LTU Daphnia – log-transformed toxic unit based on geometric mean for Daphnia species

L TU HC5 – A – log-transformed toxic unit based on hazard concentration for 5% of algal species

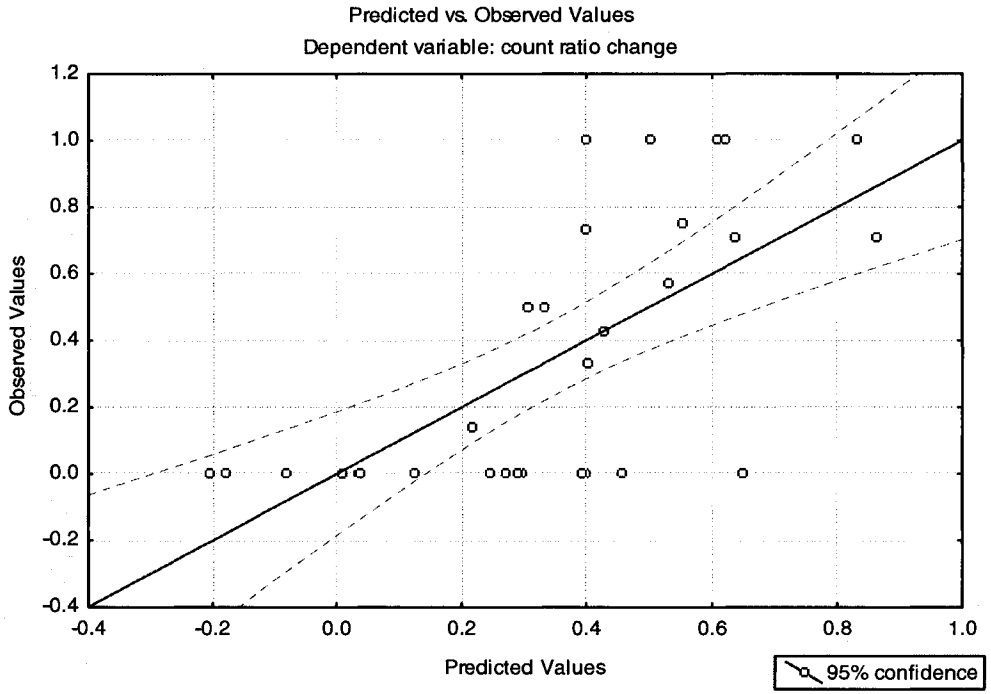
L TU HC5 – C – log-transformed toxic unit based on hazard concentration for 5% of crustacea species

L Volume - log-transformed volume

L WPHL – log-transformed water photolysis half-life

log Koc – log-transformed organic carbon absorption coefficient

Figure 5. 1– The best algae model (based on 30 herbicide entries) – L TSA/VR, L TU HC5-A & L AAB.



Key

LAAB – log-transformed aerobic aquatic biotransformation

LTSA/V R – log-transformed total surface area to volume ratio

L TU HC5 – A – log-transformed toxic unit based on hazard concentration for 5% of algal speci

CHAPTER 6 - General Discussions and Conclusions

6.1 General Discussions

This research generated predictive linear models for three major groups of freshwater organisms – crustacea, insecta and algae. Crustacea analyses were done at three levels - all crustacea species, cladocera and copepods; the latter two categories make up a large proportion of the freshwater invertebrates. Modelling was done using data from lentic systems and reflects abundance changes (expressed as count ratio of effects and abundance change ratio) caused by single applications of pesticides.

6.1.2 Predictability of Single Species Laboratory Toxicity Data

The results of this study show that laboratory single species toxicity data such as geometric means of Daphnia species or in the form of a Hazard Concentration for five percent of the species (crustacea - HC5-C, insecta – HC5-I, algae – HC5-A) are able to produce statistically significant models capable of predicting field effects of pesticides exposure on abundance within lentic systems. The use of HC5 values were preferred to the single species values, with the sole exception being for the count ratio of effects for crustacea species (Daphnia toxicity units were preferred). Since no alga or insect single species toxicity based units were used, this conclusion therefore only applies to crustacea and insects. Most of the models that are based only on the toxicity units are poor predictors and more predictive models are produced in combinations with fate (hydrolysis half-life, water photolysis half-life, aerobic aquatic biotransformation half-

life, aerobic soil biotransformation half-life), physico-chemical (log Kow, Koc) or system properties (volume, surface area to volume ratio) variables.

Of the three groups examined (crustaceans, algae and insects), crustacea analyses produced the most predictive models with best models having adjusted R^2s of 0.55 (cladocera – L TU HC5-C & L WPHL), 0.62 (copepoda L TU HC5-C & L AAB) and 0.55 (crustacea species – L TU Daphnia & log Kow & L HHL); while best insecta and algae models have an adjusted R^2 of 0.29 and 0.39 respectively. However, the range of predictive strength varied tremendously within each group and sub-group of organisms. All the best models for crustacea were validated using an independent dataset, but this proved to be problematic for insecta and algal models, many models failed the validation tests, while there was inadequate data to validate all the best approximating insecta family models. However, it must be noted that the best insect model was statistically validated, while the best algal model shows statistical significance for validation at p=0.09.

This validation problem and relatively poor predictive ability of algal and insect models may possibly have been caused by small sample size. Small sample size, coupled with fish presence, might have also contributed to the inconsistent results (e.g. varied effects of fate parameter on toxicity) obtained for cladocera. Also, the poor performance of algal and insect models likely results from to the inadequacy of the selected laboratory predictor variables to explain the field responses observed. While, laboratory toxicity data do not take into account species interactions and abiotic environmental factors,

thereby creating the tendency to underestimate pesticide induced effects; similar laboratory toxicity values were able to yield strong models for crustaceans. So the poor predictive ability of laboratory-derived toxicity data for algae and insects probably has more to do with insufficient sampling of these diverse taxonomic groups to adequately predict their susceptibility. One reason for the lack of data may be attributed to there being few species for which standard tests have been developed, in addition regulatory agencies do not insist on single species laboratory toxicity data for aquatic insects, but instead use Daphnia values to extrapolate protectiveness. The results show that insect models predict better using crustacean-based toxicity units, which therefore seems to indicate that characterisation of insect toxicity data is rather poor.

6.1.3 Pesticide Grouping and Fish Presence

The influence of pesticide grouping on the predictive strength of crustacea and algae models (only insecticide data were used for insect models) is evident but is somewhat quite minimal; this may be due to the under representation of those pesticides which the organism groups are considered to be less sensitive to.

However, the crustacea models show that fish presence is a greater influence to model predictability than pesticide grouping; the coefficients of determination for the best models are better by 10 to 20% in those models derived from data without fish and all the best models come from fish-free insecticide data. These results therefore support the recommendation of the workshop on Community Level Aquatic System Studies – Interpretation Criteria (CLASSIC) held during May –June, 1999 in Germany - fish

should not be included in a micro- or meso- cosm study if effects on invertebrates are important endpoints.

6.1.4 System Structural Properties

Even though volume or surface area to volume ratio is included in some of the best approximating models for algae, insecta and crustacea, and is a statistically significant contributor in some cases, these structural variables were generally not included in any of the best models except for algae (surface area to volume ratio).

However, an interesting finding in our study is that the best copepod models from fish-present and fish-free combined data included a structural property, but best model from the fish-free data did not require the inclusion of a structural property. The results also indicate that more effects are expected in smaller systems or those with larger surface area to volume ratios (which is usually associated with smaller systems). The reason for inclusion of a structural property is probably because secondary effects caused by fish are more pronounced in the smaller systems.

6.1.5 Physico-chemical Properties

Both physico-chemical properties collected for analyses – Kow and Koc – relate to the hydrophobicity of the pesticide. Like system structural properties these variables are included in some of the best approximating models and are even statistically significant

contributors to a few. However, the only best model that used a physico-chemical property (Kow) came from crustacea species data.

Worthy of note, is that the few models that contained Kow as a statistically significant contributor indicate that hydrophobic insecticides are a bigger threat to crustacea species (made up of 65% cladocera and 25% copepoda species), while copepoda and cladocera models suggest that pesticides that are hydrophilic will cause more reductions. The crustacea species results are similar to those of Mohlenberg *et al.* (2001) who found that toxicity for copepoda and cladocera is higher for those insecticides that are hydrophobic.

6.1.6 Fate Properties

The best copepoda, insecta and algae models all have aerobic aquatic biotransformation half-life as a predictor variable; while the best models for cladocera and crustacea species (65% of which belong to the order cladocera) include water photolysis half-life and hydrolysis half-life respectively. In addition, the copepoda, insecta and algae best models all indicate that more impacts are likely to occur with persistent pesticides; while the cladocera and crustacea species models suggest that more impacts are predicted to occur when exposed to those chemicals that undergo rapid degradation by hydrolysis.

While it is probably possible that pesticides degrade into compounds that are more toxic than the parent material, hence pesticides with shorter half-lives would be more toxic; unfortunately this subject matter has been under-researched and several organophosphorus pesticides studies report that these degradation products are more, less

or similar to the parent in terms of its toxicity (Pehkonen & Zhang, 2002). The possibility also exists that some pesticides (like organothiophosphates, which were well-represented in the dataset used for modelling) need to be transformed to be toxic. However, a test to decide whether possible potentiation of pesticides affected toxicity did not provide support for this hypothesis since both metabolite-mediated and directly toxic insecticides produced models with shorter hydrolysis half-lives leading to more effects.

In essence, these results reiterate the fact that the actual system characteristics and application method of the pesticide can greatly influence the toxic effects seen, since 1) hydrolysis is affected by temperature and pH; 2) photolysis can be accelerated or inhibited depending on depth and availability of sunlight; and 3) aerobic aquatic biotransformation is influenced by the physico-chemical properties of the system.

6.1.7 Protection being offered at the Standard Threshold Limits

Our results show that a threshold level of 0.01 * Daphnia L(E)C50 can only protect against hydrophilic (or intermediate hydrophobic) insecticides that are resistant to hydrolysis degradation, otherwise at least 37% of crustacea species to be affected. Insecticides with a half-life of about 35.5 days will cause about a 50% decrease while those that undergo rapid hydrolysis such as organophosphates can lead to as much as 63% of species being affected. Considering that only statistically significant changes were regarded as effects and that most studies only produced statistical significance when there was a change of approximately 70% in magnitude, these losses may be considered

unacceptable. This contradicts Brock *et al.* (2000a) who support that a threshold level at 0.01 * Daphnia LC50 will be protective of crustacea.

Although, Daphnia toxicity unit based models are not considered as the best for the other groups or sub-groups of organisms, it is worthy to note that with a few exceptions (temephos, azinphos-methyl) all other insecticides in our database show that 0.1 * HC5-C values are between 1.28 (chlorpyrifos) and 246 (lindane) times more conservative than the 0.01 * Daphnia values.

Threshold levels that are set at 0.1 * HC5 based on acute data, appear to be protective of cladocera, copepoda, insects and algae, except under worse case scenarios. Cladocera can be at risk (abundance decreased by as much as 74%) when exposed to insecticides which have short water photolysis half-lives (such as pyrethroids), while an insecticide that lingers within the ecosystem can cause a 19% change in copepoda abundance and affect 24% of insecta families. Persistent herbicides are predicted to affect 30% of algal species if they contaminate small systems. A 19% abundance change in copepod may go undetected and be possibly biologically insignificant but having almost a quarter of insect families or almost a third of algal species affected should not be.

Brock *et al.* (2006) suggest that using a medium HC5 based on acute invertebrate toxicity data should be protective; however the best models produced in this research do not fully support this. Our models suggest using a HC5 threshold concentration would allow any insecticide (most likely pyrethroids and organophosphates) with a water photolysis half-

life of less than 12 days to cause at least a 50% change in cladocera abundance. However, these impacts should be short-lived since these insecticides degrade rapidly and this group of organisms have fast generation times.

The best copepoda model predicts that while the HC5 threshold concentration would not allow an insecticide with an aerobic aquatic biotransformation half-life of less than 27days to impact copepoda abundance, a persistent insecticide could lead to as much as a 79% decrease. The insecta model predicts that an insecticide with a half-life over 10days will affect 27% of families, while the algal model predicts that a persistent herbicide can affect 22% (large systems) to 51% (small systems) of algal species. Given the general phase out of persistent pesticides a threshold at the HC5 concentration may be protective of copepods and algae; however it may not be for insects. But, given the poor predictive power (adjusted $R^2 = 0.29$) and the fact that the models did not contain responses at very low toxicity units, it can not be conclusively stated that this threshold is not protective of aquatic insects.

Aquatic communities are dynamic and consequently a certain amount of change is expected. So in order to decipher whether these predicted changes at the currently used threshold levels are a matter of concern would be based on the normal changes that are expected within the community at a given time and, of course, on the potential for system recovery – which was not looked at in this study.

6.2 Conclusions

Acute laboratory single species toxicity data as geometric L(E)C50s or Hazard Concentrations can produce models capable of explaining the effects of pesticides on crustacea, insects and algae in ecosystem studies. Despite the data hungry and labour intensive nature of attaining Hazard Concentrations values, it seems worthwhile since they were shown to be better predictors than the geometric mean single species values. Crustacea models are the most predictive, followed by algae and then insects. However, small sample size may have had a role in model prediction, selection of variables and validation with an independent dataset. In addition, characterisation of insect toxicity data seems rather poor.

The addition of fate variables improved model predictions and proved to be invaluable in explaining the responses in the studies, since they help to pinpoint under what conditions more effects can occur and to some extent which types of pesticides pose greater threats to which groups of organisms. Cladocera are possibly most sensitive to organothiophosphates and pyrethroids, while insects and copepods are more sensitive to more persistent insecticides. Algae are more affected by herbicides that are persistent; and with the exception of copepoda, all other groups – crustacea species, cladocera, insecta, algae – are most affected by hydrophobic chemicals.

Use of data from ecosystems studies that contained fish to make predictions about the likely impacts of pesticides on crustaceans is not recommended, since fish presence seems to confound toxicity effects and leads to less predictive models. However, there

may be something gained from just using the impacts of herbicides or insecticides on their most sensitive species group (algae for herbicides, crustacea for insecticides).

A threshold value of 0.01 * Daphnia L(E)C50 does not appear to offer enough protection to crustaceans; however a concentration at 0.1 times the median Hazard Concentration for five percent of crustacea based on acute toxicity data can protect copepoda (except against persistent insecticides) and cladocera (except against insecticides with fast water photolysis half-lives, like pyrethroids). This threshold concentration can also offer protection for insects, except against extremely persistent insecticides; while a threshold value at the 0.1 times the median Hazard Concentration for five percent of algae would sufficiently protect algae, except against persistent herbicides in small aquatic systems. While algae and copepods can probably still be protected (given the phasing out of persistent pesticides) at the median Hazard Concentration values for five percent of algae or crustacea respectively, it is quite inadequate for cladocera and aquatic insects.

In conclusion, the use of single species laboratory toxicity data can be used to predict actual field effects, but there seems to be poor characterisation of insecta data. Model predictability is better when a hazard concentration value derived from species sensitivity distribution is used. Single species laboratory toxicity data by themselves are poor predictors of ecosystem effects, therefore using a tool or attempting to apply thresholds of acceptability without taking into account fate characteristics could severely underestimate the effects of worse case scenarios and result in poor predictability of effects.

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AGRITOX
http://www.dive.afssa.fr/

Animal Diversity Web – University of Michigan Museum of Zoology
http://animaldiversity.ummz.umich.edu/site/index.html

Compendium of Pesticide Common Names
http://www.hclrss.demon.co.uk/sitemap.html

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EXtention TOXicology NETwork
http://extoxnet.orst.edu/

European Commission Pesticide Review reports
http://ec.europa.eu/

InChem
http://www.inchem.org/

Pesticide Action Network Pesticides Database
http://www.pesticideinfo.org/Index.html

Pesticide Management Regulatory Agency (Canada) fate database.

The Taxonomicon & Systema Naturae 2000.
http://sn2000.taxonomy.nl/Taxonomicon/Default.aspx

United States Department of Agriculture – Natural Resources Conservation Service
http://www.nrcs.usda.gov/

United States Environmental Protection Agency
http://www.epa.gov/pesticides/about/

United States Environmental Protection Agency - Pesticide Fate Database
http://cfpub.epa.gov/pfate/home.cfm

United States Environmental Protection Agency - Pesticide Registration (PR) Notice 2001-5. http://ceris.purdue.edu/info/prnotice/pr2001-5.pdf

Software

BurrliOZ - Environment Australia. Department of Environment and Heritage
http://www.cmis.csiro.au/Envir/burrlioz/

Microsoft® Excel

STATISTICA® 6.0 – StatSoft

ETX 2.0 - van Vlaardingen *et al.* (2004)

Appendix 1 – Data used to model Crustacea species responses

Key

Type – type of pesticides
H - herbicide **I** – insecticide **F** - fish toxicant / fungicide

Fish – the presence of fish within the systems
F - fish was present **NF** – no fish was present

Structural Properties of the System
LTSA/V R – log-transformed total surface area to volume ratio

L Volume - log-transformed volume (m^3)

Toxicity Units
LTU Daphnia – log-transformed toxic unit based on geometric means for Daphnia species

L TU HC5 – C – log-transformed toxic unit based on hazard concentration for 5% of crustacea species

Physico-Chemical Pesticide Properties
log Koc – log-transformed organic carbon absorption coefficient

log kow – log-transformed octanol-water coefficient

Fate Properties of the Pesticide
LAAB – log-transformed aerobic aquatic biotransformation (days)
L HHL - log-transformed hydrolysis half-life (days)

L ASB - log-transformed aerobic soil biotransformation (days)
L WPHL - log-transformed water photolysis half-life (days)

Common name	Type	L Volume	L TSA/V R	Effect Concentration (ug/l)	count ratio change	Fish	L TU Daphnia	L TU HC5 - C	log Kow	log Koc	LASB	L WPHL	LAAB	L HHL	Source
atrazine	H	2.0512	0.0792	100	0	F	-2.3507	-1.2307	2.5	1.939	1.6435	2.525	2.2782	2.301	Hamilton et al. (1988)
azinphos-methyl	I			0.2	0.06	NF	-0.7449	0.1549	2.96	2.8974	1.5051	0.5046	0.557	1.5682	Sierszen & Lozano (1998)
azinphos-methyl	I			1	0.22	NF	-0.0459	0.8539	2.96	2.8974	1.5051	0.5046	0.557	1.5682	
azinphos-methyl	I			4	0.5	NF	0.5562	1.4559	2.96	2.8974	1.5051	0.5046	0.557	1.5682	
azinphos-methyl	I			20	0.83	NF	1.2551	2.1549	2.96	2.8974	1.5051	0.5046	0.557	1.5682	
bendiocarb	I	5.301		24	0.5	NF	-0.0852	1.7106	1.72	2.5855	0.2586	1.1551	0.8733	0.5315	Lahr et al (2000)

carbaryl	I	1.4969	0.3444	1000	0.75	NF	-0.0247	2.6397	4.5	5.0646	1.2788	1.6812	1.9418	1.3054	Hanazato & Yasuno (1998)
carbendazim	F	0.4771	0.8692	2.17	0.75	NF	-2.5158	-0.7631	1.38	2.4735	2.5051	3.0394	1.7853	3.2613	Slijkerman et al (2004)
carbendazim	F	0.4771	0.8692	20.67	0.75	NF	-1.5369	0.2157	1.38	2.4735	2.5051	3.0394	1.7853	3.2613	
carbendazim	F	0.4771	0.8692	226	1	NF	-0.4981	1.2545	1.38	2.4735	2.5051	3.0394	1.7853	3.2613	
carbofuran	I	0.1847	0.7451	6.3	1	NF	-0.7429	1.5451	1.52	1.4065	1.0414	0.7482	0.301	-0.168	Wayland (1991)
carbofuran	I	0.1847	0.7451	22.5	1	NF	-0.1901	2.0979	1.52	1.4065	1.0414	0.7482	0.301	-0.168	
carbofuran	I			9	1	NF	-0.588	1.7	1.52	1.4065	1.0414	0.7482	0.301	-0.168	Wayland & boad (1990)
carbofuran	I			14	1	NF	-0.3961	1.8919	1.52	1.4065	1.0414	0.7482	0.301	-0.168	
carbofuran	I			32	1	NF	-0.0371	2.2509	1.52	1.4065	1.0414	0.7482	0.301	-0.168	
carbofuran	I	0.1038	0.7938	6.3	1	NF	-0.7429	1.5451	1.52	1.4065	1.0414	0.7482	0.301	-0.168	
carbofuran	I	0.1038	0.7938	22.5	1	NF	-0.1901	2.0979	1.52	1.4065	1.0414	0.7482	0.301	-0.168	
chlorpyrifos	I	-0.2147	0.8831	5	1	NF	0.8942	2	4.7	3.688	2.2022	1.4771	1.7076	1.8573	Brock et al (1992)
chlorpyrifos	I	-0.2147	0.8831	35	1	NF	1.7393	2.8451	4.7	3.688	2.2022	1.4771	1.7076	1.8573	
chlorpyrifos	I	-0.2147	0.8831	35	1	NF	1.7393	2.8451	4.7	3.688	2.2022	1.4771	1.7076	1.8573	
chlorpyrifos	I	1.7404	0.3838	0.51	0.8	F	-0.0972	1.0086	4.7	3.688	2.2022	1.4771	1.7076	1.5459	Siefert et al (1989)
chlorpyrifos	I	1.7404	0.3838	6.29	0.8	F	0.9939	2.0997	4.7	3.688	2.2022	1.4771	1.7076	1.5459	
chlorpyrifos	I	1.7404	0.3838	32	0.8	F	1.7004	2.8062	4.7	3.688	2.2022	1.4771	1.7076	1.5459	
chlorpyrifos	I	1.7782	0.721	0.1	0.33	NF	-0.8048	0.301	4.7	3.688	2.2022	1.4771	1.7076	1.8573	Van den Brink et al (1996)
chlorpyrifos	I	1.7782	0.721	0.9	0.67	NF	0.1495	1.2553	4.7	3.688	2.2022	1.4771	1.7076	1.8573	
chlorpyrifos	I	1.7782	0.721	6	1	NF	0.9734	2.0792	4.7	3.688	2.2022	1.4771	1.7076	1.8573	
chlorpyrifos	I	1.7782	0.721	44	1	NF	1.8387	2.9445	4.7	3.688	2.2022	1.4771	1.7076	1.8573	
chlorpyrifos	I	0.9731	0.8136	1.7	1	NF	0.4257	1.5315	4.7	3.688	2.2022	1.4771	1.7076	1.1159	Lucassen & Leeuwangh

chlorpyrifos	I	0.9731	0.8136	16.5	1	NF	1.4127	2.5185	4.7	3.688	2.2022	1.4771	1.7076	1.1159	(1994)
cyfluthrin	I	0.6532	0.5587	0.22	1	F	-0.1715	2.0882	6	4.8981	1.7738	1.0864	1.6681	2.2856	Heimbach et al (1992)
cyfluthrin	I	0.6532	0.5587	1.8	1	F	0.7413	3.0011	6	4.8981	1.7738	1.0864	1.6681	2.2856	
cypermethrin	I	-0.699	1.0752	0.08	0.8	NF	-1.7957	1.0795	6.6	4.627	1.7782	0.2782	1.7762	0.2553	Wendt-Rasch (2003)
cypermethrin	I	-0.699	1.0752	0.3	1	NF	-1.2217	1.6535	6.6	4.627	1.7782	0.2782	1.7762	0.2553	
cypermethrin	I	-0.699	1.0752	1.6	1	NF	-0.4947	2.3805	6.6	4.627	1.7782	0.2782	1.7762	0.2553	
cypermethrin	I	-0.699	1.0752	3.2	1	NF	-0.1937	2.6816	6.6	4.627	1.7782	0.2782	1.7762	0.2553	
deltamethrin	I	5.301		0.64	0.25	NF	-0.0247	2.6397	4.5	5.0646	1.2788	1.6812	1.9418	1.3054	Lahr et al (2000)
diflubenzuron	I	1.0086	0.7435	10	1	NF	0.3683	1.5062	3.89	3.8663	0.6021	1.4472	1.0534	1.9243	Ali & Kok-Yokomi (1989)
diflubenzuron	I	5.301		10.4	0.86	NF	0.3853	1.5233	3.89	3.8663	0.6021	1.4472	1.0534	1.9243	
esfenvalerate	I			0.01	0.39	F	-1.5886	0.122	6.22	4	1.8692	1	1.7182	3.2613	Lozano et al (1992)
esfenvalerate	I			0.08	0.5	F	-0.6855	1.0251	6.22	4	1.8692	1	1.7182	3.2613	
esfenvalerate	I			0.2	0.44	F	-0.2876	1.423	6.22	4	1.8692	1	1.7182	3.2613	
esfenvalerate	I	0.9731	0.7451	1	0.72	F	0.4114	2.122	6.22	4	1.8692	1	1.7182	3.2613	
esfenvalerate	I	0.9731	0.7451	5	0.66	F	1.1104	2.821	6.22	4	1.8692	1	1.7182	3.2613	
fenitrothion	I	5.301		80	0.57	NF	0.8061	2.7653	3.43	3.0519	1.5563	0.5623	1.1021	2.2695	Lahr et al (2000)
fenthion	I	0.1761	0.8615	23	0.75	NF	1.5693	2.5373	4.84	3.2553	0	-1.983	0.7526	1.7544	Hanazato & Kasai (1995)
fenthion	I	0.1761	0.8615	175	1	NF	2.4506	3.4186	4.84	3.2553	0	-1.983	0.7526	1.7544	
glufosinate-ammonium	H	1.2586	0.4942	1	0	NF	-5.841	-3.6543	-4.81	1.8412	1.0837	0.832	0.4771	2.5623	Faber et al (1996)
glufosinate-ammonium	H	1.2586	0.4942	10	0	NF	-4.841	-2.6543	-4.81	1.8412	1.0837	0.832	0.4771	2.5623	
glufosinate-ammonium	H	1.2586	0.4942	100	0	NF	-3.841	-1.6543	-4.81	1.8412	1.0837	0.832	0.4771	2.5623	

glufosinate-ammonium	H	1.2586	0.4942	1000	1	NF	-2.841	-0.6543	-4.81	1.8412	1.0837	0.832	0.4771	2.5623	
glufosinate-ammonium	H	1.2586	0.4942	10000	1	NF	-1.841	0.3457	-4.81	1.8412	1.0837	0.832	0.4771	2.5623	
hexazinone	H	1.9469	0.1303	32	0	NF	-3.7913	-2.658	1.2	1.4232	2.2908	3.0394	1.7782	3.2613	Thompson et al (1993)
hexazinone	H	1.9469	0.1303	102	0	NF	-3.2878	-2.1546	1.2	1.4232	2.2908	3.0394	1.7782	3.2613	
hexazinone	H	1.9469	0.1303	1106	1	NF	-2.2527	-1.1194	1.2	1.4232	2.2908	3.0394	1.7782	3.2613	
hexazinone	H	1.9469	0.1303	11276	1	NF	-1.2443	-0.111	1.2	1.4232	2.2908	3.0394	1.7782	3.2613	
lindane	I	0	0.7482	4	0.33	NF	-2.4499	0.941	3.5	3.0414	2.9912	3.0394	2.3068	2.2375	Peither et al (1996)
lindane	I	0	0.7482	8	0.33	NF	-2.1489	1.2421	3.5	3.0414	2.9912	3.0394	2.3068	2.2375	
lindane	I	0	0.7482	16	0.33	NF	-1.8479	1.5431	3.5	3.0414	2.9912	3.0394	2.3068	2.2375	
lindane	I	0	0.7482	24	0.33	NF	-1.6718	1.7192	3.5	3.0414	2.9912	3.0394	2.3068	2.2375	
lindane	I	0	0.7482	32	0.33	NF	-1.5468	1.8441	3.5	3.0414	2.9912	3.0394	2.3068	2.2375	
lindane	I	0	0.7482	64	0.33	NF	-1.2458	2.1452	3.5	3.0414	2.9912	3.0394	2.3068	2.2375	
linuron	H	0.0086		1000	1	NF	0.3501	1.3771	3	2.3918	1.6902	1.6902	1.6243	3.2613	Stephenson & Kane (1984)
methabenzthiazuron	H	0.7782		10	0	NF	-2.9585	-1.7507	2.64	3.2916				2.5623	Wellmann et al (1998)
methabenzthiazuron	H	0.7782		21	0	NF	-2.6363	-1.4285	2.64	3.2916				2.5623	
methabenzthiazuron	H	0.7782		43	0	NF	-2.3251	-1.1172	2.64	3.2916				2.5623	
methabenzthiazuron	H	0.7782		89	0	NF	-2.0092	-0.8013	2.64	3.2916				2.5623	
methabenzthiazuron	H	0.7782		184	0.5	NF	-1.6937	-0.4859	2.64	3.2916				2.5623	
methabenzthiazuron	H	0.7782		380	0.5	NF	-1.3788	-0.1709	2.64	3.2916				2.5623	
methabenzthiazuron	H	0.7782		787	0.5	NF	-1.0626	0.1453	2.64	3.2916				2.5623	
methabenzthiazuron	H	0.7782		1629	0.5	NF	-0.7466	0.4612	2.64	3.2916				2.5623	
methabenzthiazuron	H	0.7782		3371	0.5	NF	-0.4308	0.7771	2.64	3.2916				2.5623	
methyl parathion	I	0.699	0.415	100	1	F	2.0503	2.6875	3	3.6776	1.0106	1	0.6128	1.3222	Crossland (1984)

methyl parathion	I	1.6021	0.5119	8	0.2	F	0.9534	1.5906	3	3.6776	1.0106	1	0.6128	1.3222	Crossland (1988)
methyl parathion	I	1.6021	0.5119	29	0.2	F	1.5127	2.1499	3	3.6776	1.0106	1	0.6128	1.3222	
methyl parathion	I	0.0086		2500	1	NF	3.4482	4.0854	3	3.6776	1.0106	1	0.6128	1.3222	Stephenson & Kane (1984)
metribuzin	H	-0.3565	0.8927	1.8	0	NF	-3.8858	-3.0952	1.6	1.2304	2.25	-0.747	2	3.2613	Brock et al (2004)
metribuzin	H	-0.3565	0.8927	5.6	0	NF	-3.3929	-2.6022	1.6	1.2304	2.25	-0.747	2	3.2613	
metribuzin	H	-0.3565	0.8927	18	0	NF	-2.8858	-2.0952	1.6	1.2304	2.25	-0.747	2	3.2613	
metribuzin	H	-0.3565	0.8927	56	0.67	NF	-2.3929	-1.6022	1.6	1.2304	2.25	-0.747	2	3.2613	
metribuzin	H	-0.3565	0.8927	180	0.67	NF	-1.8858	-1.0952	1.6	1.2304	2.25	-0.747	2	3.2613	
metsulfuron methyl	H	1.9469	0.1303	10	0	NF	-4.1761	-2.9682	0.018	0.8129	2.0478	3.0394	1.6392	3.2613	Thompson et al (1993)
metsulfuron methyl	H	1.9469	0.1303	100	0	NF	-3.1761	-1.9682	0.018	0.8129	2.0478	3.0394	1.6392	3.2613	
metsulfuron methyl	H	1.9469	0.1303	500	0	NF	-2.4771	-1.2693	0.018	0.8129	2.0478	3.0394	1.6392	3.2613	
metsulfuron methyl	H	1.9469	0.1303	1000	0	NF	-2.1761	-0.9682	0.018	0.8129	2.0478	3.0394	1.6392	3.2613	
permethrin	I	2.0969	0.0792	0.5	0.67	NF	-0.611	1.5491	6.1	4.6114	1.4771	-0.903	1.5125	3.2613	Kaushik et al (1987)
permethrin	I	2.0969	0.0792	5	0.89	NF	0.389	2.5491	6.1	4.6114	1.4771	-0.903	1.5125	3.2613	
permethrin	I	0.4771	0.6561	0.75	0.67	NF	-0.4349	1.7252	6.1	4.6114	1.4771	-0.903	1.5125	3.2613	Yasuno et al (1988)
permethrin	I	0.4771	0.6561	1.5	1	NF	-0.1338	2.0263	6.1	4.6114	1.4771	-0.903	1.5125	3.2613	
phorate	I	1.6532	0.4914	23	1	NF	0.8282	3.5454	3.92	2.6575	0.4771	0.0414	-0.34	0.5051	Dieter (1996)
phorate	I	1.6532	0.4914	45	1	NF	1.1197	3.8369	3.92	2.6575	0.4771	0.0414	-0.34	0.5051	
pyridaben	I	1.2304	0.534	1.06	0.2	F	0.2057	2.0162	6.1	4.8451	2.1145	-1.681	1.8469	3.2613	Rand et al (2000)
pyridaben	I	1.2304	0.534	4.85	0.4	F	0.8661	2.6766	6.1	4.8451	2.1145	-1.681	1.8469	3.2613	
pyridaben	I	1.2304	0.534	50.98	0.6	F	1.8878	3.6983	6.1	4.8451	2.1145	-1.681	1.8469	3.2613	
rotenone	F	2.3927		6	1	F	-0.4515	0.4752	4.16	4	0.4771		0.9879	0.1139	Beal & Anderson (1993)
rotenone	F	4.8651	0.179	3000	0.83	NF	2.2475	3.1742	4.16	4	0.4771		0.9879	0.1139	Melaas et al (2001)

tebufenozide	I	1.7959	0.2041	70	0.33	F	-1.1435	0.486	4.25	2.6799	2.4344	2.5136	2	3.0145	Kreutzweiser et al (1995)
tebufenozide	I	1.7959	0.2041	130	0.67	F	-0.8746	0.7549	4.25	2.6799	2.4344	2.5136	2	3.0145	
tebufenozide	I	1.7959	0.2041	330	0.67	F	-0.4701	1.1594	4.25	2.6799	2.4344	2.5136	2	3.0145	
tebufenozide	I	1.7959	0.2041	660	0.67	F	-0.169	1.4605	4.25	2.6799	2.4344	2.5136	2	3.0145	
tebufenozide	I	1.3979	0.4472	9	0.38	NF	-2.0343	-0.4048	4.25	2.6799	2.4344	2.5136	2	3.0145	Kruetzweiser & Faber (1999)
tebufenozide	I	1.3979	0.4472	157	0.25	NF	-0.7927	0.8368	4.25	2.6799	2.4344	2.5136	2	3.0145	
temephos	I	0.9731	0.7451	58.6	1	NF	3.7265	3.4887	4.91	5	1.4771	1.176	1.2355	2.6628	Hanazato et al (1989)
temephos	I	0.9731	0.7451	77.6	1	NF	3.8485	3.6106	4.91	5	1.4771	1.176	1.2355	2.6628	
triazamate	I	-0.1024	0.7782	0.08	0	NF	-3.4184	-1.1758	2.15	3.0039		2.1761			Toy (1994)
triazamate	I	-0.1024	0.7782	0.228	0	NF	-2.9636	-0.721	2.15	3.0039		2.1761			
triazamate	I	-0.1024	0.7782	0.852	0	NF	-2.3911	-0.1485	2.15	3.0039		2.1761			
triazamate	I	-0.1024	0.7782	1.885	0	NF	-2.0462	0.1964	2.15	3.0039		2.1761			
triazamate	I	-0.1024	0.7782	5.5	0	NF	-1.5812	0.6615	2.15	3.0039		2.1761			

Appendix 2 – Data used to model Copepoda responses

Key

Type – type of pesticides
H - herbicide **I** – insecticide **F** - fish toxicant / fungicide

Fish – the presence of fish within the systems
F - fish was present **NF** – no fish was present

LAR - log-transformed abundance ratio

Structural Properties of the System
LTSA/V R – log-transformed total surface area to volume ratio

L Volume - log-transformed volume (m^3)

Toxicity Units
LTU Daphnia – log-transformed toxic unit based on geometric means for Daphnia species

L TU HC5 – C – log-transformed toxic unit based on hazard concentration for 5% of crustacea species

Physico-Chemical Pesticide Properties
log Koc – log-transformed organic carbon absorption coefficient

log kow – log-transformed octanol-water coefficient

Fate Properties of the Pesticide
LAAB – log-transformed aerobic aquatic biotransformation (days)
L HHL - log-transformed hydrolysis half-life (days)

L ASB - log-transformed aerobic soil biotransformation (days)
L WPHL - log-transformed water photolysis half-life (days)

Common name	Type	L Volume	L TSA/V R	Effect Concentration (ug/l)	L A R	Fish	L TU Daphnia spp GM L/EC50	L TU HC5 - C	log Kow	log Koc	LASB	L WPHL	LAAB	L HHL	Source
atrazine	H			20	0.0000	F	-3.0496	-1.9297	2.5000	1.9390	1.6435	2.5250	2.2782	2.3010	DeNoyelles et al (1982)
atrazine	H	2.0512	0.0934	100	0.0000	F	-2.3507	-1.2307	2.5000	1.9390	1.6435	2.5250	2.2782	2.3010	
azinphos-methyl	I			0.2	0.0000	NF	-0.7449	0.1549	2.9600	2.8974	1.5051	0.5046	0.5570	1.5682	Sierszen & Lozano (1998)
azinphos-methyl	I			1	0.0000	NF	-0.0459	0.8539	2.9600	2.8974	1.5051	0.5046	0.5570	1.5682	
azinphos-methyl	I			4	0.0000	NF	0.5562	1.4559	2.9600	2.8974	1.5051	0.5046	0.5570	1.5682	
azinphos-methyl	I			20	0.0000	NF	1.2551	2.1549	2.9600	2.8974	1.5051	0.5046	0.5570	1.5682	
carbaryl	I	1.4969	0.3444	1000	1.3979	NF	1.6636	2.7150	1.8500	2.3010	1.0167	1.0709	1.2738	0.2355	Hanazato & Yasuno (1998)
carbendazim	F	0.4771	0.8692	2.17	0.0000	NF	-2.5158	-0.7631	1.3800	2.4735	2.5051	3.0394	1.7853	3.2613	Slijkerman et al (2004)
carbendazim	F	0.4771	0.8692	20.67	0.2923	NF	-1.5369	0.2157	1.3800	2.4735	2.5051	3.0394	1.7853	3.2613	

carbendazim	F	0.4771	0.8692	226	0.5378	NF	-0.4981	1.2545	1.3800	2.4735	2.5051	3.0394	1.7853	3.2613	
chlorpyrifos	I	-0.2147	0.8831	5	0.0000	NF	0.8942	2.0000	4.7000	3.6880	2.2022	1.4771	1.7076	1.8573	Borck et al (1992)
chlorpyrifos	I	-0.2147	0.8831	35	1.7800	NF	1.7393	2.8451	4.7000	3.6880	2.2022	1.4771	1.7076	1.8573	
chlorpyrifos	I	-0.2147	0.8831	35	1.7534	NF	1.7393	2.8451	4.7000	3.6880	2.2022	1.4771	1.7076	1.8573	
chlorpyrifos	I	1.0492	0.4440	0.03	0.0000	F	-1.3276	-0.2218	4.7000	3.6880	2.2022	1.4771	1.7076	1.2041	Biever et al (1994)
chlorpyrifos	I	1.0492	0.4440	0.088	0.0000	F	-0.8603	0.2455	4.7000	3.6880	2.2022	1.4771	1.7076	1.2041	
chlorpyrifos	I	0.4771	0.7267	28	1.0792	NF	1.6424	2.7482	4.7000	3.6880	2.2022	1.4771	1.7076	1.8573	Hughes et al (1980)
chlorpyrifos	I	1.7404	0.3838	0.51	0.0000	F	-0.0972	1.0086	4.7000	3.6880	2.2022	1.4771	1.7076	1.5459	Siefert et al (1989)
chlorpyrifos	I	1.7404	0.3838	6.29	0.0000	F	0.9939	2.0997	4.7000	3.6880	2.2022	1.4771	1.7076	1.5459	
chlorpyrifos	I	1.7404	0.3838	32	0.0000	F	1.7004	2.8062	4.7000	3.6880	2.2022	1.4771	1.7076	1.5459	
chlorpyrifos	I	0.9731	0.8136	1.7	1.6532	NF	0.4257	1.5315	4.7000	3.6880	2.2022	1.4771	1.7076	1.1159	Lucassen & Leeuwangh (1994)
chlorpyrifos	I	0.9731	0.8136	16.5	1.6990	NF	1.4127	2.5185	4.7000	3.6880	2.2022	1.4771	1.7076	1.1159	
cypermethrin	I	-0.6990	1.0752	0.08	1.1761	NF	-1.7957	1.0795	6.6000	4.6270	1.7782	0.2782	1.7762	0.2553	Wendt-Rasch et al (2003a)
cypermethrin	I	-0.6990	1.0752	0.3	1.4771	NF	-1.2217	1.6535	6.6000	4.6270	1.7782	0.2782	1.7762	0.2553	
cypermethrin	I	-0.6990	1.0752	1.6	2.1761	NF	-0.4947	2.3805	6.6000	4.6270	1.7782	0.2782	1.7762	0.2553	
cypermethrin	I	-0.6990	1.0752	3.2	2.1761	NF	-0.1937	2.6816	6.6000	4.6270	1.7782	0.2782	1.7762	0.2553	
cypermethrin	I	-1.0862	1.1166	0.05	0.2742	NF	-1.9998	0.8754	6.6000	4.6270	1.7782	0.2782	1.7762	0.2553	Wendt-Rasch et al (2003b)
deltamethrin	I	1.2041	0.7559	13	2.1987	NF	1.2831	3.9475	4.5000	5.0646	1.2788	1.6812	1.9418	1.3054	Tidou et al (1992)
diflubenzuron	I	2.6021		30	1.2338	F	0.8454	1.9834	3.8900	3.8663	0.6021	1.4472	1.0534	1.7475	Ludwig (1993)
diflubenzuron	I	1.6335	0.4654	3.36	1.0000	F	-0.1054	1.0326	3.8900	3.8663	0.6021	1.4472	1.0534	1.9243	Tanner & Moffett (1995)
diflubenzuron	I	1.6335	0.4654	27.2	2.0000	F	0.8028	1.9408	3.8900	3.8663	0.6021	1.4472	1.0534	1.9243	
esfenvalerate	I			0.01	0.0000	F	-1.5886	0.1220	6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	Lozano et al (1992)
esfenvalerate	I			0.08	0.0000	F	-0.6855	1.0251	6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
esfenvalerate	I			0.2	0.0000	F	-0.2876	1.4230	6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
esfenvalerate	I			1	0.0000	F	0.4114	2.1220	6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
esfenvalerate	I			5	0.0000	F	1.1104	2.8210	6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
esfenvalerate	I	-0.7447	1.0318	0.005	0.4564	NF	-1.8896	-0.1790	6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	Samsoe-Peterson et al (2001)
esfenvalerate	I	-0.7447	1.0318	0.18	1.0000	NF	-0.3333	1.3773	6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
esfenvalerate	I	-0.7447	1.0318	0.61	2.0000	NF	0.1967	1.9073	6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
esfenvalerate	I	-0.7447	1.0318	2.1	2.0000	NF	0.7336	2.4442	6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
esfenvalerate	I	-0.7447	1.0318	7.5	2.0000	NF	1.2865	2.9971	6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
esfenvalerate	I	-0.7447	1.0318	26	2.0000	NF	1.8264	3.5370	6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
fenthion	I	0.1761	0.8615	23	0.6021	NF	1.5693	2.5373	4.8400	3.2553	0.0000	-1.9830	0.7526	1.7544	Hanazato &

fenthion	I	0.1761	0.8615	175	0.9031	NF	2.4506	3.4186	4.8400	3.2553	0.0000	-1.9830	0.7526	1.7544	Kasai (1995)
fenthion	I	2.6021		250	0.3424	F	2.6055	3.5735	4.8400	3.2553	0.0000	-1.9830	0.7526	1.7544	Ludwig (1993)
fenvalerate	I	2.0969	0.0792	0.05	0.8451	NF	-1.0885	0.8303	5.0100	3.7243	2.2524	0.7780	1.9192	1.4771	Day & Kaushik (1987)
fenvalerate	I	2.0969	0.0792	0.01	0.0000	NF	-1.7875	0.1313	5.0100	3.7243	2.2524	0.7780	1.9192	1.4771	
glufosinate-ammonium	H	1.2586	0.4942	1	0.0000	NF	-5.8410	-3.6543	-4.8100	1.8412	1.0837	0.8320	0.4771	2.5623	Faber et al (1998)
glufosinate-ammonium	H	1.2586	0.4942	10	0.0000	NF	-4.8410	-2.6543	-4.8100	1.8412	1.0837	0.8320	0.4771	2.5623	
glufosinate-ammonium	H	1.2586	0.4942	100	0.0000	NF	-3.8410	-1.6543	-4.8100	1.8412	1.0837	0.8320	0.4771	2.5623	
glufosinate-ammonium	H	1.2586	0.4942	1000	1.2739	NF	-2.8410	-0.6543	-4.8100	1.8412	1.0837	0.8320	0.4771	2.5623	
glufosinate-ammonium	H	1.2586	0.4942	10000	4.5784	NF	-1.8410	0.3457	-4.8100	1.8412	1.0837	0.8320	0.4771	2.5623	
hexazinone	H	1.9469	0.1303	32	0.0000	NF	-3.7913	-2.6580	1.2000	1.4232	2.2908	3.0394	1.7782	3.2613	Thompson et al (1993)
hexazinone	H	1.9469	0.1303	102	0.0000	NF	-3.2878	-2.1546	1.2000	1.4232	2.2908	3.0394	1.7782	3.2613	
hexazinone	H	1.9469	0.1303	1106	0.1761	NF	-2.2527	-1.1194	1.2000	1.4232	2.2908	3.0394	1.7782	3.2613	
hexazinone	H	1.9469	0.1303	11276	0.9031	NF	-1.2443	-0.1110	1.2000	1.4232	2.2908	3.0394	1.7782	3.2613	
isoproturon	H	-0.6021		10	0.0000	NF	-2.8228	-1.6150	2.5000	2.0864	1.6609	1.9031	1.6089	3.1930	Traunspurger et al (1993)
isoproturon	H	-0.6021		30	0.0000	NF	-2.3457	-1.1378	2.5000	2.0864	1.6609	1.9031	1.6089	3.1930	
isoproturon	H	-0.6021		90	0.0000	NF	-1.8686	-0.6607	2.5000	2.0864	1.6609	1.9031	1.6089	3.1930	
lambda-cyhalothrin	I	1.3979	0.4472	0.017	0.0000	NF	-1.0376	1.4575	7.0000	5.0969	1.5441	1.3617	1.5476	0.8451	Hamer & Hill (1994)
lambda-cyhalothrin	I	1.3979	0.4472	0.17	0.0000	NF	-0.0376	2.4575	7.0000	5.0969	1.5441	1.3617	1.5476	0.8451	
lindane	I	-0.5229		4	0.0000	NF	-2.4499	0.9410	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	Fliedner & Klein (1996)
lindane	I	-0.5229		8	0.6021	NF	-2.1489	1.2421	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	
lindane	I	-0.5229		16	1.4472	NF	-1.8479	1.5431	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	
lindane	I	-0.5229		32	1.7482	NF	-1.5468	1.8441	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	
lindane	I	-0.5229		64	2.4472	NF	-1.2458	2.1452	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	
lindane	I	-0.5229		128	2.4472	NF	-0.9448	2.4462	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	
lindane	I	-0.5229		256	2.4472	NF	-0.6437	2.7472	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	
lindane	I	-0.5229		512	2.4472	NF	-0.3427	3.0483	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	
lindane	I	1.0000	0.7782	321	2.5999	NF	-0.5455	2.8455	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	Tidou et al (1992)
linuron	H	0.0086		1000	2.5484	NF	0.3501	1.3771	3.0000	2.3918	1.6902	1.6902	1.6243	3.2613	Stephenson & Kane (1984)
methabenzthiazuron	H	0.7782		10	0.0000	NF	-2.9585	-1.7507	2.6400	3.2916				2.5623	Wellmann et al (1998)

methabenzthiazuron	H	0.7782		21	0.0000	NF	-2.6363	-1.4285	2.6400	3.2916				2.5623	
methabenzthiazuron	H	0.7782		43	0.0000	NF	-2.3251	-1.1172	2.6400	3.2916				2.5623	
methabenzthiazuron	H	0.7782		89	0.0000	NF	-2.0092	-0.8013	2.6400	3.2916				2.5623	
methabenzthiazuron	H	0.7782		184	0.0000	NF	-1.6937	-0.4859	2.6400	3.2916				2.5623	
methabenzthiazuron	H	0.7782		380	0.0000	NF	-1.3788	-0.1709	2.6400	3.2916				2.5623	
methabenzthiazuron	H	0.7782		787	0.0000	NF	-1.0626	0.1453	2.6400	3.2916				2.5623	
methabenzthiazuron	H	0.7782		1629	0.0000	NF	-0.7466	0.4612	2.6400	3.2916				2.5623	
methabenzthiazuron	H	0.7782		3371	0.0000	NF	-0.4308	0.7771	2.6400	3.2916				2.5623	
methoxychlor	I	2.0334	0.1430	2.9	0.0000	NF	-0.6301	0.6510	5.0800	4.9165	1.9542	1.8360	1.7628	2.5647	Stephenson et al (1989)
methoxychlor	I	2.0334	0.1430	321	2.4559	NF	1.4140	2.6952	5.0800	4.9165	1.9542	1.8360	1.7628	2.5647	
methoxychlor	I	2.0334	0.1430	4.5	0.5441	NF	-0.4393	0.8419	5.0800	4.9165	1.9542	1.8360	1.7628	2.5647	
methoxychlor	I	2.0334	0.1430	46.5	2.1461	NF	0.5749	1.8561	5.0800	4.9165	1.9542	1.8360	1.7628	2.5647	
methyl parathion	I	0.6990	0.4150	100	0.2122	F	2.0503	2.6875	3.0000	3.6776	1.0106	1.0000	0.6128	1.3222	Crossland (1984)
methyl parathion	I	0.0086		2500	1.7035	NF	3.4482	4.0854	3.0000	3.6776	1.0106	1.0000	0.6128	1.3222	Stephenson & Kane (1984)
metsulfuron methyl	H	1.9469	0.1303	10	0.0000	NF	-4.1761	-2.9682	0.0180	0.8129	2.0478	3.0394	1.6392	3.2613	Thompson et al (1993)
metsulfuron methyl	H	1.9469	0.1303	100	0.0000	NF	-3.1761	-1.9682	0.0180	0.8129	2.0478	3.0394	1.6392	3.2613	
metsulfuron methyl	H	1.9469	0.1303	500	0.0000	NF	-2.4771	-1.2693	0.0180	0.8129	2.0478	3.0394	1.6392	3.2613	
metsulfuron methyl	H	1.9469	0.1303	1000	0.0000	NF	-2.1761	-0.9682	0.0180	0.8129	2.0478	3.0394	1.6392	3.2613	
metsulfuron methyl	H	-1.0862	1.1166	1	0.0000	NF	-5.1761	-3.9682	0.0180	0.8129	2.0478	3.0394	1.6392	3.2613	
metsulfuron methyl	H	-1.0862	1.1166	5	0.0000	NF	-4.4771	-3.2693	0.0180	0.8129	2.0478	3.0394	1.6392	3.2613	
metsulfuron methyl	H	-1.0862	1.1166	20	0.0000	NF	-3.8751	-2.6672	0.0180	0.8129	2.0478	3.0394	1.6392	3.2613	
pyridaben	I	1.2304	0.5340	1.06	0.0000	F	0.2057	2.0162	6.1000	4.8451	2.1145	-1.6812	1.8469	3.2613	Rand et al (200)
pyridaben	I	1.2304	0.5340	4.85	0.9823	F	0.8661	2.6766	6.1000	4.8451	2.1145	-1.6812	1.8469	3.2613	
pyridaben	I	1.2304	0.5340	50.98	1.5051	F	1.8878	3.6983	6.1000	4.8451	2.1145	-1.6812	1.8469	3.2613	
rotenone	F	2.3927		6	2.6990	F	-0.4515	0.4752	4.1600	4.0000	0.4771		0.9879	0.1139	Beal & Anderson (1993)
rotenone	F	4.8651	0.1790	3000	1.9652	NF	2.2475	3.1742	4.1600	4.0000	0.4771		0.9879	0.1139	Melass et al (2001)

tebufenozide	I	1.7959	0.2041	70	0.0000	F	-1.1435	0.4860	4.2500	2.6799	2.4344	2.5136	2.0000	3.0145	Hughes et al (1980)
tebufenozide	I	1.7959	0.2041	130	0.0000	F	-0.8746	0.7549	4.2500	2.6799	2.4344	2.5136	2.0000	3.0145	
tebufenozide	I	1.7959	0.2041	330	0.0000	F	-0.4701	1.1594	4.2500	2.6799	2.4344	2.5136	2.0000	3.0145	
tebufenozide	I	1.7959	0.2041	660	0.0000	F	-0.1690	1.4605	4.2500	2.6799	2.4344	2.5136	2.0000	3.0145	
temephos	I	0.4771	0.7267	9	1.0792	NF	2.9128	2.6750	4.9100	5.0000	1.4771	1.1760	1.2355	2.6628	Hughes et al (1980)

Appendix 3 – Data used to model Cladocera responses

Key

Type – type of pesticides
H - herbicide **I** – insecticide **F** - fish toxicant / fungicide

Fish – the presence of fish within the systems
F - fish was present **NF** – no fish was present

LAR – log-transformed abundance ratio

Structural Properties of the System
LTSA/V R – log-transformed total surface area to volume ratio

L Volume - log-transformed volume (m^3)

Toxicity Units
LTU Daphnia – log-transformed toxic unit based on geometric means for Daphnia species

L TU HC5 – C – log-transformed toxic unit based on hazard concentration for 5% of crustacea species

Physico-Chemical Pesticide Properties
log Koc – log-transformed organic carbon absorption coefficient

log kow – log-transformed octanol-water coefficient

Fate Properties of the Pesticide
LAAB – log-transformed aerobic aquatic biotransformation (days)
L HHL - log-transformed hydrolysis half-life (days)

L ASB - log-transformed aerobic soil biotransformation (days)
L WPHL - log-transformed water photolysis half-life (days)

Common name	Type	L Volume	L TSA/V R	Effect Concentration (ug/l)	L A R	Fish	L TU Daphnia spp GM L/EC50	L TU HC5 - C	log Kow	log Koc	LASB	L WPHL	LAAB	L HHL	Source
azinphos-methyl	I	1.5682	0.5172	1.17	0.0000	F	0.0223	0.9221	2.9600	2.8974	1.5051	0.5046	0.5570	1.5682	Tanner & Knuth (1995)
carbaryl	I	1.4969	0.3444	1000	1.5563	NF	1.6636	2.7150	1.8500	2.3010	1.0167	1.0709	1.2738	0.2355	Hanazato & Yasuno (1998)
carbendazim	F	0.4771	0.8692	2.17	0.6385	NF	-2.5158	-0.7631	1.3800	2.4735	2.5051	3.0394	1.7853	3.2613	Slijkerman et al (2004)
carbendazim	F	0.4771	0.8692	20.67	0.6021	NF	-1.5369	0.2157	1.3800	2.4735	2.5051	3.0394	1.7853	3.2613	
carbendazim	F	0.4771	0.8692	226	1.5441	NF	-0.4981	1.2545	1.3800	2.4735	2.5051	3.0394	1.7853	3.2613	
chlorpyrifos	I	-0.2147	0.8831	5	1.0000	NF	0.8942	2.0000	4.7000	3.6880	2.2022	1.4771	1.7076	1.8573	Brock et al (1992)
chlorpyrifos	I	-0.2147	0.8831	35	2.0000	NF	1.7393	2.8451	4.7000	3.6880	2.2022	1.4771	1.7076	1.8573	
chlorpyrifos	I	-0.2147	0.8831	35	2.4771	NF	1.7393	2.8451	4.7000	3.6880	2.2022	1.4771	1.7076	1.8573	
chlorpyrifos	I	1.0492	0.4440	0.03	0.0000	F	-1.3276	-0.2218	4.7000	3.6880	2.2022	1.4771	1.7076	1.2041	Biever et al (1994)
chlorpyrifos	I	1.0492	0.4440	0.088	0.0000	F	-0.8603	0.2455	4.7000	3.6880	2.2022	1.4771	1.7076	1.2041	

chlorpyrifos	I	0.4771	0.7267	28	1.2553	NF	1.6424	2.7482	4.7000	3.6880	2.2022	1.4771	1.7076	1.8573	Hughes et al (1980)
chlorpyrifos	I	0.9731	0.8136	1.7	2.3010	NF	0.4257	1.5315	4.7000	3.6880	2.2022	1.4771	1.7076	1.1159	Lucassen & Leeuwangh (1994)
chlorpyrifos	I	0.9731	0.8136	16.5	2.3010	NF	1.4127	2.5185	4.7000	3.6880	2.2022	1.4771	1.7076	1.1159	
cypermethrin	I	-0.6990	1.0752	0.08	0.9489	NF	-1.7957	1.0795	6.6000	4.6270	1.7782	0.2782	1.7762	0.2553	Wendt-Rasch et al (2003)
cypermethrin	I	-0.6990	1.0752	0.3	2.6021	NF	-1.2217	1.6535	6.6000	4.6270	1.7782	0.2782	1.7762	0.2553	
cypermethrin	I	-0.6990	1.0752	1.6	2.7404	NF	-0.4947	2.3805	6.6000	4.6270	1.7782	0.2782	1.7762	0.2553	
cypermethrin	I	-0.6990	1.0752	3.2	2.8451	NF	-0.1937	2.6816	6.6000	4.6270	1.7782	0.2782	1.7762	0.2553	
cypermethrin	I	1.4771	0.3927	0.07	0.0000	NF	-1.8537	1.0215	6.6000	4.6270	1.7782	0.2782	1.7762	0.2553	Hamer & Hill (1994)
deltamethrin	I	1.2041	0.7559	13	2.1987	NF	1.2831	3.9475	4.5000	5.0646	1.2788	1.6812	1.9418	1.3054	Tidou et al (1992)
diflubenzuron	I	1.0086	0.7435	10	2.0000	NF	0.3683	1.5062	3.8900	3.8663	0.6021	1.4472	1.0534	1.9243	Ali & Kok-Yokomic (1989)
diflubenzuron	I	2.6021		30	0.2355	F	0.8454	1.9834	3.8900	3.8663	0.6021	1.4472	1.0534	1.7475	Ludwig (1993)
diflubenzuron	I	1.6335	0.4654	3.36	1.0969	F	-0.1054	1.0326	3.8900	3.8663	0.6021	1.4472	1.0534	1.9243	Tanner & Moffett (1995)
diflubenzuron	I	1.6335	0.4654	27.2	1.0969	F	0.8028	1.9408	3.8900	3.8663	0.6021	1.4472	1.0534	1.9243	
esfenvalerate	I			0.01	0.0000	F	-1.5886	0.1220	6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	Lozano et al (1992)
esfenvalerate	I			0.08	0.0000	F	-0.6855	1.0251	6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
esfenvalerate	I			0.2	0.0000	F	-0.2876	1.4230	6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
esfenvalerate	I			1	0.0000	F	0.4114	2.1220	6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
esfenvalerate	I			5	0.0000	F	1.1104	2.8210	6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
esfenvalerate	I	-0.7447	1.0318	0.005	0.3010	NF	-1.8896	-0.1790	6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	Samsoe-Peterson et al (2001)
esfenvalerate	I	-0.7447	1.0318	0.18	1.0000	NF	-0.3333	1.3773	6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
esfenvalerate	I	-0.7447	1.0318	0.61	2.0000	NF	0.1967	1.9073	6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
esfenvalerate	I	-0.7447	1.0318	2.1	2.0000	NF	0.7336	2.4442	6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
esfenvalerate	I	-0.7447	1.0318	7.5	2.0000	NF	1.2865	2.9971	6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
esfenvalerate	I	-0.7447	1.0318	26	2.0000	NF	1.8264	3.5370	6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
fenthion	I	2.6021		250	1.0175	F	2.6055	3.5735	4.8400	3.2553	0.0000	-1.9830	0.7526	1.7544	Ludwig (1993)
fenvalerate	I	2.0969	0.0792	0.05	0.8938	NF	-1.0885	0.8303	5.0100	3.7243	2.2524	0.7780	1.9192	1.4771	Day & Kaushil (1987)
fenvalerate	I	2.0969	0.0792	0.01	0.0000	NF	-1.7875	0.1313	5.0100	3.7243	2.2524	0.7780	1.9192	1.4771	
glufosinate-ammonium	H	1.2586	0.4942	1000	0.4624	NF	-2.8410	-0.6543	-4.8100	1.8412	1.0837	0.8320	0.4771	2.5623	Faber et al (1998)
glufosinate-ammonium	H	1.2586	0.4942	10000	3.4591	NF	-1.8410	0.3457	-4.8100	1.8412	1.0837	0.8320	0.4771	2.5623	
hexazinone	H	1.9469	0.1303	32	0.0000	NF	-3.7913	-2.6580	1.2000	1.4232	2.2908	3.0394	1.7782	3.2613	Thompson et al (1993)
hexazinone	H	1.9469	0.1303	102	0.0000	NF	-3.2878	-2.1546	1.2000	1.4232	2.2908	3.0394	1.7782	3.2613	
lambda-cyhalothrin	I	1.3979	0.4472	0.017	0.0000	NF	-1.0376	1.4575	7.0000	5.0969	1.5441	1.3617	1.5476	0.8451	Hamer et Hill (1994)

lambda-cyhalothrin	I	1.3979	0.4472	0.17	0.0000	NF	-0.0376	2.4575	7.0000	5.0969	1.5441	1.3617	1.5476	0.8451	
lindane	I	-0.5229		4	0.0000	NF	-2.4499	0.9410	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	Fliedner & Klein (1996)
lindane	I	-0.5229		8	0.0000	NF	-2.1489	1.2421	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	
lindane	I	-0.5229		16	0.0000	NF	-1.8479	1.5431	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	
lindane	I	-0.5229		32	0.0000	NF	-1.5468	1.8441	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	
lindane	I	-0.5229		64	0.0000	NF	-1.2458	2.1452	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	
lindane	I	-0.5229		128	1.0607	NF	-0.9448	2.4462	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	
lindane	I	-0.5229		256	1.0607	NF	-0.6437	2.7472	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	
lindane	I	-0.5229		512	1.0607	NF	-0.3427	3.0483	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	
lindane	I	1.0000	0.7782	321	0.0000	NF	-0.5455	2.8455	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	Tidou et al (1992)
metamitron	H	0.6532	0.5065	400	0.0000	F	-2.4497	-1.2308	0.8300	3.5156	1.1611		1.3467	0.8751	Heimbach (1994)
metamitron	H	0.6532	0.5065	20000	0.3010	F	-0.7507	0.4682	0.8300	3.5156	1.1611		1.3467	0.8751	
metamitron	H	0.6532	0.5065	16	0.0000	F	-3.8477	-2.6287	0.8300	3.5156	1.1611		1.3467	0.8751	Heimbach et al (1994)
metamitron	H	0.6532	0.5065	170	0.0000	F	-2.8213	-1.6024	0.8300	3.5156	1.1611		1.3467	0.8751	
metamitron	H	1.8791	0.2201	32	0.0000	F	-3.5466	-2.3277	0.8300	3.5156	1.1611		1.3467	0.8751	
metamitron	H	1.8791	0.2201	330	0.0000	F	-2.5333	-1.3143	0.8300	3.5156	1.1611		1.3467	0.8751	
methabenzthiazuron	H	0.7782		10	0.0000	NF	-2.9585	-1.7507	2.6400	3.2916				2.5623	Wellmann et al (1998)
methabenzthiazuron	H	0.7782		21	0.0000	NF	-2.6363	-1.4285	2.6400	3.2916				2.5623	
methabenzthiazuron	H	0.7782		43	0.0000	NF	-2.3251	-1.1172	2.6400	3.2916				2.5623	
methabenzthiazuron	H	0.7782		89	0.0000	NF	-2.0092	-0.8013	2.6400	3.2916				2.5623	
methoxychlor	I	2.0334	0.1430	2.9	0.0000	NF	-0.6301	0.6510	5.0800	4.9165	1.9542	1.8360	1.7628	2.5647	Stephenson et al (1989)
methoxychlor	I	2.0334	0.1430	321	2.2219	NF	1.4140	2.6952	5.0800	4.9165	1.9542	1.8360	1.7628	2.5647	
methoxychlor	I	2.0334	0.1430	4.5	0.9031	NF	-0.4393	0.8419	5.0800	4.9165	1.9542	1.8360	1.7628	2.5647	
methoxychlor	I	2.0334	0.1430	46.5	2.0843	NF	0.5749	1.8561	5.0800	4.9165	1.9542	1.8360	1.7628	2.5647	
metsulfuron methyl	H	1.9469	0.1303	10	0.0000	NF	-4.1761	-2.9682	0.0180	0.8129	2.0478	3.0394	1.6392	3.2613	Thompson et al (1993)
metsulfuron methyl	H	1.9469	0.1303	100	0.0000	NF	-3.1761	-1.9682	0.0180	0.8129	2.0478	3.0394	1.6392	3.2613	
metsulfuron methyl	H	1.9469	0.1303	500	0.0000	NF	-2.4771	-1.2693	0.0180	0.8129	2.0478	3.0394	1.6392	3.2613	
metsulfuron methyl	H	1.9469	0.1303	1000	0.0000	NF	-2.1761	-0.9682	0.0180	0.8129	2.0478	3.0394	1.6392	3.2613	
permethrin	I	2.0969	0.0792	0.5	1.7782	NF	-0.6110	1.5491	6.1000	4.6114	1.4771	-0.9031	1.5125	3.2613	Kaushik et al (1987)
permethrin	I	2.0969	0.0792	5	2.7782	NF	0.3890	2.5491	6.1000	4.6114	1.4771	-0.9031	1.5125	3.2613	

pyridaben	I	1.2304	0.5340	1.06	0.0000	F	0.2057	2.0162	6.1000	4.8451	2.1145	-1.6812	1.8469	3.2613	Rand et al (2000)
pyridaben	I	1.2304	0.5340	4.85	0.0000	F	0.8661	2.6766	6.1000	4.8451	2.1145	-1.6812	1.8469	3.2613	
pyridaben	I	1.2304	0.5340	50.98	0.0000	F	1.8878	3.6983	6.1000	4.8451	2.1145	-1.6812	1.8469	3.2613	
rotenone	F	2.3927		6	1.7782	F	-0.4515	0.4752	4.1600	4.0000	0.4771		0.9879	0.1139	Beal & Anderson (1989)
tebufenozide	I	1.7959	0.2041	70	0.0000	F	-1.1435	0.4860	4.2500	2.6799	2.4344	2.5136	2.0000	3.0145	Kreutzweiser & Thompson (1995)
tebufenozide	I	1.7959	0.2041	130	1.9570	F	-0.8746	0.7549	4.2500	2.6799	2.4344	2.5136	2.0000	3.0145	
tebufenozide	I	1.7959	0.2041	330	2.3549	F	-0.4701	1.1594	4.2500	2.6799	2.4344	2.5136	2.0000	3.0145	
tebufenozide	I	1.7959	0.2041	660	3.2000	F	-0.1690	1.4605	4.2500	2.6799	2.4344	2.5136	2.0000	3.0145	
temephos	I	0.4771	0.7267	9	1.2553	NF	2.9128	2.6750	4.9100	5.0000	1.4771	1.1760	1.2355	2.6628	Hughes et al (1980)
triazamate	I	-0.1024	0.7782	0.08	0.0000	NF	-3.4184	-1.1758	2.1500	3.0039		2.1761			Toy (1994)
triazamate	I	-0.1024	0.7782	0.228	0.0000	NF	-2.9636	-0.7210	2.1500	3.0039		2.1761			
triazamate	I	-0.1024	0.7782	0.852	0.0000	NF	-2.3911	-0.1485	2.1500	3.0039		2.1761			
triazamate	I	-0.1024	0.7782	1.885	0.0000	NF	-2.0462	0.1964	2.1500	3.0039		2.1761			
triazamate	I	-0.1024	0.7782	5.5	0.0000	NF	-1.5812	0.6615	2.1500	3.0039		2.1761			

Appendix 4 – Data used to model Insecta Species responses

Key

Type – type of pesticides
H - herbicide **I** – insecticide **F** - fish toxicant / fungicide

Fish – the presence of fish within the systems
F - fish was present **NF** – no fish was present

Structural Properties of the System
LTSA/V R – log-transformed total surface area to volume ratio

L Volume - log-transformed volume (m^3)

Toxicity Units
LTU Daphnia – log-transformed toxic unit based on geometric means for Daphnia species

L TU HC5 – C – log-transformed toxic unit based on hazard concentration for 5% of crustacea species

L TU HC5 – I – log-transformed toxic unit based on hazard concentration for 5% of insecta species

Physico-Chemical Pesticide Properties
log Koc – log-transformed organic carbon absorption coefficient

log kow – log-transformed octanol-water coefficient

Fate Properties of the Pesticide
LAAB – log-transformed aerobic aquatic biotransformation (days)
L HHL - log-transformed hydrolysis half-life (days)

L ASB - log-transformed aerobic soil biotransformation (days)
L WPHL - log-transformed water photolysis half-life (days)

Common name	L Volume	L TSA/V R	Effect Concentration (ug/l)	count ratio change	L TU Daphnia spp	L TU HC5 - C	L TU HC5 - I	log Kow	log Koc	LASB	L WPHL	LAAB	L HHL	Source
bendiocarb	5.3010		24	0	-0.0852	1.7106	-0.3223	1.7200	2.5855	0.2586	1.1551	0.8733	0.5315	Lahr et al (2000)
carbaryl	1.4969	0.3444	1000	1	1.6636	2.7150	2.2034	1.8500	2.3010	1.0167	1.0709	1.2738	0.2355	Hanazato & Yasuno (1995)
carbofuran	0.1038	0.7938	6.3	0	-0.7429	1.5451	0.7938	1.5200	1.4065	1.0414	0.7482	0.3010	-0.1675	Wayland & Boag (1995)
carbofuran			9	1	-0.5880	1.7000	0.9487	1.5200	1.4065	1.0414	0.7482	0.3010	-0.1675	
carbofuran			14	0.5	-0.3961	1.8919	1.1406	1.5200	1.4065	1.0414	0.7482	0.3010	-0.1675	
carbofuran	0.1038	0.7938	22.5	1	-0.1901	2.0979	1.3466	1.5200	1.4065	1.0414	0.7482	0.3010	-0.1675	
carbofuran			32	1	-0.0371	2.2509	1.4996	1.5200	1.4065	1.0414	0.7482	0.3010	-0.1675	
chlorpyrifos	1.0492	0.4440	0.03	0	-1.3276	-0.2218	-1.0669	4.7000	3.6880	2.2022	1.4771	1.7076	1.2041	Biever et al (1994)

chlorpyrifos	1.0492	0.4440	0.088	0.67	-0.8603	0.2455	-0.5996	4.7000	3.6880	2.2022	1.4771	1.7076	1.2041	
chlorpyrifos	1.0492	0.4440	0.25	0.67	-0.4068	0.6990	-0.1461	4.7000	3.6880	2.2022	1.4771	1.7076	1.2041	
chlorpyrifos	1.0492	0.4440	0.83	0.67	0.1143	1.2201	0.3750	4.7000	3.6880	2.2022	1.4771	1.7076	1.2041	
chlorpyrifos	1.0492	0.4440	2.7	0.67	0.6266	1.7324	0.8873	4.7000	3.6880	2.2022	1.4771	1.7076	1.2041	
chlorpyrifos	-0.2147	0.8831	5	1	0.8942	2.0000	1.1549	4.7000	3.6880	2.2022	1.4771	1.7076	1.8573	Brock et al (1992)
chlorpyrifos	-0.2147	0.8831	35	1	1.7393	2.8451	2.0000	4.7000	3.6880	2.2022	1.4771	1.7076	1.8573	
chlorpyrifos	-0.2147	0.8831	35	1	1.7393	2.8451	2.0000	4.7000	3.6880	2.2022	1.4771	1.7076	1.8573	
chlorpyrifos	1.7404	0.3838	0.51	1	-0.0972	1.0086	0.1635	4.7000	3.6880	2.2022	1.4771	1.7076	1.5459	Siefert et al (1989)
chlorpyrifos	1.7404	0.3838	6.29	1	0.9939	2.0997	1.2546	4.7000	3.6880	2.2022	1.4771	1.7076	1.5459	
chlorpyrifos	1.7404	0.3838	32	1	1.7004	2.8062	1.9611	4.7000	3.6880	2.2022	1.4771	1.7076	1.5459	
chlorpyrifos	1.7782	0.7210	0.1	0.18	-0.8048	0.3010	-0.5441	4.7000	3.6880	2.2022	1.4771	1.7076	1.8573	Van den Brink et al (1996)
chlorpyrifos	1.7782	0.7210	0.9	0.64	0.1495	1.2553	0.4102	4.7000	3.6880	2.2022	1.4771	1.7076	1.8573	
chlorpyrifos	1.7782	0.7210	6	0.91	0.9734	2.0792	1.2341	4.7000	3.6880	2.2022	1.4771	1.7076	1.8573	
chlorpyrifos	1.7782	0.7210	44	1	1.8387	2.9445	2.0994	4.7000	3.6880	2.2022	1.4771	1.7076	1.8573	
deltamethrin	5.3010		0.64	1	-0.0247	2.6397	1.7211	4.5000	5.0646	1.2788	1.6812	1.9418	1.3054	Lahr et al (2000)
deltamethrin	0.5740		0.23	1	-0.4691	2.1953	1.2767	4.5000	5.0646	1.2788	1.6812	1.9418	1.3054	Morrill & Neal (1990)
deltamethrin	1.2041	0.7559	13	1	1.2831	3.9475	3.0289	4.5000	5.0646	1.2788	1.6812	1.9418	1.3054	Tidou et al (1992)
deltamethrin	1.2041	0.7559	0.5	1	-0.1319	2.5325	1.6139	4.5000	5.0646	1.2788	1.6812	1.9418	1.3054	
diflubenzuron	1.0086	0.7435	10	1	0.3683	1.5062	-0.6679	3.8900	3.8663	0.6021	1.4472	1.9243	1.0534	Ali & Kok-Yokomi (1989)
diflubenzuron	5.3010		10.4	0	0.3853	1.5233	-0.6509	3.8900	3.8663	0.6021	1.4472	1.0534	1.9243	Lahr et al (2000)
esfenvalerate			0.01	0	-1.5886	0.1220		6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	Lozano et al (1992)
esfenvalerate			0.08	1	-0.6855	1.0251		6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
esfenvalerate			0.2	1	-0.2876	1.4230		6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
esfenvalerate			1	1	0.4114	2.1220		6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
esfenvalerate			5	1	1.1104	2.8210		6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
fenitrothion	5.3010		80	1	0.8061	2.7653	1.6611	3.4300	3.0519	1.5563	0.5623	1.1021	2.2695	Lahr et al (2000)
lindane	0.0000	0.7482	4	1	-2.4499	0.9410	0.1423	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	Peither et al (1996)
lindane	0.0000	0.7482	8	1	-2.1489	1.2421	0.4434	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	
lindane	0.0000	0.7482	16	1	-1.8479	1.5431	0.7444	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	
lindane	0.0000	0.7482	24	1	-1.6718	1.7192	0.9205	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	
lindane	0.0000	0.7482	32	1	-1.5468	1.8441	1.0454	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	
lindane	0.0000	0.7482	64	1	-1.2458	2.1452	1.3464	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	
lindane	1.0000	0.7782	321	0	-0.5455	2.8455	2.0468	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	Tidou et al (1992)
lindane	1.0000	0.7782	103.6	1	-1.0366	2.3543	1.5556	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	Caquet et al (1992)

methyl parathion	1.6021	0.5119	8	1	0.9534	1.5906	0.9691	3.0000	3.6776	1.0106	1.0000	0.6128	1.3222	Crossland (1988)
methyl parathion	1.6021	0.5119	29	1	1.5127	2.1499	1.5284	3.0000	3.6776	1.0106	1.0000	0.6128	1.3222	
permethrin	0.4771	0.6561	0.75	1	-0.4349	1.7252	0.8178	6.1000	4.6114	1.4771	-0.9031	1.5125	3.2613	Yasuno et al (1988)
permethrin	0.4771	0.6561	1.5	1	-0.1338	2.0263	1.1188	6.1000	4.6114	1.4771	-0.9031	1.5125	3.2613	
phorate	1.6532	0.4914	45	1	0.8282	3.5454	1.7320	3.9200	2.6575	0.4771	0.0414	-0.3401	0.5051	Dieter et al (1996)
phorate	1.6532	0.4914	45	1	1.1197	3.8369	2.0235	3.9200	2.6575	0.4771	0.0414	-0.3401	0.5051	

Appendix 5 – Data used to model Insecta Family responses

Key

Type – type of pesticides
H - herbicide **I** – insecticide **F** - fish toxicant / fungicide

Fish – the presence of fish within the systems
F - fish was present **NF** – no fish was present

Structural Properties of the System
LTSA/V R – log-transformed total surface area to volume ratio

L Volume - log-transformed volume (m^3)

Toxicity Units
LTU Daphnia – log-transformed toxic unit based on geometric means for Daphnia species
L TU HC5 – I – log-transformed toxic unit based on hazard concentration for 5% of insecta species

L TU HC5 – C – log-transformed toxic unit based on hazard concentration for 5% of crustacea species

Physico-Chemical Pesticide Properties
log Koc – log-transformed organic carbon absorption coefficient

log kow – log-transformed octanol-water coefficient

Fate Properties of the Pesticide
LAAB – log-transformed aerobic aquatic biotransformation (days)
L HHL - log-transformed hydrolysis half-life (days)

L ASB - log-transformed aerobic soil biotransformation (days)
L WPHL - log-transformed water photolysis half-life (days)

Common name	L Volume	L TSA/V R	Effect Concentration (ug/l)	count ratio change	L TU Daphnia spp GM L/EC50	L TU HC5 - C	L TU HC5 - I	log Kow	log Koc	LASB	L WPHL	L AAB	L HHL	
Bendiocarb	5.3010		24	0	-0.0852	1.711	-0.322	1.7200	2.5855	0.2586	1.1551	0.8733	0.5315	Lahr et al (2000)
Carbaryl	1.4969	0.3444	1000	1	1.6636	2.715	2.203	1.8500	2.3010	1.0167	1.0709	1.2738	0.2355	Hanazato & Yasuno (1995)
Carbofuran			9.2	0.57	-0.5784	1.71	0.958	1.5200	1.4065	1.0414	0.7482	0.3010	-0.1675	Wayland & Boag (1995)
Carbofuran			14.4	0.57	-0.3839	1.904	1.153	1.5200	1.4065	1.0414	0.7482	0.3010	-0.1675	
Carbofuran			32.5	0.57	-0.0304	2.258	1.506	1.5200	1.4065	1.0414	0.7482	0.3010	-0.1675	
Carbofuran			32.6	0.57	-0.0290	2.259	1.508	1.5200	1.4065	1.0414	0.7482	0.3010	-0.1675	
Carbofuran	0.1038	6.2200	6.3	0	-0.7429	1.545	0.794	1.5200	1.4065	1.0414	0.7482	0.3010	-0.1675	Wayland & Boag (1990)
Carbofuran			9	1	-0.5880	1.7	0.949	1.5200	1.4065	1.0414	0.7482	0.3010	-0.1675	
Carbofuran			14	0.5	-0.3961	1.892	1.141	1.5200	1.4065	1.0414	0.7482	0.3010	-0.1675	
Carbofuran	0.1038	0.7938	22.5	1	-0.1901	2.098	1.347	1.5200	1.4065	1.0414	0.7482	0.3010	-0.1675	
Carbofuran			32	1	-0.0371	2.251	1.5	1.5200	1.4065	1.0414	0.7482	0.3010	-0.1675	
Chlorpyrifos	1.0492	0.4440	0.03	0	-1.3276	-0.222	-1.067	4.7000	3.6880	2.2022	1.4771	1.7076	1.2041	Biever et al (1994)

Chlorpyrifos	1.0492	0.4440	0.088	0.5	-0.8603	0.246	-0.6	4.7000	3.6880	2.2022	1.4771	1.7076	1.2041	
Chlorpyrifos	1.0492	0.4440	0.25	0.5	-0.4068	0.699	-0.146	4.7000	3.6880	2.2022	1.4771	1.7076	1.2041	
Chlorpyrifos	1.0492	0.4440	0.83	0.5	0.1143	1.22	0.375	4.7000	3.6880	2.2022	1.4771	1.7076	1.2041	
Chlorpyrifos	1.0492	0.4440	2.7	0.5	0.6266	1.732	0.887	4.7000	3.6880	2.2022	1.4771	1.7076	1.2041	
Chlorpyrifos	-0.2147	0.8831	5	1	0.8942	2	1.155	4.7000	3.6880	2.2022	1.4771	1.7076	1.8573	Brock et al (1992)
Chlorpyrifos	-0.2147	0.8831	35	1	1.7393	2.845	2	4.7000	3.6880	2.2022	1.4771	1.7076	1.8573	
Chlorpyrifos	-0.2147	0.8831	35	1	1.7393	2.845	2	4.7000	3.6880	2.2022	1.4771	1.7076	1.8573	
Chlorpyrifos	1.7404	0.3838	0.51	1	-0.0972	1.009	0.164	4.7000	3.6880	2.2022	1.4771	1.7076	1.8573	Siefert et al (1989)
Chlorpyrifos	1.7404	0.3838	6.29	1	0.9939	2.1	1.255	4.7000	3.6880	2.2022	1.4771	1.7076	1.8573	
Chlorpyrifos	1.7404	0.3838	32	1	1.7004	2.806	1.961	4.7000	3.6880	2.2022	1.4771	1.7076	1.8573	
Chlorpyrifos	1.7782	0.7210	0.1	0.22	-0.8048	0.301	-0.544	4.7000	3.6880	2.2022	1.4771	1.7076	1.8573	
Chlorpyrifos	1.7782	0.7210	0.9	0.67	0.1495	1.255	0.41	4.7000	3.6880	2.2022	1.4771	1.7076	1.8573	Van den Brink et al (1996)
Chlorpyrifos	1.7782	0.7210	6	88.89	0.9734	2.079	1.234	4.7000	3.6880	2.2022	1.4771	1.7076	1.8573	
Chlorpyrifos	1.7782	0.7210	44	1	1.8387	2.944	2.099	4.7000	3.6880	2.2022	1.4771	1.7076	1.8573	
Cypermethrin	4.3979	2.47	0.07	1	-1.8537	1.022	0.668	6.6000	4.6270	1.7782	0.2782	1.7762	0.2553	Farmer et al (1994)
Deltamethrin	5.3010		0.64	1	-0.0247	2.64	1.721	4.5000	5.0646	1.2788	1.6812	1.9418	1.3054	Lahr et al (2000)
Deltamethrin	0.5740		0.23	1	-0.4691	2.195	1.277	4.5000	5.0646		1.6812			Morrill & Neal (1990)
Deltamethrin	1.2041	0.7559	13	1	1.2831	3.948	3.029	4.5000	5.0646	1.2788	1.6812	1.9418	1.3054	Tidou et al (1992)
Deltamethrin	1.2041	0.7559	0.5	1	-0.1319	2.533	1.614	4.5000	5.0646		1.6812			
Diflubenzuron	1.0086	0.7435	10	1	0.3683	1.506	-0.668	3.8900	3.8663	0.6021	1.4472	1.0534	1.9243	Ali & Kok-Yokomi (1989)
Diflubenzuron	5.3010		10.4	0	0.3853	1.523	-0.651	3.8900	3.8663	0.6021	1.4472	1.0534	1.9243	Lahr et al (2000)
Diflubenzuron	1.6335	0.4654	3.36	1	-0.1054	1.033	-1.142	3.8900	3.8663	0.6021	1.4472	1.0534	1.9243	Tanner & Moffett (1995)
Diflubenzuron	1.6335	0.4654	27.2	1	0.8028	1.941	-0.233	3.8900	3.8663	0.6021	1.4472	1.0534	1.9243	
Diflubenzuron	1.8129	0.3304	0.7	0	-0.7866	0.351	-1.823	3.8900	3.8663	0.6021	1.4472	1.0534	1.7503	Liber et al (1998)
Diflubenzuron	1.8129	0.3304	2.5	0.25	-0.2338	0.904	-1.27	3.8900	3.8663	0.6021	1.4472	1.0534	1.7503	
Diflubenzuron	1.8129	0.3304	7	0.25	0.2134	1.351	-0.823	3.8900	3.8663	0.6021	1.4472	1.0534	1.7503	
Diflubenzuron	1.8129	0.3304	30	0.75	0.8454	1.983	-0.191	3.8900	3.8663	0.6021	1.4472	1.0534	1.7503	
Esfenvalerate			0.01	0	-1.5886	0.122		6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	Lozano et al (1992)
Esfenvalerate			0.08	0.5	-0.6855	1.025		6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
Esfenvalerate			0.2	0.5	-0.2876	1.423		6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
Esfenvalerate	0.9731	0.7451	1	0.75	0.4114	2.122		6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
Esfenvalerate	0.9731	0.7451	5	1	1.1104	2.821		6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
Fenitrothion	5.3010		80	1	0.8061	2.765	1.661	3.4300	3.0519	1.5563	0.5623	1.1021	2.2695	Lahr et al (2000)
lambda-cyhalothrin	1.3979	0.4472	0.017	1	-1.0376	1.458		7.0000	5.0969	1.5441	1.3617	1.5476	0.8451	Hamer & Hill (1994)

lambda-cyhalothrin	1.3979	0.4472	0.17	1	-0.0376	2.458		7.0000	5.0969	1.5441	1.3617	1.5476	0.8451	
Lindane	0.0000	0.7482	4	1	-2.4499	0.941	0.142	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	Peither et al (1996)
Lindane	0.0000	0.7482	8	1	-2.1489	1.242	0.443	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	
Lindane	0.0000	0.7482	16	1	-1.8479	1.543	0.744	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	
Lindane	0.0000	0.7482	24	1	-1.6718	1.719	0.92	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	
Lindane	0.0000	0.7482	32	1	-1.5468	1.844	1.045	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	
Lindane	0.0000	0.7482	64	1	-1.2458	2.145	1.346	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	
Lindane	1.0000	0.7782	321	0	-0.5455	2.845	2.047	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	
Lindane	1.0000	0.7782	103.6	1	-1.0366	2.354	1.556	3.5000	3.0414	2.9912	3.0394	2.3068	2.2375	
methyl parathion	0.6990	0.4150	100	0.5	2.0503	2.687	2.066	3.0000	3.6776	1.0106	1.0000	0.6128	1.5682	Crossland (1984)
methyl parathion	1.6021	0.5119	8	0.6	0.9534	1.591	0.969	3.0000	3.6776	1.0106	1.0000	0.6128	1.5682	Crossland (1988)
methyl parathion	1.6021	0.5119	29	0.6	1.5127	2.15	1.528	3.0000	3.6776	1.0106	1.0000	0.6128	1.5682	
methyl parathion	0.0086		2500	1	3.4482	4.085	3.464	3.0000	3.6776	1.0106	1.0000	0.6128	1.5682	Stephenson & Kane (1984)
Permethrin			1	0	-0.3099	1.85	0.943	6.1000	4.6114	1.4771	-0.9031	1.5125	3.2613	Conrad et al (1999)
Permethrin			10	1	0.6901	2.85	1.943	6.1000	4.6114	1.4771	-0.9031	1.5125	3.2613	
Permethrin			50	1	1.3890	3.549	2.642	6.1000	4.6114	1.4771	-0.9031	1.5125	3.2613	
Permethrin			100	1	1.6901	3.85	2.943	6.1000	4.6114	1.4771	-0.9031	1.5125	3.2613	
Permethrin	0.4771	0.6561	0.75	1	-0.4349	1.725	0.818	6.1000	4.6114	1.4771	-0.9031	1.5125	3.2613	Yasano et al (1988)
Permethrin	0.4771	0.6561	1.5	1	-0.1338	2.026	1.119	6.1000	4.6114	1.4771	-0.9031	1.5125	3.2613	
Phorate	1.6532	0.4914	23	1	0.8282	3.545	1.732	3.9200	2.6575	0.4771	0.0414	-0.3401	0.5051	Dieter et al (1996)
Phorate	1.6532	0.4914	45	1	1.1197	3.837	2.023	3.9200	2.6575	0.4771	0.0414	-0.3401	0.5051	
Trichlorphon	0.2900	0.6946	2.5	0	0.6448		0.038	0.4300	0.2014	1.4314	2.0414	-0.4815	0.1461	Cook et al (1995)
Trichlorphon	0.2900	0.6946	25	0	1.6448		1.038	0.4300	0.2014	1.4314	2.0414	-0.4815	0.1461	
Trichlorphon	0.2900	0.6946	250	0	2.6448		2.038	0.4300	0.2014	1.4314	2.0414	-0.4815	0.1461	

Appendix 6 – Data used to model Algal Species responses

Key

Type – type of pesticides
H - herbicide **I** – insecticide **F** - fish toxicant / fungicide

Fish – the presence of fish within the systems
F - fish was present **NF** – no fish was present

Structural Properties of the System
LTSA/V R – log-transformed total surface area to volume ratio

L Volume - log-transformed volume (m^3)

Toxicity Units
LTU Daphnia – log-transformed toxic unit based on geometric means for Daphnia species
L TU HC5 – A – log-transformed toxic unit based on hazard concentration for 5% of algae species

L TU HC5 – C – log-transformed toxic unit based on hazard concentration for 5% of crustacea species

Physico-Chemical Pesticide Properties
log Koc – log-transformed organic carbon absorption coefficient

log kow – log-transformed octanol-water coefficient

Fate Properties of the Pesticide
LAAB – log-transformed aerobic aquatic biotransformation (days)
L HHL - log-transformed hydrolysis half-life (days)

L ASB - log-transformed aerobic soil biotransformation (days)
L WPHL - log-transformed water photolysis half-life (days)

Common name	Type	L Volume	L TSA/V R	Effect Concentration (ug/l)	count ratio change	L TU Daphnia spp	L TU HC5 - C	L TU HC5 - A	log Kow	log Koc	LASB	L WPHL	LAAB	L HHL	Source
alachlor	H	-0.9431	1.4158	0.89	0.14	-4.1070	-2.9201	0.1354	3.0900	2.2304	1.0502	3.0394	1.2885	3.2613	Spawn et al (1997)
alachlor	H	-0.9431	1.4158	9	0.43	-3.1021	-1.9152	1.1402	3.0900	2.2304	1.0502	3.0394	1.2885	3.2613	
alachlor	H	-0.9431	1.4158	27.2	0.57	-2.6218	-1.4349	1.6205	3.0900	2.2304	1.0502	3.0394	1.2885	3.2613	
alachlor	H	-0.9431	1.4158	84.9	0.71	-2.1275	-0.9406	2.1149	3.0900	2.2304	1.0502	3.0394	1.2885	3.2613	
alachlor	H	-0.9431	1.4158	1027	0.71	-1.0448	0.1421	3.1975	3.0900	2.2304	1.0502	3.0394	1.2885	3.2613	
atrazine	H	2.0969	0.0792	140	0.5	-2.2046	-1.0846	1.0666	2.5000	1.9390	1.6435	2.5250	2.2782	2.3010	Stephenson et al (1999)
atrazine	H	2.0969	0.0792	1560	0.75	-1.1576	-0.0376	2.1136	2.5000	1.9390	1.6435	2.5250	2.2782	2.3010	
atrazine	H	2.0512	0.0934	100	0.5	-2.3507	-1.2307	0.9205	2.5000	1.9390	1.6435	2.5250	2.2782	2.3010	Yasuno et al (1988)
atrazine	H	-0.8038	1.0212	200	0	-2.0496	-0.9297	1.2215	2.5000	1.9390	1.6435	2.5250	2.2782	2.3010	Lay et al (1984)
cypermethrin	I	-0.6990	1.0752	0.08	0.81	-1.7957	1.0795	-3.9341	6.6000	4.6270	1.7782	0.2782	1.7762	0.2553	Webdt-Rasch et al (2003)
cypermethrin	I	-0.6990	1.0752	0.3	1	-1.2217	1.6535	-3.3601	6.6000	4.6270	1.7782	0.2782	1.7762	0.2553	

cypermethrin	I	-0.6990	1.0752	1.6	1	-0.4947	2.3805	-2.6331	6.6000	4.6270	1.7782	0.2782	1.7762	0.2553	
cypermethrin	I	-0.6990	1.0752	3.2	1	-0.1937	2.6816	-2.3321	6.6000	4.6270	1.7782	0.2782	1.7762	0.2553	
cypermethrin	I	1.4771	0.3927	0.07	0	-1.8537	1.0215	-3.9921	6.6000	4.6270	1.7782	0.2782	1.7762	0.2553	Farmer et al (1994)
dichlobenil	H	-0.8038	1.0212	4300	0	-0.1730	0.4405	0.6963	2.7000	2.0414	1.9590	1.1790	0.3802	3.2613	Lay et al (1984)
fenthion	I	0.1761	0.8615	23	0.5	1.5693	2.5373	-0.5169	4.8400	3.2553	0.0000	-1.9830	0.7526	1.7544	Hanazato & Kasai (1995)
fenthion	I	0.1761	0.8615	175	0.5	2.4506	3.4186	0.3644	4.8400	3.2553	0.0000	-1.9830	0.7526	1.7544	
hexazinone	H	1.9469	0.1303	32	0	-3.7913	-2.6580	1.6012	1.2000	1.4232	2.2908	3.0394	1.7782	3.2613	Thompson et al (1993)
hexazinone	H	1.9469	0.1303	102	0.33	-3.2878	-2.1546	2.1046	1.2000	1.4232	2.2908	3.0394	1.7782	3.2613	
hexazinone	H	1.9469	0.1303	1106	1	-2.2527	-1.1194	3.1398	1.2000	1.4232	2.2908	3.0394	1.7782	3.2613	
hexazinone	H	1.9469	0.1303	11276	1	-1.2443	-0.1110	4.1482	1.2000	1.4232	2.2908	3.0394	1.7782	3.2613	
lambda-cyhalothrin	I	1.4771	0.3927	0.017	0	-1.0376	1.4575	-3.2582	7.0000	5.0969	1.5441	1.3617	1.5476	0.8451	Farmer et al (1994)
lambda-cyhalothrin	I	1.4771	0.3927	0.17	0	-0.0376	2.4575	-2.2582	7.0000	5.0969	1.5441	1.3617	1.5476	0.8451	
metamitron	H	0.6532	0.5065	16	0	-3.8477	-2.6287	-0.5355	0.8300	3.5156	1.1611	-1.6990	1.3467	0.8751	Heimbach et al (1994)
metamitron	H	1.8791	0.2201	32	0	-3.5466	-2.3277	-0.2345	0.8300	3.5156	1.1611	-1.6990	1.3467	0.8751	
metamitron	H	0.6532	0.5065	170	0	-2.8213	-1.6024	0.4908	0.8300	3.5156	1.1611	-1.6990	1.3467	0.8751	
metamitron	H	1.8791	0.2201	330	0	-2.5333	-1.3143	0.7789	0.8300	3.5156	1.1611	-1.6990	1.3467	0.8751	
metribuzin	H	-0.3565	0.8927	1.8	0	-2.8858	-2.0952	0.6402	1.6000	1.2304	2.2500	-0.7467	2.0000	3.2613	Brock et al (2004)
metribuzin	H	-0.3565	0.8927	5.6	0	-3.3929	-2.6022	0.1332	1.6000	1.2304	2.2500	-0.7467	2.0000	3.2613	
metribuzin	H	-0.3565	0.8927	18	0.73	-2.8858	-2.0952	0.6402	1.6000	1.2304	2.2500	-0.7467	2.0000	3.2613	
metribuzin	H	-0.3565	0.8927	56	1	-2.3929	-1.6022	1.1332	1.6000	1.2304	2.2500	-0.7467	2.0000	3.2613	
metribuzin	H	-0.3565	0.8927	180	1	-1.8858	-1.0952	1.6402	1.6000	1.2304	2.2500	-0.7467	2.0000	3.2613	
metsulfuron methyl	H	1.9469	0.1303	10	0	-4.1761	-2.9682	0.5854	0.0180	0.8129	2.0478	3.0394	1.6392	3.2613	Thompson et al (1993)
metsulfuron methyl	H	1.9469	0.1303	100	0	-3.1761	-1.9682	1.5854	0.0180	0.8129	2.0478	3.0394	1.6392	3.2613	
metsulfuron methyl	H	1.9469	0.1303	500	0	-2.4771	-1.2693	2.2843	0.0180	0.8129	2.0478	3.0394	1.6392	3.2613	
metsulfuron methyl	H	1.9469	0.1303	1000	0	-2.1761	-0.9682	2.5854	0.0180	0.8129	2.0478	3.0394	1.6392	3.2613	
metsulfuron methyl	H	-1.0862	1.1166	1	0	-5.1761	-3.9682	-0.4146	0.0180	0.8129	2.0478	3.0394	1.6392	3.2613	Wendt-Rasch et al (2003)
metsulfuron methyl	H	-1.0862	1.1166	5	0	-4.4771	-3.2693	0.2843	0.0180	0.8129	2.0478	3.0394	1.6392	3.2613	
metsulfuron methyl	H	-1.0862	1.1166	20	1	-3.8751	-2.6672	0.8864	0.0180	0.8129	2.0478	3.0394	1.6392	3.2613	
permethrin	I	0.4771	0.6561	0.75	0.25	-0.4349	1.7252	-0.8989	6.1000	4.6114	1.4771	-0.9031	1.5125	3.2613	Yasuno et al (1998)
permethrin	I	0.4771	0.6561	1.5	0.25	-0.1338	2.0263	-0.5979	6.1000	4.6114	1.4771	-0.9031	1.5125	3.2613	

atrazine	H			20	0	-3.0496	-1.9297	0.2215	2.5000	1.9390	1.6435	2.5250	2.2782	2.3010	DeNoyelles et al (1982)
atrazine	H			500	1	-1.6517	-0.5317	1.6195	2.5000	1.9390	1.6435	2.5250	2.2782	2.3010	
esfenvalerate	I	1.670246		0.035	0.25	-1.0445	0.6661	-1.1996	6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	Schroll & Jespersen (1998)
esfenvalerate	I	1.670246		0.077	0.25	-0.7021	1.0085	-0.8572	6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	
esfenvalerate	I	1.670246		0.132	0.25	-0.4680	1.2426	-0.6231	6.2200	4.0000	1.8692	1.0000	1.7182	3.2613	

Appendix 7 – Types of Pesticides in Database

Insecticides : 25
Herbicides : 20 (2 types of 2,4 D)
Fish Toxicant : 1
Fungicide : 1

Herbicides

Pesticide	Chemical Group	Code	Mode of Action	Code
2,4 D (DMA & BEE)	Phenoxy	PX	Synthetic auxin	S A
Alachlor	Amide	A	Inhibition of protein synthesis and root elongation	P S
Atrazine	Triazine	T	Photosynthetic electron transport inhibitor at the photosystem II receptor site.	P
Dichlobenil	Nitrile	N	Inhibition of cell wall (cellulose) biosynthesis	C B
Diquat	Quaternary ammonium	Q	During photosynthesis, superoxide is generated, which damages cell membranes and cytoplasm	S G
Glyphosate	Op	OP	Inhibits 5-enolpyruvylshikimate-3-phosphate synthase (epsps), an enzyme of the aromatic acid biosynthetic pathway	EPSPS
Glufosinate-ammonium	Op	OP	Glutamine synthetase inhibitor	G S
Hexazinone	Triazinone	TN	Photosynthetic electron transport inhibitor at the photosystem II receptor site.	P
Isoproturon	Urea	U	Photosynthetic electron transport inhibitor at the photosystem II receptor site.	P
Linuron	Urea	U	Photosynthetic electron transport inhibitor at the photosystem II receptor site.	P
Metamitron	Triazinone	TN	Photosynthetic electron transport inhibitor at the photosystem II receptor site.	P
Methabenzthiazuron	Urea	U	Photosynthetic electron transport inhibitor at the photosystem II receptor site.	P
Metsulfuron methyl	Urea	U	Through inhibition of the enzyme acetolactate synthase	A S

Metribuzin	Triazinone	TN	Photosynthetic electron transport inhibitor at the photosystem II receptor site.	P
Nicosulfuron	Urea	U	Through inhibition of the enzyme acetolactate synthase	A S
Simazine	Triazine	T	Photosynthetic electron transport inhibitor at the photosystem II receptor site.	P
Tebuthiuron	Urea	U	Photosynthetic electron transport inhibitor at the photosystem II receptor site.	P
Terbutryn	Triazine	T	Photosynthetic electron transport inhibitor at the photosystem II receptor site.	P
Triclopyr	Pyridine	PD	Synthetic auxin	S A

Insecticides

Pesticide	**Chemical Group**	**Code**	**Mode of Action**	**Code**
Aminocarb	Carbamate	C	Cholinesterase inhibitor	C
Azinphosmethyl	Organothiophosphate	OP	Cholinesterase inhibitor	C
Bendiocarb	Carbamate	C	Cholinesterase inhibitor	C
Carbaryl	Carbamate	C	Cholinesterase inhibitor	C
Carbofuran	Carbamate	C	Oxidative phosphorylation uncoupler, leading to membrane disruption	O
Chlorpyrifos	Organothiophosphate	OP	Cholinesterase inhibitor	C
Cyfluthrin	Pyrethroid	P	Sodium channel disruption of neurons	S
Cypermethrin	Pyrethroid	P	Sodium channel disruption of neurons	S
Deltamethrin	Pyrethroid	P	Sodium channel disruption of neurons	S
Diflubenzuron	Insect Growth Regulator	IGR	Chitin synthesis inhibitor	Ch
Esfenvalerate	Pyrethroid	P	Sodium channel agonist	S
Fenitrothion	Organothiophosphate	OP	Cholinesterase inhibitor	C
Fenthion	Organothiophosphate	OP	Cholinesterase inhibitor	C
Fenvalerate	Pyrethroid	P	Sodium channel disruption of neurons	S
Lambda-cyhalothrin	Pyrethroid	P	Sodium channel disruption of neurons	S

Lindane	Organochlorine	OC	Antagonist of the GABA receptor-chloride channel complex	A
Methoxychlor	Organochlorine	OC		U
Methyl parathion	Organothiophosphate	OP	Cholinesterase inhibitor	C
Permethrin	Pyrethroid	P	Sodium channel disruption of neurons	S
Phorate	Organothiophosphate	OP	Cholinesterase inhibitor	C
Pyridaben	Pyridazinone	PZ	Inhibitor of mitochondrial electron transport at complex	M
Tebufenozide	Moulting hormone agonist	IGR	Moulting hormone agonist	H
Temephos	Organothiophosphate	OP	Cholinesterase inhibitor	C
Triazamate	Carbamoyl triazole	CT	Cholinesterase inhibitor	C
Trichlorphon	Phosphonate	PP	Cholinesterase inhibitor	C

Fungicide & Fish Toxicant

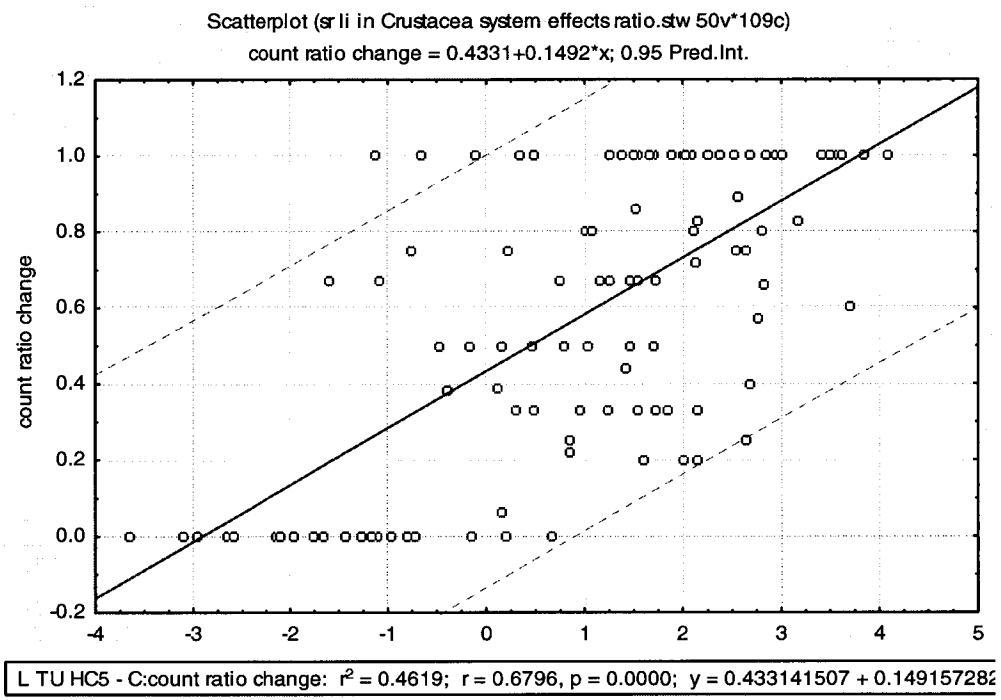
Pesticide	**Chemical Group**	**Code**	**Mode of Action**	**Code**
Carbendazim	Carbamate	C	Inhibits beta-tubulin synthesis	BT
Rotenone	Botanical	B	Respiratory inhibitor acting by inhibiting electron transport at NADH-ubiquinone oxidoreductase (complex I).	R

Appendix 8 - Study Sites, Application Months and Application Methods used in Ecosystem Studies

Country	**No of Studies**	**Application Month**	**No of Studies**	**Application Type**	**No of Studies**
Canada	27	January	1	Surface application - spray - spray drift	54
Denmark	3	February	1		27
France	4	April	4		10
Germany	5	May	13	Sub-surface application	12
Japan	3	June	22		
Senegal	1	July	16	Not Stated	18
Sweden	1	August	6		
The Netherlands	5	September	3		
UK	6	October	7		
USA	22	December	1		
Not Stated	8	Not Stated	17		
Total	**84**			**Total**	**84**

* some studies had multiple experiments with differing application dates.

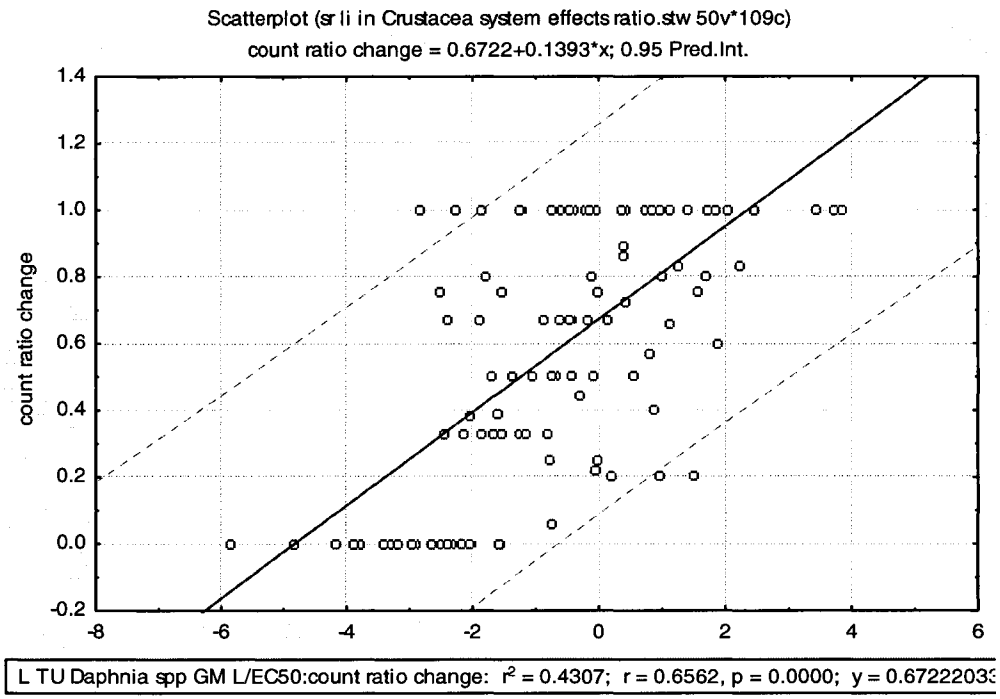
Appendix 9 – Scatterplot of Crustacea count ratio of effect and L TU HC5-C



Key

L TU HC5 – C – log-transformed toxic unit based on hazard concentration for 5% of crustacea species

Appendix 10 – Scatterplot of Crustacea count ratio of effect and L TU Daphnia



Key

LTU Daphnia – log-transformed toxic unit based on geometric mean for Daphnia species