AN ABSTRACT OF THE THESIS OF

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Abstract approved:

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Two pesticides used in forestry were tested against native, field collected stream invertebrates representative of Pacific Northwest streams. A flask assay system was developed to provide the high oxygenation and cold water conditions required by native organisms. The acute toxicity to six indigenous macroinvertebrates (*Ameletus* sp., *Brachycentrus americanus*, *Calineuria californica*, *Cinyma* sp., *Lepidostoma unicolor*, and *Psychoglypha* sp. (early and late instar)) to formulated triclopyr ester (herbicide) and carbaryl (insecticide) was determined. Toxicity was expressed as LC_{50} and LC_1 values based on 96-hr survival. Carbaryl was found to be 1000 times more toxic than triclopyr for all the organisms tested. LC_1 values (7.5, 28.8, 9.0, 3.0, 9.5, 14.8, 33.8 µg/L), respectively for carbaryl and 1.8, 3.9, 4.0, 4.2, 29.0, 16.1 mg/L respectively, triclopyr) were used in the calculation of hazardous concentration to 5% of the community based on the lower 95% confidence limit ($HC_5 / 95$) for carbaryl (0.43 – 0.66 µg/L) and triclopyr (0.11 mg/L). Pulsed exposures (15, 30 and 60 minutes) with carbaryl assessed the effect of exposure duration on mortality using two of the six species tested, *C. californica* and *Cinygma* sp. Significant differences in effects between the two species during pulsed exposure was noted, with *Cinygma* sp. being significantly more affected in

all combinations of dose and exposure time than *Calineuria californica*. A probit plane model, $Y = -10.86 + 4.83 (\ln C) + 3.0 (\ln T)$ developed for *Cinygma* sp. predicted probit mortality (Y) at different combinations of dose (C) and duration of exposure (T).

A rule-based model was developed to incorporate characteristics of morphology, behavior and life history for aquatic invertebrates that may determine the potential for field effects in the short-term (exposure and uptake) and long-term (recovery). Frequency distributions were generated that explored variation in these characteristics between genera within 8 aquatic orders (Plecoptera, Trichoptera, Ephemeroptera, Diptera, Coleoptera, Odonata, Hemiptera and Megaloptera), and organisms were identified that had an increased risk of effects. Model analysis distinguished the potential for effects between Oregon streams that had different insect communities. Stream insect communities in the Cascades exhibited a higher potential for short-term effects than Willamette Valley streams.

The Use of Native Macroinverebrates to Assess Pesticide Risk to Oregon Streams

by
Jennifer L. Peterson

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CONTRIBUTION OF AUTHORS

Dr. Paul Jepson and Dr. Jeffery Jenkins were involved in the design, analysis, and writing of each manuscript. The Oregon Department of Forestry was involved in proposing the testing addressed in chapter 2. Dr. Phil Heneghan was involved in the design and construction of the database developed in chapter 4. Dr. Judith Li and Dr. Alan Herlihy provided stream data used in chapter 5.

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DEDICATION

I would like to dedicate this work to my husband Jim and daughter Kendra for their support in attending graduate school. Thank you Jim for putting in many years of commuting to and from Portland in order for me to attend Oregon State University. Thank you for working so hard in your career so that we could afford it. Thank you Kendra for coming along in the midst of laboratory bioassays and analysis and putting all the hard work in perspective. You instantly taught me patience and what real achievement consisted of. Thanks for giving me such a new perspective on life and a more focused drive for my work than I had previously. Thanks for the many hours you spent with me in the "office", and for trying your best to understand that when the door was closed Mommy was working.

The Use of Native Macroinvertebrates to Assess Pesticide Risk to Oregon Streams

Chapter 1

Introduction and Literature Review

OVERVIEW

This research examined potential effects on stream macroinvertebrate communities from pesticide applications used in forest practices. The first part of the review includes an overview on Oregon forestry and forest practices, pesticide use and distribution, and chemical properties and behavior in the environment. Second, macroinvertebrate importance in stream systems, and justification for selection as test organisms for this research is described. Third, a general overview of current risk assessment process is outlined, including problem formulation, analysis, and risk characterization. Properties related to exposure assessment, including physical properties of stream habitat and ecology of the organisms, and effects assessment, describing methods for characterizing pesticide effects (i.e. current test methodology) and analysis are reviewed. An ecotoxicological profile is provided for the two chemicals used in this research, triclopyr and carbaryl. The endpoints of the risk assessment process is reviewed, including uncertainty analysis, and the implications of the results are reviewed as they pertain to risk managers, such as the Oregon Department of Forestry.

OREGON FORESTRY

Oregon provides an optimal environment for timber production. Areas close to the ocean receive an average of 115 inches of precipitation each year, resulting in dense and massive forests. Forest land covers 28.5 million of the state's 62 million acres land base, or about 46 percent of the state's total land mass (Oregon Department of Forestry, 1995). Over $40 million worth of timber is harvested from state forests each year, comprising approximately 23 percent of the nation's softwood timber inventory, and up to 20 percent of the nation's harvest (Green, 1982). Of these forest lands, 57 percent is owned by the federal

government, 38 percent is held by various private owners, 3 percent by the State of Oregon, and 2 percent is publicly owned (Oregon Department of Forestry, 1995).

The Department of Forestry regulates chemical and other petroleum product use on these lands under the authority of the Oregon Forest Practices Act. This act was adopted in 1971 to protect the soil, air and water quality of forest resources by outlining guidelines for forest practices, including pesticide use. This act applies to all private, state, county and most city forest lands, covering approximately 11.7 million acres. Annually there are approximately 20,000 forest operations on these lands (Oregon Department of Forestry, 1997). Included in this act are the Chemical Rules, which set down specific guidelines for the use of pesticides in the forest environment. These include guidelines for the proper mixing of chemicals, the required buffer zones widths around streams and other bodies of water, and the appropriate weather conditions for applications (Oregon Department of Forestry, 1997). The riparian zone provides canopy cover for the stream as well as being an important energy and nutrient source of allochthonous input for macroinvertebrate communities. Because of its important interactions with the stream system, the Oregon Forest Practices Act requires that streams have a 100 ft. riparian buffer zone if the land is privately owned, and a 300 ft. buffer if the land is publicly owned.

Chemicals that fall under the forest practice rules are herbicides, insecticides, rodenticides, fungicides, petroleum products (used as carriers for pesticides), adjuvants (surfactants, drift control additives, anti-foam agents, wetting agents, and spreading agents), and fertilizers. The Chemical Rules were revised in January of 1997, introducing a need to test their effectiveness in protecting riparian function and water quality.

PESTICIDE USE IN FORESTRY

CHEMICAL USE AND DISTRIBUTION

Pesticides are an effective tool in profitable timber production by protecting forest trees from damaging competitive vegetation, insects, and diseases (Newton, 1981). Methods of pesticide application include ground foliar spray, basal spray, injections to cut surfaces, and aerial spray. However, aerial applications usually present the greatest danger to aquatic systems because only a portion of the chemical is deposited on the target area while the rest of the chemical is lost as drift. Drift can be minimized by carefully addressing operational parameters including spray formulation, droplet size, flow rate, release height, and meteorological conditions (Maksymiuk, 1971).

Small, charged droplets tend to be more susceptible to wind or thermal currents and have the potential to move the greatest distances (Matthews, 1992). For coniferous forests, it has been determined that droplet size spectrum in the spray should be kept within the 15 um to 55 um range (after evaporation) (Picot et. al, 1986). Reducing droplet size can be accomplished by selecting nozzles with large orifices, and positioning them toward the rear of the trust line of the aircraft to minimize turbulence. In addition, increasing viscosity and surface tension while reducing temperature favors larger drops (Maksymiuk, 1971).

Droplet dispersal is further influenced by local environmental conditions such as mean wind velocity and direction, relative humidity, temperature and turbulence (Matthews, 1992; Christensen et al., 1971). Morning application times often provide optimal conditions for spray applications because of low wind velocities and ground temperature. An increase in ground temperature later in the day can result in increased convection and thermal currents (Maksymiuk, 1971).

PROPERTIES AND BEHAVIOR

Pesticides used in forestry include herbicides, fungicides, and insecticides Herbicides are widely used for the control of grasses, broad-leaved weeds, and hardwood trees that can reduce the survival and early growth of the harvest tree, resulting in longer establishment periods (Willoughby & Dewar, 1995; Campbell, 1991; Green, 1982). Herbicides are used for site preparation, where: 1) vegetation is controlled so that seedlings can be established; 2) conifer release, where competing forest weeds are removed from a stand of harvest trees; or 3) for timber stand improvement, where the composition of the harvest stand is improved by eliminating competing trees (Newton, 1981). Herbicide applications occur both in the spring and the fall in the Pacific Northwest. Common names of the herbicides currently used in forest practices are listed in Table 1.1, as of April, 2000.

Table 1.1: Generic names of herbicides currently registered for use in forestry (Oregon Department of Forestry, 2000).

2,4-D
2,4-D Picloram
Atrazine
Glyphosate
Hexazinone
Imazapyr
Metsulfuron methyl
Sulfometuron methyl
Triclopyr
Clopyralid

Insect and disease infestations annually claim an estimated 1.6 billion board feet of Oregon's board feet of timber (Oregon Department of Forestry, 1997).

However, insecticide use must be carefully monitored in forest practices because of potential impact on non-target insects. Therefore, they are used only in response to uncontrollable outbreaks of insects, such as the gypsy moth (*Lymantria dispar*), the spruce budworm (*Choristoneura fumiferana*), the western spruce budworm (*Choristoneura occidentalis*), and the tussock moth (*Orgyia pseudotsugata*) (Norris et al., 1991). Insecticides currently registered for use in Oregon are included in Table 1.2 as of April 2000.

Table 1.2: Generic names of insecticides currently registered for use in Oregon forestry (Oregon Department of Forestry, 2000).

3-methyl-cyclohexen-1-one
Acephate
Bacillus thuringiensis
Carbaryl
Chlorothalonil
Diflubenzuron
Nuclear polyhedrosis virus

The pesticides identified in this study represent the products most widely used today and of concern to the Department of Forestry, and does not include all pesticides that are used on forest lands that may present a hazard to aquatic communities. The pesticides identified by the Department of Forestry include the herbicides 2,4-D ester, glyphosate (w/o surfactant), atrazine, and triclopyr ester, the insecticide carbaryl, and the fungicide chlorothalonil. Fungicides are used when needed to control diseases in forest stands. Because these chemicals were considered for study, more information on their properties is provided below (Table 1.3). Due to resource constraints it was not possible to test all six of these chemicals. One herbicide, triclopyr, and one insecticide, carbaryl, were chosen for

this research. Carbaryl, while not in wide use today, was selected because of its known toxicological effects on insects (Kuhr & Dorough, 1976). Triclopyr has been shown to be one of the more toxic herbicides to aquatic organisms, namely the salmonid fish (Mayes et al., 1986; Servizi et al., 1987; Wan et al., 1987; Morgan et al., 1991). Its toxicity, in combination with its wide use in forestry, made it an excellent choice for this study.

Table 1.3: Chemical properties and toxicity data for pesticides widely used in Pacific Northwest forest practices.

Chemical Properties	Glyphosate	2,4-D Ester	Triclopyr Ester	Atrazine	Chlorothalonil	Carbaryl
Water Solubility	12,000 mg/L	890 mg/L	400 mg/L	33 mg/L	0.6 mg/L	120 mg/L
Organic Solubility	Insoluble	9.5 g / 100g Ethalnol	989,000 ppm Acetone	52,000 ppm Chloroform	20,000 ppm Acetone	79,000 Methanol
Log K_{ow} – Octanol Water	-1.6	0.27		2.68	2.88	2.31
K_{oc} – Soil Sorption	2100		1.5 - 160	100 g/ml	5000	205 – 457.1
Henry's Law Constant	1.04×10^{-7}	1.1×10^{-7}	4.3×10^{-8}	1.06×10^{-7}	0.22	4.5×10^{-4}
Vapor Pressure	1.94×10^{-7} mmHG @ 45°C	8×10^{-6}	1.26×10^{-6}	3.0×10^{-7} mmHg @ 20°C	1.3 Pa @ 40 °C	0.005 mmHg @ 26 °C
Melting Point (°C)	200	138	148 - 150	175 - 177	250 - 251	141 - 142
Boiling Point		156 – 162 @ 1.5 mmHg	290		350	N/A
Molecular Weight	169.1	221.04	256.48	215.68	265.92	201.22
Photolysis-Soil	Stable @ 22 C°		0.00034 @ 25 °C	0.015 @ 25 °C	Stable	

Table 1.3 (Continued)

Photolysis - Water	Stable @ 24.5 °C		2 @ 25 °C	0.002 @ 12-44 °C	0.011	0.0154 @ 25 °C
Hydrolysis	Stable @ pH 3,6,9		Stable	Stable @ 25 °C	Stable @ pH 5-9	0.066 @ 25 °C
Northwest Application Rate	1.0 – 5.0 pds / acre	0.5 – 3.0 pds / acre	0.3 – 2.0 pds / acre	0.5 – 5.0 pds / acre		
Toxicity (Acute)						
Rat LC_{50}	5,600 mg/kg	375-666 mg/kg	630 – 729 mg/kg	622 – 3,000 mg/kg	10,000 mg/kg	400-850 mg/kg
Rainbow Trout LC_{50} (96 h)	140 ppm; 8.3 ppm Roundup[a]	1ppm (fingerlings)	117 ppm	4.5 ppm	0.25 ppm	1.75-4.25 mg/kg @ 24 h
Daphnia magna	5.3 ppm Roundup[a] (48 h)	417 ppm (96 h)	1170 mg/L – Salt (48 h)		70 ppb (48 h)	
Honeybee LC_{50}	100 µg/bee	11.5 µg/bee		Nontoxic	181 µg/bee	

California Department of Transportation, 1991; EXTOXNET, 1994; USDA, 1984, 1985; Weed Science Society of America, 1989
[a]formulated product

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2,4-D ester

2,4-D ester is a phenoxy herbicide used in the treatment of herbaceous and woody broadleaf plants. It is absorbed mainly through the leaves of the plant, where it mimics many of the plant hormones. The ester form of 2,4-D tends to resist washing from the leaf surface but is rapidly converted to the acid form by the plant (Aherns, 1994). A number of plant processes are affected, resulting in abnormal growth, uneven cellular elongation, and decreased respiration (Willoughby and Dewar, 1995). In soil, the disappearance of 2,4-D is attributed to soil microbes.

2,4-D has a low solubility in water (ester formulations). Toxicity to aquatic organisms varies with formulation type, but ester formulations are more toxic to invertebrates and fish than acid formulations (Aherns, 1994). Hydrolysis in water from the ester to the acid form occurs in 4.5 to 44.7 days depending on water temperature, which can reduce its potential toxicity (Norris, 1981). Half lives range from 10-50 days, depending on environmental conditions, with microbial breakdown being the primary degradation process. Nutrient poor water may slow its removal, while more basic conditions can accelerate it. It does not bioaccumulate, but is more toxic under conditions of lower pH (OSU Extension, 1996). It can create severe taste and odor problems in drinking water (Willoughby & Dewar, 1995).

Atrazine

Atrazine is used widely as a selective herbicide for grass and pre-emergent weed control. It is absorbed by both roots and foliage and is translocated to the meristem and leaves where it accumulates and inhibits photosynthesis.

Atrazine has a 13-day half-life on foliage, and a 66 day half life on leaf litter. It can be washed off foliage with rain. Atrazine is highly mobile in soil and water, therefore presenting a threat to groundwater as well as to surface water by run-off events. It is readily adsorbed in clay soils with organic matter (Aherns, 1994) but does not adsorb strongly to soil particles (Koc 100g/ml). It is toxic to insects at concentrations of 50 parts per billion and aquatic plants at 100 parts per billion (OSU Extension, 1996).

Glyphosate

Glyphosate is a broad spectrum herbicide, and its nonselective nature will likely damage all vegetation it comes in contact with to some degree. It is taken up by the foliage and conveyed to the roots, where it inhibits amino acid synthesis, resulting in chlorosis, and death of the leaves, roots, and shoots (Willoughby & Dewar, 1995; Caltran, 1991). It is used to control a wide range of different weeds, brush, and deep-rooted species. The major formulation used in forestry practices is Roundup (41% of the isopropylamine salt of glyphosate with surfactants), and it is usually applied in the Northwest at 1.0 to 5.0 pounds of active ingredient per acre (OSU Extension, 1996).

Glyphosate is relatively immobile in soil as a result of its strong adsorption to soil particles, especially those with high organic content. This property reduces its chance of being transported to groundwater and streams by leaching from sprayed areas. Degradation occurs primarily by microorganisms in about 14 to 21 days depending on soil properties (Caltran, 1991; OSU Extension, 1996). In the aquatic environment, glyphosate is likely to adsorb onto the sediment because of its tendency to sorb to soil particles (Feng, et al., 1990; Newton et al., 1984). It has been found to accumulate in the bottom sediments and remain persistent, with the highest levels being detected following storm events (Feng et al., 1989, 1990).

Accumulation was more prevalent in streams with low flow rates that provide more depositional areas for sediment bound particles than high flow streams that flush accumulated sediment during storm events (Feng, et al., 1990).

Chlorothalonil

Chlorothalonil is a broad spectrum organochlorine fungicide used in forestry applications for disease control as well as other vegetable and fruit crops. It tends to persist on foliage, and does not readily translocate into plants from surface or soil. It has a higher degree of binding in silty loam and clay soils, and has a half-life of 1 to 3 months depending on moisture and temperature.

It has a low solubility in water and may tend to accumulate on the air/water interface of aquatic systems or associate with suspended material in the water column (Davies, 1988). Therefore, organisms inhabiting the surface of the water may be at a greater risk. Chlorothalonil and its metabolites are highly toxic to fish and aquatic invertebrates. Fish have been found to be noticeably affected even when chlorothalonil concentrations are low (less than 1 ppm) (EXONET, 1997).

Triclopyr

Triclopyr ester is a plant growth regulating herbicide that is rapidly absorbed by the foliage, roots, and stems. It is a selective herbicide used to control most herbaceous and woody plants, providing superior control of root sprouting species (Aherns, 1994). The formulation most used in forestry practices is GARLON 4, containing 0.14 kg triclopyr per liter as a butoxyethyl ester.

Triclopyr rapidly adsorbs onto soil particles. The degree of adsorption depends on soil type, with organic matter being the primary parameter involved

(USDA, 1984). Its half life in dry tree leaves is 2 to 3 months, and in soil is 30 to 46 days depending on climatic conditions (OSU Extension, 1996). It is only slightly water soluble, and in aquatic environments may be expected to adsorb to the sediments or organic matter reserves such as leaf packs (Thompson, 1995). Triclopyr concentrations have been found to be rapidly dissipated in the water column of forest streams (Thompson et al., 1991). Strong adsorption to sediment and other organic matter such as leaf litter has been determined (Thompson et al., 1991), suggesting organisms inhabiting or feeding leaf litter or other organic deposits may be at a greater risk (Thompson et al, 1995). The ester form of triclopyr is more toxic to aquatic organisms than the acid form, which has been attributed to the more lipophilic nature of the ester (Mayes, 1986).

Carbaryl

Carbaryl is a wide spectrum carbamate insecticide that has been used for more than 30 years to control insect infestations on forest stands and other crops such as cotton, ornamentals, lawns, fruits, and nuts. In forestry it is used to control defoliating insects, with application rates under 1.12 kg/hectare (U.S. Forest Service, 1977). Once applied, carbaryl is bound to organic matter and can be transported by soil runoff. Carbaryl is rapidly metabolized and degraded in the environment, with a soil half-life of 7-28 days depending on conditions.

This chemical is classified as moderately to very toxic to mammals with an oral LD_{50} of 250 mg/kg to 850 mg/kg for rats (National Library of Medicine, 1992), and has been found to be toxic to many non-target invertebrates such as bees, fish and invertebrates. It has bioaccumulation factor of 28.2 – 28.8 (EXONET, 1994), and has been shown to bioaccumulate in fish, crayfish, snails, and algae (National Library of Medicine, 1992).

Significant increased drift and mortality has been found to occur in streams exposed to carbaryl, with aquatic insects from the orders of Ephemeroptera, Diptera, Plecoptera and Diptera being especially susceptible (Courtemanch & Gibbs, 1980; Burdick et al. 1960). Its effect on stream invertebrate communities can be severe, and have been show to exceed 1 year (Courtemanch & Gibbs, 1980). Courtemanch (1980) found stonefly (Plecoptera) populations especially slow at repopulating treated streams, exhibiting low numbers 2 years after the initial disturbance.

BENTHIC MACROINVERTEBRATES

Stream macroinvertebrates were selected as study organisms in the evaluation of stream ecological condition because of their important link between organic matter such as leaf litter and detritus, and organisms higher in the food chain such as fish (Hauer & Resh, 1996). Macroinvertebrate families including the Hydropsychidae and Limnephilidae (Trichoptera), Perlidae (Plecoptera), Tipulidae (Diptera) convert allochthonous detritus, or coarse particulate organic matter (CPOM), to fine particulate organic matter (FPOM) and dissolved particulate organic matter (DOM) (Fisher & Likens, 1973; Wallace et al., 1982; Cuffney et al., 1984; Cummins et al., 1989). These fine materials are exported and become an important source of energy to filter and deposit feeders further downstream, which include families such as the Baetidae and Heptageniidae (Ephemeroptera), Hydropyschidae and Glossosomatidae (Trichoptera), and Simuliidae and Chironominae (Diptera) (Merritt & Cummins, 1996; Wallace & Merritt, 1980; Anderson & Sedell, 1979;). This forms the basis for theoretical concepts in stream ecology, such as the River Continuum Concept (Vannote et al., 1980) and nutrient spiraling (Newbold, 1982).

In addition, macroinvertebrates are important sources of food for fish populations. The adult and nymphal stages of aquatic macroinvertebrates comprise the diet of many fish species (Healey, 1984). In fact, when the diets of 42 families of freshwater fish in the world were examined, macroinvertebrates were the main food of 29 families, and among the food of all 42 (Sterba, 1962; Scott & Crossman,1973). For these reasons, it can be said that the health of the macroinvertebrate community reflects the health of the entire stream ecosystem (Reice & Wohlenberg, 1993).

RISK ASSESSMENT

Chemical release in the environment can result in direct or indirect effects on individuals, communities and populations within the ecosystem. The inability to accurately predict effects resulting from environmental disturbances necessitates regulatory decisions to be made based on incomplete scientific information, highlighting the need for a risk assessment process (Ruckelshaus, 1983; Moghissi, 1984). Risk assessment allows data on environmental effects to be organized and analyzed in order to rigorously evaluate the likelihood of adverse effects (U.S. EPA, 1992). This includes the development of methodologies and tools to estimate the magnitude of expected effects, as well as an evaluation of uncertainty and variation in the generated estimates (U.S. EPA OPP, 1997).

Probabilistic risk assessment is based on the premise that certainty is impossible, and instead an accurate probability of risk is deemed a sufficient basis for decision making. This is in contrast to deterministic risk assessment, where the environmental parameters are assumed to be constant and accurately specified. In the past, the EPA Office of Pesticide Programs (USEPA OPP) has relied on deterministic methods in order to assess the effects of pesticides on non-target organisms. However, current protocol calls for the development and validation of probabilistic methodology to assess chemicals of concern (U.S. EPA OPP, 1997; U.S. EPA, 1998). Therefore, this research will uphold the current position of the EPA by utilizing probabilistic methodology to describe the pesticide exposure patterns of aquatic macroinvertebrates.

The risk components of an assessment can be divided into three parts: problem formulation, analysis, and risk characterization (U.S. EPA, 1998). In problem formulation, the purpose of the assessment and the problem is defined, and relevant endpoints are selected. An assessment endpoint is defined as "a quantitative or quantifiable expression of the environmental value considered to be

at risk in a risk assessment" (Suter, 1993). Problem formulation for this project included a meeting with the Department of Forestry, which identified a need to perform a risk assessment to evaluate chemical effects on stream systems. Chemicals of concern and assessment endpoints were identified. The result was a project evaluating the effects of forest chemicals on macroinvertebrate susceptibility as a measure of health in Oregon streams.

One or more measurement endpoints were used to make inferences about the assessment endpoint. A measurement endpoint is "a quantitative summary of the results of a toxicity test, a biological monitoring study, or other activity intended to reveal the effects of a substance" (Suter, 1993). Criteria have been outlined by the EPA for endpoint selection, and include ecological relevance, susceptibility to known or potential stressors, and relevance to management goals (U.S. EPA, 1998). One of the endpoints for this project was susceptibility distributions, determined through toxicity tests, of ecologically relevant macroinvertebrates.

The analysis phase follows problem formulation. This phase evaluates how exposure to a chemical is likely to occur, and given this exposure, what kind of effects are expected. The third step, risk characterization, assimilates information on exposure and stressor-response profiles to describe risk. This allows for the evaluation of the relationship between exposure and effects in order to reach management conclusions on the predicted risk.

EXPOSURE CHARACTERIZATION

Exposure is a function of chemical use and distribution, the properties of the chemical, characteristics of the stream environment, and biological characteristics of the organisms. Risk is estimated based on an evaluation of the exposure / effects relationship. The goal of the analysis phase is to provide the data necessary to

predict ecological responses under relevant exposure conditions. To achieve this goal, the amount of contact and dose an organism may receive from chemical exposure is estimated. This includes estimating the transport, fate, and uptake of the chemical by the organism. Key factors to determine pesticide fate and environmental concentration include sorption, dissipation, hydrology and management practices (ECOFRAM, 1999). Exposure assessment is designed to refine the understanding of exposures so that the exposure magnitude and duration can be predicted accurately and realistically. Temporal variations in exposure may include duration, frequency above a certain magnitude, intervals between chemical pulses, and seasonal differences in exposure (ECOFRAM, 1999). In addition to the chemical properties of the pollutant, a knowledge of the natural history, behavior and physiology of the organisms in the community is required. The various components of exposure assessment, from both the chemical and biological perspective, will be discussed in the following sections.

Stream Habitat and Physical Characteristics

Sorption is the degree of interaction between the pesticide and the soil, and is primarily determined by the adsorption properties of the pesticide and the composition of the soil. This primarily involves the organic matter and clay components. The dissipation rate is a function of its overall transformation rate due to microbial degradation, hydrolysis and photolysis as well as loss mechanisms such as volatilization, runoff, erosion and leaching. The hydrology of a stream system is a function of interactions with the landscape, climate, and bio-geography. Regular seasonal changes in discharge volumes and water quality can have profound effects on ecological processes and pesticide persistence. The influence of management practices such as rate of application, method, timing of application, and vegetative filter/buffer strip characteristics also must be considered (Table 1.4).

Table 1.4: Ecological and physical parameters affecting macroinvertebrate exposure and recovery (after Jepson, 1988).

Operational	Habitat	Biological
CHEMICAL EXPOSURE		
During Spraying	During Spraying	During Spraying
• Application method • Type of nozzle used • Droplet size and spectrum • Formulation • Local meterological conditions (e.g. wind speed and direction, humidity, temperature) • Seasonal timing of spray	• Composition of buffer zone (amount of overstory and species make-up) • Width of buffer zone	• Organism distribution in the environment (leaf litter, sediment, water column, hyporheic zone)
Following Application	Following Application	Following Application
• Physicochemcial properties (e.g. water solubility, Kow, Kom) • Breakdown rates (soil, water, organic matter)	• Stream bank gradient • Degree of stream bank groundcover • Soil properties • Stream substrate composition (% boulder, cobble, fine sediment) • Stream Gradient • Stream Discharge / velocity • Proportion of pools and runs (chemical holdup)	• Organism distribution in the environment

Table 1.4 (Continued)

SUSCEPTIBILITY

- Formulation (degree of particulates)
- Intrinsic toxicity of chemcial (mode of action)

- Degree of refuge habitat available

- Life history characteristics
- Life stage present
- Body size
- Functional feeding strategy
- Respiratory strategy – appendage / morphology
- Behavior – drift, rheotaxis, foraging time

RECOVERY / RECOLONIZATION

- Chemical properties (breakdown rates)

- Frequency of ideal habitat (substrate particle size) for recolonization
- Proximity to nearby streams – sources for recolonizing organsims

- Number of generations per year
- Number of offspring per generation
- Degree of adult dispersal during reproduction
- Drift behavior

The constituents of the habitat exposed to chemical spray, such as the riparian zone, stream bank and physical characteristics, can influence the degree and length of exposure. For example, streams with steep banks on either side may be more susceptible to additional influxes of chemical through runoff processes, especially if ground cover is limited. Stream physical characteristics such as velocity and discharge, and the proportion of areas with fast (riffles) versus slower water (pools) will help determine how fast a chemical may move downstream as well as help identify areas of possible accumulation. Substrate composition is also important in determining possible adsorption sites. A stream composition of mostly large cobble and boulders may have less available surface area for chemical adsorption than streams containing a higher degree of fine sediment.

First and second order streams make up approximately 73% of the total stream length in the United States (Leopold et al., 1964). Of these streams, those in forested regions receive large inputs of autumn shed leaves (e.g. Fisher & Likens, 1973; Cummins, 1973; Webster & Patten, 1979). Leaf fall in the Pacific Northwest, depending on elevation, starts in the early fall, corresponding with the chemical application season for herbicides. Leaves can accumulate chemical residues while still intact, and some chemicals have been found to have rather long half-lives on leaves (Derr, 1974). These contaminated leaves have the potential to accumulate in stream systems through leaf fall. Stream leaf litter already in the stream system can also become contaminated when chemicals present in the water column partition into organic matter (Kreutzweiser et al., 1994; Thompson et al., 1991, 1995). Both of these mechanisms can lead to an important route of exposure for macroinvertebrates, especially shredder organisms.

Sediment may also be an important route of exposure for macroinvertebrates. Some chemicals have the potential to partition and accumulate in the sediments, as discussed earlier. For example, glyphosate strongly binds to soil and sediment particles and can be quite persistent (Feng, et al., 1989, 1990; Newton et al., 1984). Many macroinvetertebrates live in and feed on the sediment

in the rich depositional regions of streams. These chemicals may be bioavailable and represent an important exposure pathway for chemical toxicity.

Biological Characteristics

Risk to macroinvertebrate communities varies as a function of chemical bioavailability. Although one key determinant of the degree of exposure is how the chemical behaves in the environment, it is necessary to identify characteristics that put certain taxa at an increased risk to chemical exposure (Table 1.4). These may include: season present —different life histories may exclude certain taxa from the stream environment during the seasonal chemical application periods; the life stage that is present during the application season— different instars may be more sensitive chemical impacts; organism distribution in the environment —certain habitats, such as leaf litter pack or sediment pools, are more likely to be exposed (depending the chemical's physical properties); behavior, such as positive or negative rheotaxis, may increase the rate of exposure, while organisms that are strictly night foragers may decrease their risk; morphology —some smaller body forms and those that have a reduction in integument, increases in spiracle openings, or have extensive respiratory and feeding appendages may be more susceptible to chemical uptake; food source —chemical accumulation in the organisms dietary medium (organic matter, soil, etc.) identifies strong uptake pathways.

Life History

The timing of life cycles can be used to predict which organisms will be present during different times of the year. Life history characteristics govern the reproduction and survival of macroinvertebrates and can vary widely between

different species (Wallace & Anderson, 1996). Life cycle length varies considerably, and may range from one life cycle per year (univoltine) which includes most mayflies, caddisflies, and some stoneflies in the area, to organisms like some of the flies (Diptera) that can complete two life cycles in one year (bivoltine). Some organisms may require 2 to 3 years to complete a life cycle (Hauer & Resh, 1996), such as the stonefly *Calineuria californica*. In addition, adult emergence, that can occur at different times of the year depending on the taxa, may correspond with chemical applications that usually occur in the spring (March/April) and again in the fall (Sept./Oct). Identifying the community structure during the spring and fall spray seasons can help identify organisms that may be impacted.

Behavior

Behavior can influence rate of encounter and exposure to pesticides. For example, organisms that exhibit high degrees of positive rheotaxis, or a tendency to move up stream within the substrate, may increase their rate of encounter of the pesticide. This behavior has been found in most major invertebrate taxa, and has been thought to help organisms search for new resources, avoid unfavorable conditions, and increase dispersal (Allan, 1995). Upstream migrations by the mayfly *Leptophlebia cupida* were reported by Neave (1930) to cover 1.6 km, at a rate of about 200 m per day. Similar behaviors were observed for the mayfly *Baetis rhodani* (Elliott, 1971), the clam *Campeloma decisum* (Brown, 1991) and the amphipod *Gammarus bousfieldi* (Mickley, 1964).

Other behaviors may decrease rates of chemical encounter, such as night foraging and negative phototaxis. Negative phototaxis occurs in many species (Williams, 1981), and because chemical applications occur during daylight hours, this behavior may decrease exposure to initial chemical pulses in the water column.

Morphology

The morphological adaptations of some macroinvertebrates to obtain oxygen from the aquatic environment may increase chemical exposure and uptake. Taxa that rely on obtaining dissolved oxygen from the water may be at more of a risk than those that utilize temporary stores of atmospheric oxygen. For example, smaller taxa such as some species of Diptera utilize a small body size to provide a sufficient uptake of dissolved oxygen. As body size increases and surface area decreases, additional respiratory surfaces are required in order to obtain sufficient oxygen. As a result, some organisms have developed relatively large respiratory appendages such as large, thin, tracheal gills and increases in spiracle openings (Merritt & Cummins, 1996). The respiratory system of aquatic insects may be particularly sensitive to chemicals because these surfaces serve as both as oxygen exchange sites as well as sites for the active uptake of ions (Komnick, 1977)

Specialized feeding appendages, such as those found in filter feeding organisms including Ephemeroptera, Trichoptera, and Diptera, may present the same increased risk. Specialized anatomical structures act in much the same way to increase an organisms contact with the surrounding medium. These may include long leg setae in some mayfly and caddisfly genera, mouth brushes such as those found in mosquito larvae, head fans found in *Simulium,* or silk nets used by some caddisfly and chironomid larvae (Merritt & Cummins, 1996). Particulate pesticides especially, can become trapped in fans and brushes and ingested. For example, the control of the blackfly *Simulium damnosum* is achieved through particulate pesticides in the Onchocerciasis Control Programme in West Africa. These particulate larvicides accumulate in the fans of *Simulium* and effectively target this organism (Laird, 1981).

Food Source

How and what an organism feeds on plays a major role in determining exposure and possible chemical uptake. Macroinvertebrate exposure in the stream ecosystem may involve contact with contaminated leaf litter (shredders), sediment, or the water column (filter feeders). If the chemical accumulates in the dietary medium, some organisms may be at a greater risk for exposure and ingestion. For example, leaves may represent an important pathway for toxicity in those organisms that ingest them (shredding macroinvertebrates), or those that are in constant contact with the leaf surfaces.

CHARACTERIZATION OF PESTICIDE EFFECTS

In a perfect world, the assessment of the effects of a chemical to an environmental community would involve a field-based study to evaluate the long-term effects of pesticide treatments. Field collected data would provide highly relevant information that would most accurately predict effects at the individual, population, and community levels. However, there are some disadvantages to using field-based tests as a means of evaluating chemical effects. First, the variation inherent in field tests makes the determination of chemical effects from background noise extremely difficult. Second, field-based test results encompass a mixture of direct and indirect effects, many of which cannot be directly attributed to the chemical without further analysis or experimentation. Third, replication of these studies is very difficult because of the high variability of natural ecosystems. Replication is important in order to compare the effects of different chemicals, and in order to determine the range of variation in receiving systems. Fourth, the manpower and cost involved in the set-up of the field experiment, and the

taxonomy involved in identifying the organisms, makes them difficult to use. Fifth, field based test results are very site specific, and cannot be used to extrapolate to a whole region.

For these reasons, laboratory based testing is often used to evaluate effects on non-target organisms. Limitations of laboratory tests have been criticized for their lack of real world realism and ability to extrapolate results to field situations (Cairns, 1984; Kimball & Levin, 1985). However, laboratory data are not site specific and the sensitivity data can be assessed and used in many different ways. Advantages to using laboratory methodology are: a) the toxic effect can be measured directly in the laboratory using conditioned water, b) a range of organisms can be examined in a repeatable manner, c) the cost involved in running the tests is low, d) the level of effect is allowed to vary by organism, and e) the collection and testing of representative organisms allows extrapolation to the natural environment.

Available Test Methods

A wide variety of methods are available to evaluate chemical effects on non-target organisms. These range from single species acute and chronic tests, to multi-species tests involving microcosm and mesocosms, to the manipulation of whole natural systems. Each test has different key features, endpoints, and advantages and limitations, and varies on how well it can predict effects in the natural environment, the complexity of statistical analysis needed to provide useful endpoints, and the cost associated with their use (Table 1.5).

Standardized methods available in the testing of chemical effects include: a) microbial tests, b) tests which evaluate effects on primary production, such as algae, c) single species tests which use invertebrates and fish, d) sediment tests which evaluate the biological effects of chemicals on organisms which are in

contact with contaminated sediment, and multi-species tests which consider more than one trophic level. Standardized methods are published by the Environment Protection Agency (EPA) (U.S. EPA, 1982), Organization for Economic Cooperation and Development (OECD 1981; 1984), American Society for Testing and Material (ASTM, 1993), American Public health Association (APHA, 1989), and various other government agencies.

Table 1.5: Summary of tests conducted to assess chemical effects to aquatic systems

Test Used[a]	Key Features and Endpoints	Advantages	Limitations
Microbial Tests	Tests are conducted under constant conditions including stable growth rates, constant level of limiting and non-limiting nutrients, constant biomass, and constant environmental conditions of temp, pH, etc. Endpoints include changes in growth rates, biomass, number of cells, and biochemical properties.[2]	Microorganisms occupy important roles in ecosystem, are easy to culture in large numbers, and may be early indicators of effects.[1] Tests are rapid, simple, inexpensive, easily replicated, and simple to analyze.[2]	Extrapolation to organisms at higher trophic levels may be difficult
Primary Producers	Algal Toxicity Tests: Effects assessed on a rapidly growing population in a nutrient-enriched medium for 3-4 days under constant light conditions. Endpoints are effects on biomass and growth – can be inhibition or stimulation.[3] Vascular Plants: Exposed to toxicants in water column and by contaminated sediments. Endpoints include changes in growth and photosynthesis	Algae have a rapid reproductive rate. Algal tests are simple, reliable, inexpensive, and sensitive Vascular plants concentrate toxicants and nutrients in their tissues[3] – good for detecting contaminated sediments	Differences in physiology and morphology make responses unpredictable. Predictability is compound specific, making extrapolations difficult Vascular plants reproduce slowly, are more difficult to handle, and validated test methods are lacking.[3]

Table 1.5 (Continued)

Single Species Invertebrate Toxicity Tests	Tests carried out with a standardized organism, water conditions are static or flow-through, duration is 48 to 96 hours for acute tests, and 7 to 21 days for chronic, water conditions and light cycle are controlled.[4] Endpoints include mortality in acute tests, and changes in reproduction or behavior in chronic tests.	Tests are highly reproducible between laboratories. Organisms used are broadly distributed, occupy important links in aquatic food chains, and are small in size with short life cycles making culture and testing easy. Data from these single species tests may be best used for the screening chemicals both in ranking chemical toxicity and organism sensitivity. [4]	Organisms used may not be representative of the wide range of aquatic communities. Tests don't consider species interactions
Fish Tests	Field and laboratory test methodology available. Laboratory fish exposed in static, semi-static, and flow-through tests. Tests include acute lethal toxicity, subchronic lethal toxicity, sublethal toxicity, bioccumulation/bioconcentration, and early life stage tests. Endpoints include mortality, changes in growth rate, and effects on the metabolic processes of early life stages.	Diversity of physiology, feeding habits, and reproductive strategies, importance in natural systems, and economic value. Good sentinel organisms, for evaluating longer term changes in aquatic systems, and in evaluating bioaccumulation.[5]	Complex testing systems Laboratory tests don't consider species interactions Longer reproduction times compared to other organisms May not be best early indicator of early ecosystem level effects.[6]

Table 1.5	(Continued)		
Sediment Tests	Field and laboratory sediment bioassays/toxicity tests protocol available In laboratory tests animals exposed to spiked sediments for a variable amount of time (depending on metholodogy) and constant conditions. Test organisms include bacteria, protozoa, phytoplankton, zooplankton, benthic invertebrates, and fish. Field tests may utilize organisms in different trophic levels Endpoints include survival, growth, and reproduction	Standardized methodology available for lab tests, ability to rank sediments, ability to compare species from different trophic levels, and allows for direct evidence of sediments as being the causative agent of toxicity. Good for evaluating effects of highly persistent, hydrophobic chemicals that accumulate in sediments.[7]	Lack realism can lead to an inability to predict effects in the field Results may apply only to organism tested Not applicable for chemicals that don't partition into sediments.[7]
Multi-Species Tests	Microcosms Mesocosms Enclosures of natural systems / whole ecosystem manipulations Endpoints may include the evaluation of predator-prey interactions, behavior, competition, and chemical fate	More realism to natural communities, may be more sensitive than other tests because more endpoints are evaluated, and community responses can be analyzed.[8] May provide valuable information in site specific impacts	More components, higher complexity, higher cost, hard to replicate and compare results over time, hard to differentiate effects from background, and difficult to define a specific level of stress that will initiate management actions.[8]

[a]Categories from Handbook of Ecotoxicology, 1993; [1]Kelly & Harwell, 1989; [2]Mayfield, 1993; [3]Lewis, 1993; [4]Persoone & Janssen, 1993; [5]De L.G. Solbe, 1993; [6]Schindler, 1987; [7]Reynoldson & Day, 1993; [8]Cairns & Cherry, 1993.

Continuous Exposure Testing

Single species standardized tests, utilizing a range of standardized organisms, have been developed and are agreed on internationally for use in the screening of possible toxicants (Calow, 1993). These tests are often used to rank chemicals based on their toxicity or determining the range of organism sensitivity. Acute tests measure mortality of the test organisms over a range of concentrations, such that lethal concentrations to 50% of the test organisms (LC_{50} values) can be determined statistically. More sensitive endpoints, such as reproduction, behavior, and growth and are usually used to calculate no observed effect concentrations (NOEC).

Standardized continuous tests usually consist of the following components (Suter, 1993): a) different exposure concentrations and durations of the chemical are evaluated with the test organism for effects such as mortality or other significant endpoints, b) statistical models are used to evaluate the test data to calculate dose-response relationships and subsequent relevant test endpoints, and c) effects models can be generated from test endpoints which relate the effects found in the laboratory to population, community or ecosystem level processes.

Time Varying Exposure Testing

Pesticide contamination of surface waters usually occurs in a single or repeated pulse due to spray drift, run-off, or intermittent applications. These input patterns result in a period of high concentration, which gradually decreases due to hydrological dilution, degradation, or partitioning from water to air or sediments (Bath et al, 1970). As a result, pesticide concentration in smaller streams has been found to be of a shorter duration, but reach higher maximum concentrations than larger streams (Richards & Baker, 1993).

Toxicity is a function of both the concentration and duration of chemical exposure. Although both of these factors are important in determining effects, traditional toxicity testing has been focused on evaluating the effect of varying concentration, and not exposure duration. Continuous laboratory testing, as described above, most often uses constant chemical concentrations for a preset exposure duration (i.e. 24, 48, 96 hours) (ASTM, 1993).

Many studies suggest that organisms may reach a critical threshold after shorter exposure durations, which result in adverse affects (Abel, 1980; Pascoe & Shazili, 1986). Researchers have observed adverse effects after brief exposures to CPF, endrin, and fenvalerate (Jarvinen et al, 1988) lindane and copper (Abel, 1980), bromoxynil (Buhl et al., 1993), carbaryl (Parsons & Surgeoner, 1991), and cadmium, zinc, and phenol (Brent & Herricks, 1998). Additional studies have shown additional effects can occur after the exposure period at concentrations assumed to be safe in standardized tests, and highlights the importance of including post-exposure observation periods in order to accurately assess toxicity (Wright, 1976; Hansen & Kawatski, 1976).

The results of time varying tests, and those that incorporate a post-exposure component, show that standard toxicity testing may not accurately assess effects that may occur in the field. For example, studies have shown that endpoints (i.e. lethal estimates) generated from continuous exposures may be orders of magnitude more protective than those generated from pulsed exposures (Hosmer et al., 1998). Standardized tests can be customized to evaluate these differences by adding additional exposure concentrations, time periods, or endpoints. Consideration of the temporal pattern of mortality as it relates to expected environmental concentrations provides the risk manager with a more complete picture of the risks associated with the actual use of the pesticide, and increases the confidence of extrapolating the results of laboratory tests to field conditions.

Test Organisms

Invertebrates occupy import links between organisms at lower trophic levels and those at higher levels, such as fish, which make them good indicators of the health of both. Their small size, high reproductive rates, and ease of culture make them ideal for testing. For these reasons, invertebrates are most often used in standardized tests. This has led to a distinct group of taxa being used to represent aquatic invertebrate chemical sensitivity in most ecosystems. A list of these organisms is presented in Table 1.6 (after Persoone & Janssen, 1993), along with the recommending agency. Their use has been encouraged by regulatory agencies in order to help standardize aquatic tests and allow better reproducibility between laboratories. The most widely used for toxicity testing is undisputedly the daphnids. Advantages to their use include their broad distribution in freshwater habitats, their relatively short life cycles, the ease with which they can be cultured in laboratory settings, and their sensitivity to a range of aquatic contaminants (Persoone & Janssen, 1993).

Table 1.6: Freshwater invertebrates commonly used in toxicity testing

Organism Class:	Species:	Recommended By:
Ciliates	*Tetrahymena pyriformis*	APHA
Platyhelminthes	*Dugesia tigrina*	ASTM
Annelida	*Limnodrilus hoffmeisteri*	APHA, FAO
	Tubifex tubifex	APHA, FAO
	Branchiura sowerbyi	APHA, FAO
	Stylodrilus heringianus	APHA
Gastropoda	*Physa integra*	ASTM
	Physa heterostropha	ASTM
	Amnicola limosa	ASTM
Branchiopoda	*Daphnia magna*	APHA, ASTM, FAO,
	Daphnia pulex	USEPA, OECD
	Daphnia pulicaria	ASTM, US EPA, OECD
	Daphnia spp.	ASTM

Table 1.6 (Cont.)

	Cerodaphnia spp.	AECD, US EPA
Amphipoda	*Gammarus lacustris*	APHA, ASTM, FAO, US EPA
	Gammarus pseudolimnaeus	
	Gammarus fasciatus	APHA, ASTM, US EPA
	Hyalella azteca	APHA, ASTM FAO, US EPA
	Pontoporeia affinis	
	Hyalella spp.	APHA, FAO
Mysid	*Mysis relicta*	APHA, FAO
Decapod	*Palaemonetes cummingi*	APHA
	Palaeminetes kadadiensis	APHA
	Gambarus spp.	APHA, US EPA, FAO, ASTM
	Orconectes rusticus	
	Orconectes spp.	APHA
	Procambarus spp.	US EPA, ASTM
	Pacifastacus lenisculus	ASTM
Plecoptera	*Pteronarcys dorsata*	APHA
	Pteronarcys californica	APHA
	Pteronarcys spp.	ASTM, FAO
	Hesperoperla lycorias	APHA
	Hesperoperla pacifica	APHA
	Isogenus frontalis	APHA
	Isogenus spp.	FAO
	Perlesta placida	APHA
	Paragnetina media	APHA
	Paragnetina spp.	FAO
	Phasganophora capitata	APHA
	Phasganophora spp.	FAO
	Acroneuria californica	APHA
	Acroneuria spp.	FAO
Ephemeroptera	*Hexagenia bilineata*	APHA, ASTM, US EPA
	Hexagenia limbata	APHA, ASTM, US EPA
	Hexagenia regida	APHA
	Hexagenia spp.	FAO
	Ephemerella subvaria	APHA
	Ephemerella cornuta	APHA
	Ephemerella grandis	APHA
	Ephemerella doddsi	APHA
	Ephemerella needhanii	APHA
	Ephemerella tuberculata	APHA
	Ephemerella spp.	ASTM, FAO, US EPA
	Stenonema ithaca	APHA

	Stenonema spp.	FAO
	Baetis spp.	ASTM, US EPA
Trichoptera	*Brachycentrus americanus*	APHA
	Brachycentrus occidentalis	APHA
	Brachycentrus spp.	FAO
	Clistoronia magnifica	APHA
	Hydropsyche bettini	APHA
	Hydropyche bifida	APHA
	Hydropsyche spp.	FAO
	Macronemum zebratum	APHA
	Macronemum spp.	FAO
Diptera	*Chironomus plumosus*	APHA
	Chironomus attenuatus	APHA
	Chironomus tentats	APHA
	Chironomus californicus	APHA
	Chironomus spp.	ASTM, FAO, US EPA
	Glyptochironomus labiferus	APHA
	Goeldichironomus	APHA
	holoprasinus	APHA
	Tanypus grodhausi	FAO
	Tanypus spp.	APHA
	Tanytarsus dissimilis	FAO
	Tanytarsus spp.	

Table 1.6 (Cont.)

APHA – American Public Health Association; ASTM – American Society for Testing and Materials; FAO – Food and Agriculture Organization of the United Nations; OECD - Organization for Economic Cooperation and Development; US EPA – United States Environmental Protection Agency.

Species that are indigenous to the area where exposure may occur, or at least representative of the organisms that are likely to be exposed, can be selected for use in toxicity testing. However, this has not been the trend of organism selection for toxicity testing in the last three decades. Instead, only a few taxa have emerged to represent aquatic invertebrate chemical sensitivity in most ecosystems. However, using *Daphnia* or other standardized organisms alone also presents some disadvantages. Because *Daphnia* have not been selected for their functional role in the community, questions can be raised as to how representative they are to the

wide range of aquatic habitats and species assemblages present. In fact, one of the most widely used species, *Daphnia magna* is virtually nonexistent in the fauna of North America (Mount & Norberg, 1984). By not including native species, chemical effects may be under or over estimated.

By using a representative test group of native organisms, the accuracy of predictions that can be made from the laboratory to the field are increased. We assert that the test organisms should be more regionally customized to encompass the ecological uniqueness of the area. This includes selecting organisms based on their ecological function, functional feeding strategy, taxonomy, life history, and susceptibility.

EFFECTS ANALYSIS

LC_{50} Values Determined by Probit Analysis

One of the more classical techniques used to analyze toxicological data is the calculation of the lethal concentration of the test chemical to 50% of the organisms tested (LC_{50}). Other endpoints used include the median lethal dose (LD_{50}), the median effective concentration (EC_{50}), and the lethal threshold concentration (LC_{01}). In this analysis, mortality data from acute toxicity tests are fitted to measurements of dose or concentration in a dose response relationship. Values like the LC_{50} are usually derived from the regression relationship, so that the concentration eliciting the test endpoint can be calculated from the fitted model.

The analysis most often used to characterize dose response relationships is probit analysis (Finney, 1971). This analysis allows for the sensitivities of exposed organisms to chemicals to be characterized by a statistical distribution with a mean and a variance, which are independent of mechanisms involved in uptake,

translocation, and toxicity. The frequency distribution of organism sensitivity is assumed to be normal, with a graph of the percentage of responding individuals against dose resulting in a steadily rising curve. This curve is often sigmoidal, because the rate of increase in the response is low for concentrations near 0 and 100%, but higher for the values in between (Finney, 1971). This distribution allows for the calculation of LC_{50} (the lethal concentration to 50% of the organisms), or other lethality values in the sensitivity distribution. In order to produce accurate sensitivity data, it is important that the organisms selected for testing are homogeneous with regard to age, gender, exposure conditions, and exposure route and pathway. It is also required that the response measured is a quantal, or all-or nothing response, such as mortality.

Using indigenous organisms for toxicity testing, which is one of the goals of this project, can make sensitivity analysis difficult because obtaining field collected organisms of the same age and physiological condition can be a challenge. Large sample sizes of field collected organisms can compensate for the variation, but this option may not always be available when organisms are unavailable and hard to find. However, the homogeneous test conditions of single species laboratory testing allows for proper analysis even if there is some variation within organism response.

Chronic Endpoints and Calculation of the No Observed Effect Concentration (NOEC)

Chronic endpoints utilize other measurements of toxicant effects besides mortality, including reproduction, behavior, growth, and physiological and biochemical effects. These endpoints may be more sensitive indicators than mortality assessment, and lead to the evaluation of more sub-lethal effects. The goal of chronic tests usually includes the calculation of a no observed effect

concentration (NOEC). A NOEC is defined as the highest concentration of a chemical in a toxicity test that results in effects not statistically different from the controls (Suter, 1993). This type of endpoint is derived from hypothesis testing, because responses at different exposure concentrations are compared with the control responses to test the null hypothesis that they are the same. In addition to the NOEC, other endpoints used include the lowest observed effect concentration (LOEC).

Disadvantages (outlined by Stephan & Rogers, 1985) to this type of statistical analysis exist, which can lead to erroneous results. First, calculated NOEC values are heavily dependent on how the test was designed, including the number of replicates and the spacing of concentrations. Second, poor testing procedures can increase the variance in response and reduce the apparent toxicity of the chemical. Third, statistical significance may be achieved in the calculation of a threshold, but it does not correspond to a toxicological threshold or to any particular level of effect, nor does it allow for the derivation of exposure-response dynamics. Fourth, the type of statistical test used in hypothesis testing sets the significance for differences between concentrations, which can lead to type one and type two errors (rejection of the null hypothesis when it is true, and acceptance of the null hypothesis when it is false, respectively). Because of the misleading concepts associated with using NOEC, many have advocated alternatives such as the continued use of regression techniques to calculate LC_{50} and related values as described above (Hoekstra & Van Ewijk, 1993; Laskowski, 1995).

Advantage to the calculation of NOEC is that the analysis can be performed even when the test data are too poor to fit to a model. In addition, the NOEC calculation prevents the assessor from having to make decisions about what constitutes an appropriate effect level.

Despite the above limitations of NOEC values, the calculation of NOEC values for the aquatic insects and chemicals used in this research would involve a complicated test set-up. Chronic endpoints used in the calculation of the NOEC

usually involve the evaluation of the chemical effects on the fecundity of the adults. In the evaluation of stream organisms, it is the juveniles that are exposed to the chemical in the stream environment. Therefore, a test regime would have to have been developed that would allow for the exposure of juveniles to the test chemical, and then the evaluation of the effects on the future adult population. For this research, this type of study would have been extremely costly in both time and effort. In addition, the results from this kind of test may not produce the data needed to evaluate the transient toxic effects encountered by the juvenile in the stream environment. For these reasons, the tests conducted in this research will concentrate on determining the sensitivity of juvenile aquatic insects to pesticides using acute toxicity tests with distinct lethal endpoints.

The Use of Species Sensitivity Models to Establish Environmental Quality Criteria - the HC_5 Calculation

Lethal concentrations, as obtained from acute mortality data, can be used in sensitivity models in order to establish a hazardous concentration that will help establish appropriate environmental protection criteria. Distribution-based extrapolation methods allow for calculation of the hazardous concentration that theoretically protects significant proportions of the invertebrate community. This technique assumes that organisms' sensitivity follows a log-logistic distribution, or in other words, a symmetrical bell-shaped distribution on a logarithmic concentration axis (Kooijman, 1987). From this distribution, the profile of susceptibility for a complete community can be estimated from limited toxicological data sets. Protection limits can be calculated such that only 5% of the species (HC_5) will be exposed above their LC_{50} (Kooijman, 1987) or NOEC (Van Straalen & Denneman, 1989); in effect establishing a 95% protection level for the community. NOEC values have been recommended for use in the calculation of an

HC_5 because a chronic evaluation provides a more sensitive endpoint. However, endpoints that represent low effects from a mortality dose response relationship can also be used, such as the LC1 or LC10. The method was developed by Kooijman (1987), and elaborated by Van Straalen & Denneman (1989) to calculate a 95% protection level for species in soil ecosystems. Further modifications to the method have been made by Wagner and Lokke (1991), who proposed the use of the log-normal rather than the log-logistic distribution to fit single species data, and by Aldenberg and Slob (1992) who developed correction factors for sample size.

The successful use of this approach depends on certain assumptions. The first assumption is that the selection of test species be random in order to prevent the selection of a group of organisms occupying only one part of the distribution of sensitivities (Kooijman, 1987). If random selection cannot be made, which is usually the case, then selection should be based on ecological function (Van Straalen & Denneman, 1989; Wagner & Lokke, 1991). This selection process should include organisms that represent various biological and physiological aspects important to the ecosystem, as well as those representing different morphology and exposure route (Van Straalen & Denneman, 1989).

The second assumption is that a distribution model can accurately represent the sensitivities of the species in a community. The statistical model used is usually either based on the logistic distribution (Kooijman, 1987; Van Straalen & Denneman, 1989) or the normal distribution (Wagner & Lokke, 1991; Smith & Cairns, 1993), but other distributions have been tried, including triangular (Stephan et al., 1985) uniform, extreme value, and exponential distributions (Versteeg et al., 1999). It has been determined that the log-normal and log-logistic methods hardly differ in cases where the levels of confidence are the same (OECD, 1990).

The third assumption is that organism sensitivity tested in the laboratory approximates sensitivity in the field, and the fourth is that the 95% protection criterion protects an appropriate level of ecosystem function. These assumptions warrant more experimentation, but recent studies have found that laboratory-

generated single-species studies can be used to establish concentrations predictive of ecosystem level effects, and that the use of the 95% protection level is conservative when compared to model ecosystem data (Okkerman et al., 1993; Versteeg et al., 1999).

The use of the HC_5 as an analysis tool in risk assessment has fallen under considerable debate (Forbes & Forbes, 1993; Hopkin, 1993; Van Straalen, 1993). Much of the debate centers around some of the assumptions described earlier, such as the distribution used to represent toxicity data, the selection of organisms for testing, and relying on 95% protection to adequately protect ecological function. The goal of distribution based methods is to define criteria to protect ecosystem function, but many feel this task is too complex to understand from single-species laboratory data which does evaluate all aspects of the ecosystem, including abiotic components (Forbes & Forbes, 1993). However, others point out the advantages of using a quantitative and objective evaluation that takes into account the limitations of scientific knowledge (Van Straalen, 1993).

The ease with which this method can be used to take laboratory based toxicological data to develop criteria for the protection of ecosystem level effects can lead to its misuse. The HC_5 concept has been shown to be valuable if some of the assumptions are carefully followed, such as the selection of representative organisms (Okkerman et al., 1993; Versteeg et al., 1999). However, this method is still based on laboratory based single species tests, which do not take into consideration indirect effects that may occur between species, or biotic interactions with the abiotic environment, which may modify toxicity. However, considering the limitations of multi-species laboratory and field-based tests, this method can provide the next best thing as far as predicting community response.

The goal of this research is to provide sensitivity information for organisms indigenous to Pacific Northwest that can then be used by agencies such as the Department of Forestry in decision regarding pesticide use. Because HC_5 can be very useful decision making process where limited sensitivity information is

available, as is the case for sensitivity information on native invertebrates, the approach was selected to analyze the results of this research. Since it has been determined that at least five different species from the community need to be used in order to calculate an accurate HC_5 (Wagner & Lokke, 1991; Van Leeuwen & Hermens, 1995), toxicity tests using uniform conditions and uniform endpoints were conducted for at least five species indigenous to the area (see chapter 2).

BIOMONITORING

In addition to laboratory obtained sensitivity data, biological monitoring is also used to determine effects in the field. Biological monitoring uses the responses of living organisms to determine effects, rather than relying on only chemical and physical data (Rosenburg & Resh, 1993). Different taxa represent a range of environmental quality requirements such as temperature, habitat, and diet, allowing disturbance events to be characterized by examining changes in the community structure. One big advantage to using this technique in monitoring environmental changes is that it provides insight into the environmental conditions of the past, while chemical monitoring only provides a snapshot of environmental conditions at the time of sampling (Cairns & Pratt, 1993).

Certain organisms are good indicators of environmental quality because of their sensitivity to environmental perturbations. Patterns of presence or absence can tell us something about the environments in which they are found. The idea of using indicator organisms in this way was developed mostly through the work of Kolkwitz and Marsson in the early 1900's in Europe (Kolkwitz & Marsson, 1908), and Patrick and Forbes in North America (Patrick, 1949; Forbes & Richardson, 1913), who both published approaches for classifying the degree of river pollution based on species assemblages present. From this work, indicator organisms within different trophic levels such as algae, invertebrates, and fish that were found to be

sensitive to different environmental changes were identified and are still in wide use today (Carins & Pratt, 1993).

Macroinvertebrates are well suited for use in environmental biomonitoring. The ubiquity and diversity of these organisms in aquatic environments allows them to be studied in the context of many different environmental perturbations, and provides a large species base from which a spectrum of responses can be determined (Rosenberg & Resh, 1993). The life histories and long life cycles of macroinvertebrates provides opportunities for long term monitoring at varying chemical concentrations. Their sedentary nature allows for disturbance events to be analyzed spatially (Rosenberg & Resh, 1993; Hauer & Resh, 1996). Additionally, their high degree of association with sediments and organic matter increases their exposure to many contaminants and permits body concentrations to be determined (Reice & Wohlenberg, 1993).

The advantages for using macroinvertebrates in environmental monitoring led to the development of a variety of biological indices for assessing stream health. These indices attempt to use macroinvertebrate community diversity to quantify biotic integrity (Shannon & Weaver, 1949; Simpson, 1949; Cairnes & Dickson, 1971). Much of the methodology requires only that different taxa be separated, which eliminates the need for precise taxonomic identification. However, diversity within natural systems can be more complex than information gained from the statistical analysis of biological indices.

ECOTOXICOLOGICAL PROFILE FOR CARBARYL AND TRICLOPYR

A literature search was conducted to obtain sensitivity data for aquatic macroinvertebrates exposed to carbaryl and triclopyr, since these two chemicals were identified for use in testing. Attention was focused on published information on aquatic insects. These values are listed in Table 1.7 A - B for carbaryl, and

Table 1.8 for triclopyr. Data on standardized test organisms, such as *Daphnia*, are also included for comparison. For carbaryl, environmental conditions such as pH have been found to impact the toxicity of the compound. An increase in pH from 6.5 to 8.5 decreased the toxicity of carbaryl to stoneflies (Plecoptera) by 50% (Woodward & Mauck, 1968). Little or no alteration in toxicity appears to result from changes in water hardness or temperature with this compound. The toxicity of carbaryl could not be compared among species because of variation in the endpoint used (ranging from 24h to 30d). The majority of aquatic insects tested with carbaryl have been stoneflies, with seven species tested. Three species of caddisfly (Trichoptera) have been tested, and one species of mayfly (Ephemeroptera). LC_{50} values range from 1.3 to 30 μg/L for stoneflies, 2.7 to <220 μg/L for caddisflies, and 480 μg/L for the mayfly tested. This range is quite wide, and although it must depend in part upon environmental conditions and exposure time, this breadth suggests that a number of species should be tested if risk to the macroinvertebrate community is to be estimated with minimum uncertainty.

For triclopyr, environmental conditions do not seem to drastically influence toxicity. The current toxicological data for triclopyr are however, extremely difficult to utilize for comparisons of species sensitivity because of the range in endpoints that have been selected. Sensitivity data are available for twelve aquatic insect species. The majority of these data arise from 1 hour exposure assays, where effects were determined at 48h (Kreutzweiser et al., 1992). It is not valid to compare these with toxicological statistics obtained from much longer exposure periods. One LC_{50} value, for a 96 hour exposure to Garlon 4, a triclopyr ester formulated product, is reported as 1.2 mg/L for *Daphnia pulex* (Servizi et al., 1987). Clearly, additional data on organism susceptibility to triclopyr will aid in the risk assessment process.

Table 1.7 A. Susceptibility data from the literature for a range of aquatic insects exposed to carbaryl (technical grade unless otherwise specified). Values are lethal concentrations to 50% of the test organisms (LC_{50}) with confidence intervals are included (where available). Environmental conditions are included since carbaryl toxicity has been found to vary significantly with environmental conditions.

Genera / Species Tested	Exposure Duration / Endpoint	Test Conditions[1]	Temp °C	pH	Hardness (mg/L)	LC_{50} (μg/L) & 95% Conf. Intervals	References
Cloen (Ephemeroptera:Baetidae)	48 hr	-	-	-	-	480	Unpublished data in Verschueren, 1991
Acroneuria lycorias (Plecoptera:Perlidae)	30 day	-	-	-	-	2.2	Unpublished data in Verschueren, 1991
Claasenia sabulosa (Plecoptera:Perlidae)	24 hr	Static	15.5	7.1	44	12 (9.1-16.0)	Sanders&Cope, 1968
	48 hr	Static	15.5	7.1	44	6.8 (5.1-8.9)	Sanders&Cope, 1968
	96 hr	Static	15.5	7.1	44	5.6 (3.9-8.1)	Sanders&Cope, 1968
Isogenus sp. (Plecoptera:Perlodidae)	24 hr	Static	7.0	7.0	35	8.0 (5.3-12.0)	Mayer&Ellersieck, 1986
	96 hr	Static	7.0	7.0	35	2.8 (2.0-4.0)	Mayer&Ellersieck, 1986
	24 hr	Static	7.0	7.5	42	15 (8.9-24.0)[a]	Mayer&Ellersieck, 1986

	96 hr	Static	7.0	7.5	42	9.2 (7.4-12.0)[a]	Mayer&Ellersieck, 1986
Skwala sp. (Plecoptera:Perlodidae)	96 hr	Static	12.0	-	-	3.6 (2.4-5.5)	Johnson&Finley, 1980
	96 hr	Static	7.0	-	-	9.2 (7.4-12.0)[a]	Johnson&Finley, 1980
Pteronarcys dorsata (Plecoptera:Pteronarcyidae)	30 day	-	-	-	-	23.0[b]	Unpublished data in Verschueren, 1991
Pteronarcys badia (Plecoptera:Pteronarcyidae)	24 hr	Static	15.5	7.1	44	5.0 (3.6-7.0)	Sanders&Cope, 1968
	48 hr	Static	15.5	7.1	44	3.6 (29.0-4.8)	Sanders&Cope, 1968
	96 hr	Static	15.5	7.1	44	1.7 (1.4-2.4)	Sanders&Cope, 1968
	96 hr	Static	12	6.5	40	11 (9.7-13.0)	Woodward&Mauck, 1968
	96 hr	Static	12	7.5	40	13 (12.0-16.0)	Woodward&Mauck, 1968
	96 hr	Static	12	8.5	40	29 (21.0-41.0)	Woodward&Mauck, 1968
Pteronarcys californica (Plecoptera:Pteronarcyidae)	24 hr	Static	15.5	7.1	44	30 (22.0-40.0)	Sanders&Cope, 1968
	48 hr	Static	15.5	7.1	44	13 (10.0-16.0)	Sanders&Cope, 1968
	96 hr	Static	15.5	7.1	44	4.8 (3.0-7.7)	Sanders&Cope, 1968

Table 1.7 A (Continued)

Hydropsyche bettoni (Trichoptera:Hydropsychidae)	30 days	-	-	-	-	2.7[b]	Unpublished data in Verschueren, 1991
Hydropsyche (Trichoptera:Hydropsychidae)	16 hr	Static /Aeration	25-27	7.3 - 7.9	-	<220[c]	Bradt & Williams, 1980
Cheumatopsyche (Trichoptera:Hydropsychidae)	16 hr	Static /Aeration	25-27	7.3 - 7.9	-	<220[c]	Bradt & Williams, 1980

[a]exposed to oil dispersion (49% carbaryl); [b]carbaryl test material unknown; [c]exposed to Ortho-Sevin formulated product – 27% active ingredient

[1]Conditions refers to the water conditions throughout the test. Static tests are performed without the water and / or toxicant being replaced during the test. Flow-through tests are designed to replace the toxicant and the dilution water either continuously or at regular intervals.

Table 1.7 B No observed effect concentrations (NOEC) obtained from the literature for aquatic insects exposed to carbaryl.

Genera / Species Tested	Exposure Duration (days)	NOEC	Reference
Acroneuria lycorias (Plecoptera:Perlidae)	30 day	1.3 µg/L	Unpublished data in Verschueren, 1991
Hydropsyche bettoni (Trichoptera:Hydropsychidae	30 day	1.8 µg/L	Unpublished data in Verschueren, 1991
Pteronarcys dorsata (Plecoptera:Pteronarcyidae)	30 day	11.5 µg/L	Unpublished data in Verschueren, 1991

Table 1.8. Lethal concentrations to 50% of the test organisms (LC$_{50}$) obtained from the literature for aquatic insects exposed to triclopyr as Garlon 4 (61.6% triclopyr BEE/acid equivalent 44.3%), unless otherwise indicated. Environmental conditions and methodology are included for comparison.

Genera / Species Tested	Exposure Duration (hrs) (time evaluated)	Conditions[1]	Temp °C	pH	Hardness (mg/L)	LC$_{50}$ (mg/L) & 95% Conf. Intervals	Reference
Daphnia magna (Arthropoda:Crustacea)	48	Static	20	7.9	149	1170[a]	Gersich et al., 1994
Daphnia pulex (Arthropoda:Crustacea)	96	Static	21	7.8	84	1.2[b]	Servizi et al., 1987
Simulium sp. (Diptera:Simuliidae)	1 (48)	Flow	13-15	6.6-7.5	45	302.9 (249.3-370.0)	Kreutzweiser et al., 1992
Epeorus vitrea (Ephemeroptera:Heptageniidae)	1 (48)	Flow	13-15	6.6-7.5	45	>320	Kreutzweiser et al., 1992
Heptagenia flavescens (Ephemeroptera:Heptageniidae)	1 (48)	Flow	13-15	6.6-7.5	45	>320	Kreutzweiser et al., 1992
Isonychia sp (Ephemeroptera:Siphlonuridae)	1 (48)	Flow	13-15	6.6-7.5	45	>320	Kreutzweiser et al., 1992
	9 (9)	Flow	9-11	7.0-7.5	40-50	14.9/17.6	Kreutzweiser et al., 1994
	9 (9)	Flow	9-11	7.0-7.5	40-50	4.0/4.5	Kreutzweiser et al., 1994
Ophiogomphus carolus (Odonata:Gomphidae)	1 (48)	Flow	13-15	6.6-7.5	45	>320	Kreutzweiser et al., 1992

Table 1.8 (Continued)

Acroneuria abnormis (Plecoptera:Perlidae)	1 (48)	Flow	13-15	6.6-7.5	45	>320	Kreutzweiser et al., 1992
Paragnetina sp. (Plecoptera:Perlidae)	1 (48)	Flow	13-15	6.6-7.5	45	>320	Kreutzweiser et al., 1992
Isogenoides sp. (Plecoptera:Perlodidae)	1 (48)	Flow	13-15	6.6-7.5	45	61.7 (21.8-126.0)[c]	Kreutzweiser et al., 1992
Pteronarcys sp. (Plecoptera:Pteronarcyidae)	1 (48)	Flow	13-15	6.6-7.5	45	>290	Kreutzweiser et al., 1992
Dolophilodes distinctus (Trichoptera:Philopotamidae)	1 (48)	Flow	13-15	6.6-7.5	45	0.6 (.07-1.27)[c]	Kreutzweiser et al., 1992
Hydropsyche sp. (Trichoptera:Hydropsychidae)	9 (9)	Flow	9-11	7.0-7.5	40-50	37 (34.0-42.9)	Kreutzweiser et al., 1994
	24 (24)	Flow	9-11	7.0-7.5	40-50	8.8 (7.2-10.1)[c]	Kreutzweiser et al., 1994
	1 (48)	Flow	13-15	6.6-7.5	45	>310	Kreutzweiser et al., 1992
Pycnopsyche guttifer (Trichoptera:Limnephilidae)	1 (48)	Flow	13-15	6.6-7.5	45	>290	Kreutzweiser et al., 1992

[a]Exposed to triclopyr salt; [b]EC_{50}; [c]90% Confidence Intervals

[1]Conditions refers to water conditions throughout the test. Static tests are performed without the water and / or toxicant being replaced during the test. Flow-through tests are designed to replace the toxicant and the dilution water either continuously or at regular interval

NATURE OF DECISION MAKING – RISK MANAGEMENT

Risk management is the process of decision-making that attempts to minimize risks without undue harm to other societal values, and is most often requires a yes or no answer with respect to the acceptability of a chemical hazard. These decisions do not require an exact prediction of the nature and magnitude of environmental effects, but instead it is sufficient to show where a chemical would or would not be damaging to use. This type of regulation is a routine process, because chemical uses and effluents are often clearly acceptable or unacceptable.

The goal of other regulatory agencies responsible for quality standards such as the Clean Air Act, or the Federal Water Pollution Control Act, is to make decisions about where on a scale of concentrations the boundary between acceptability and unacceptability occurs (Suter, 1993). Pesticides are regulated under the Federal Insecticide and Rodenticide Act (1947 and revised in 1972) in the United States, which states that only chemicals that do not unreasonably harm the environment should be approved for use. However, there is considerable debate about the type and extent of data that need to be compiled in order to reach this decision. The current Environmental Protection Agency's OPP (Office of Pesticide Programs) protocol for assessing the effects of pesticides places an emphasis on data derived from laboratory based single-species tests rather than field studies (Touart & Maciorowski, 1997). This objective is to determine chemical effects in an expeditious and cost effective manner by establishing dose-response profiles for selected species, and exposure profiles for representative species relevant to the endpoints being considered. In addition, the EPA's current position is that additional information is not considered essential for effective risk management decisions (Touart & Maciorowski, 1997).

Risk management is policy based, and defines assessment boundaries based on socio-economic and legal values in order to protect the environment. When the scientific and societal risks are deemed unacceptable, actions are taken

through risk management to minimize exposure. An effective risk assessment addresses the issues that are relevant to the risk manager, or decision-maker. Realistically, the data must be in a form that can be properly utilized by regulatory agencies in order help in decision making. In this project, our goal is to provide new methodology in order to incorporate the sensitivity of indigenous organisms into the regulatory process. Therefore, we placed an importance in our research on developing test methodology that would be consistent with the current protocol of the EPA by using repeatable, accepted methodology such as laboratory based single species tests. This risk assessment will be used to express changes in ecological effects in stream systems as a function of changes in exposure to pesticides. These data will support decisions made on the acceptable levels of pesticides, and will provide estimates of environmental concentrations that are likely to result in undesirable effects.

UNCERTAINTY ANALYSIS

Uncertainty analysis is important to evaluate in any risk assessment because it provides insight into its strengths and weaknesses and highlights additional information needs. This increases the credibility of the assessment, and can serve as the basis for making alternative management decisions. Most of the uncertainty in effect analysis is a result of extrapolation from individuals tested in the laboratory to higher levels of ecological organization in the field. There are three major forms of uncertainly that need to be evaluated in the risk assessment process: natural stochasticity or variability, uncertainty about a quantity's true value (parameter error), and model error.

Natural stochasticity describes the temporal and spatial variations in environmental characteristics that affect the response of organisms, populations, and ecosystems to disturbance events (ECOFRAM, 1999). This type of uncertainty

cannot be reduced through additional data collection because it is a product of the true heterogeneity of the system. Examples of this kind of uncertainty include variability in responses of test organisms (due to variations in size, age, health genotype, etc.), variability in susceptibilities of habitats and species, ecosystem-to-ecosystem variation, and geographic variability. (ECOFRAM, 1999). Biological processes such as colonization, reproduction, and death are stochastic (Suter, 1993), and variability in individual response may be a function of life stage, parasites and disease, and test conditions. The best way to minimize this kind of uncertainty is to use test organisms of the same age that are free of disease, and to use uniform physical/chemical test conditions and good laboratory practices (U.S. EPA, 1998). However, heterogeneity may not reflect a lack of knowledge and cannot usually be reduced by further measurement.

Parameter error is another form of uncertainty in the risk assessment and results from imprecise measurements of environmental degradation rates, uptake rates, LC_{50}s, or other parameters used in assessment models (U.S. EPA, 1998). Unlike uncertainty that arises as a result of natural stochasticity, this type of uncertainly can be reduced by collecting additional information. Classical statistical methods such as confidence limits and percentiles can be used to describe parameter uncertainty. Examples of this source of uncertainty include species-to species extrapolation using regression models, and confidence bounds on LC_{50} or NOEC values (U.S. EPA, 1998).

Uncertainty can also arise as a result of model error. This is a result of incorrect specification of assessment models, including inappropriate selection or aggregation of variables, incorrect functional forms, and incorrect boundaries. This type of uncertainly is reducible by improving the validity of the assessment models. Examples of this source of uncertainty include extrapolation from lower to higher levels of organization, extrapolation from the laboratory to the field, and extrapolation across major taxonomic boundaries (U.S. EPA, 1998). For example, most current data on aquatic toxicity focus on pelagic species, such as *Daphnia*. To

determine potential effects on benthic organisms requires extrapolations to species with very different life history characteristics, biology and physiology (ECOFRAM, 1999). This species to species extrapolation is a major source of uncertainty, especially when it is made across taxonomic orders.

Addressing uncertainty in the risk assessment process will help risk managers make more informed decisions regarding protective environmental concentrations for pesticides in the environment. Reducing uncertainty affecting the decision making process for the Oregon Department of Forestry was a major objective of this research, which is described below in the aims of the study.

AIMS OF THE STUDY

The goal of this project is to quantify the risk to a native Pacific Northwest macroinvertebrate stream community from chemicals applied by the forest industry. Because of their importance within stream ecosystems, macroinvertebrate exposure and susceptibility data will provide valuable insight into the health of Oregon streams systems. The protection of invertebrates in these systems helps ensure the health of stream fish such as trout and salmon that depend on invertebrate populations for food. The success of these fish populations is a major concern to this region, and the subject of many current research projects.

CHAPTER 2

In order to reduce uncertainty for management decisions affecting Oregon streams, chapter 2 will evaluate the sensitivity of native invertebrates to two chemicals used in forestry using standardized, continuous laboratory tests. The use of standardized organisms, such as *Daphnia* sp., to represent the sensitivity of a

community of organisms that vary widely in development, physiology and morphology introduces uncertainty into the risk assessment process. This research will evaluate uncertainty by determining the susceptibility of a representative community of organisms ecologically relevant to Pacific Northwest streams to chemicals used in forest practices.

Using standardized single species laboratory tests for this research provides many advantages. It allows for the calculation of accurate sensitivity data for indigenous organisms by using accepted regulatory protocol. In addition, standardized methodology minimizes the effects of physiological and genetic variability found in field collected test organisms, such that statistical analysis can be used in sensitivity calculations. Toxicological lethal limits to 50% (LC_{50}) and 1% (LC1) of the organisms tested will be determined for macroinvertebrate species through acute laboratory studies. Organism selection will encompass ecological function, morphology, and possible routes of exposure.

With toxicological data for native species, it is possible to estimate environmental concentrations that will be protective of significant proportions of the community using community sensitivity analysis (HC_5). Community sensitivity data can be used by regulatory agencies in order to protect at least 95% of the invertebrate community from chemical effects.

The following questions will be addressed in this chapter: How variable is the sensitivity of a native community to pesticide exposure? How does this variation, incorporated through the use of community sensitivity analysis (HC_5), compare to detected environmental concentrations in Oregon stream systems? How do these sensitivity values compare to those of standardized organisms? What is the progression of symptomology after pesticide exposure? Over what time period do symptoms, acute lethal and sub-lethal effects, develop after pesticide exposure?

CHAPTER 3

Concentrations may vary temporally in stream environments resulting in variable exposure conditions, raising questions as to the degree of uncertainty that is associated with assessing effects under predetermined continuous exposure (i.e. 72, 96 hours) in laboratory bioassays. Chapter 3 will characterize organism responses under exposure conditions that more closely reflect the transient nature of stream exposure by assessing effects under pulsed, shorter and durations of exposure.

The following questions will be addressed in this chapter: How does susceptibility obtained under continuous exposure conditions compare to an assessment under variable exposure regimes? Do organisms differ in their response to pulsed exposures, and can differences in susceptibility be established that may not be obtained from the results of continuous exposure? What is the potential for recovery under shorter exposure conditions? How long does it take before effects (i.e. mortality) take place after a brief exposure? How long do organisms need to be exposed to a chemical before significant uptake of the chemical occurs to result in mortality?

CHAPTER 4

In addition to the laboratory based testing, Chapter 4 describes a database that will help establish differences in the potential for short- and long-term effects as a result of pesticide exposure by analyzing variation in the ecological attributes of organisms. This process will address uncertainty associated with using laboratory tests alone, which measures the intrinsic susceptibility between different organisms, to evaluate field effects will be evaluated by incorporating the biological and ecological characteristics that vary between organisms. Behavior

and morphology that may modify the potential for short-term effects in the field, and population level characteristics, that may determine the potential for long-term effects such as generations per year, adult vagility and fecundity, may have a profound impact on the severity of effects in the field.

The following questions will be addressed in this chapter: How may the inclusion of ecological characteristics alter sensitivity rankings based on susceptibility data alone? Can organisms be identified that may show contraindicative effects between laboratory testing and the field? How may combinations of an organism's potential for short-and long-term risk play out in the field?

CHAPTER 5

Based on the analysis and compilation of ecological characteristics that may determine the potential for field effects outline in chapter 4, chapter 5 will apply this knowledge to explore variation in these characteristics that may exists between different communities of organisms, and how these differences may correlate to different potentials for short-and long-term effects. Establishing how different community assemblages differ in their response to pesticide exposure will help reduce site specific assumptions of risk.

The following questions will be addressed: How do stream systems that vary in temperature, gradient, substrate type, water chemistry, riparian zone conditions vary in their macroinvertebrate community make-up? How can these differences be used to understand how different streams may vary in the potential for short-and long-term effects to pesticides? Can streams be identified using this process that may be at an increased risk for effects relative to others?

Chapter 2

A Test System to Evaluate the Susceptibility of Oregon Native Stream Invertebrates to Triclopyr and Carbaryl

Jennifer L. Peterson, Paul C. Jepson and Jeffrey J. Jenkins

ABSTRACT

The susceptibility of six indigenous macroinvertebrate species representative of U.S. Pacific Northwest streams (*Ameletus* sp., *Brachycentrus americanus*, *Calineuria californica*, *Cinygma* sp., *Lepidostoma unicolor*, *Psychoglypha* sp. early and late instar) to formulated triclopyr ester (herbicide) and carbaryl (insecticide) was determined using laboratory bioassays. Acute toxicity was expressed as the lethal concentration to 50% (LC_{50}) and 1% (LC_1) of the test population based on a 96-h exposure duration. Carbaryl was found to be 1000 times more toxic than triclopyr for all the organisms tested. LC_1 values (3.0, 7.5, 9.0, 9.5, 14.8, 28.8, 33.8 µg/L, respectively for carbaryl and 1.8, 3.9, 4.0, 4.2, 29.0, 16.1 mg/L respectively for triclopyr) were used in the calculation of hazardous concentration to 5% of the stream macroinvertebrate community (HC_5) based on the lower 95% confidence limit ($HC_5 / 95$). The hazardous concentration ($HC_5 / 95$) for triclopyr was 0.11 mg/L and for carbaryl ranged from 0.43 to 0.66 µg/L, respectively.

Triclopyr and carbaryl symptomology were analyzed for two organisms, *C. californica* and *Cinygma* sp. Carbaryl symptomology included knockdown and moribund states with severity and time of appearance being a function of dose. In triclopyr poisoning, death occurred suddenly with little or no symptomology. Time to 50% mortality (LT_{50}) values were consistently higher for *C. californica* than for *Cinygma* sp. exposed to both chemicals at similar concentrations.

INTRODUCTION

Protection of stream ecological health is a high priority for a number of reasons, including the conservation of fish populations. Evidence for declines in

wild salmon populations has lead to research into the factors that might inhibit habitat quality, including the exposure of stream organisms to pesticides. The Oregon Forest Practices Act, first developed in 1972, creates standards for pesticide use near streams that are designed to protect ecological function in freshwater systems. Written plans must be submitted for all forest pesticide sprays that are to be conducted in the vicinity of stream systems. In addition, riparian buffer zones are required along stream margins and extensive monitoring is undertaken to evaluate pesticide environmental concentrations. However, research has not been undertaken to relate the exposure of native organisms to pesticides within Oregon streams to potential toxicological impacts.

Macroinvertebrate communities exhibit diversity in taxonomy, ecological function, life history characteristics, feeding strategies, morphology and habitat. They play a critical role in nutrient cycling (Newbold et al., 1982) in addition to their role as prey for fish and other organisms (Vannote et al., 1980). Their diversity and ubiquity have made them a central component of stream ecology. For these reasons, macroinvertebrates have often been selected for the evaluation of pollutant risks to stream ecological health.

Macroinvertebrate community composition is strongly correlated with properties of the stream ecosystem (Reice & Wohlenberg, 1993). The selection of macroinvertebrate species for this research project was regionally customized to encompass the ecological uniqueness of the area. Native species were chosen to provide a good representation of the range of ecological roles, functional feeding strategies, taxonomy, life history and physiologies of forest stream macroinvertebrates in Oregon.

Single-species laboratory tests are adopted internationally as tools for the comparison of chemical toxicities (Persoone & Jansen, 1993). These tests provide homogeneous conditions and repeatable methodologies that ensure the rates and routes of exposure to the chemical agent are as repeatable and

predictable as possible. The results from single species tests are used by the U.S. Environmental Protection Agency's Office of Pesticide Programs to make regulatory decisions about pesticides in the United States. The ability of standardized single-species tests to provide an accurate index of pesticide risks in the field has been the focus of considerable debate (Kimball & Levin, 1985). Field-based tests and multi-species tests may provide additional data on potential indirect ecological effects that may occur as a result of chemical disturbance. However, considerable costs are involved in the set-up and analysis of field based studies and the results may be difficult to interpret and site-specific in nature (Persoone & Janssen, 1993), which limits their use in regulatory testing. Given the objective of the present study, to develop test methods and select native species that could be used to compare the toxicities of the pesticides used within Oregon forestry, a single species test methodology was developed. The limitations of these tests with regard to prediction of risk was acknowledged, but the selection of native species added a tier of realism that is not normally represented within these testing regimes.

The lethal endpoint was chosen, given the novelty of some of the species tested, as an easily measured all-or-nothing response that could be determined regardless of the species under observation. The lethal concentration to 50% of the test population (LC_{50}) is the standard endpoint value for toxicity tests, and was therefore reported in order to permit comparisons with LC_{50} values in the literature. The lethal concentration to 1% of the test population (LC_1), calculated from the probit regression model, is however more indicative of early toxic effects, and was selected as a more appropriate endpoint in the assessment of community sensitivity.

Triclopyr, formulated as the butoxyethyl ester (3,5,6-trichloro-2-pyridinyloxyacetic acid, butoxyethyl ester) is a herbicide widely used in forestry for the control of woody plants and broadleaved weeds in forest site preparation and conifer release programs. It was selected as the main test compound in this

study. It is also registered for use in rights-of way, non-cropland and industrial vegetation management. Carbaryl (1-napthyl-N-methyl carbamate), a carbamate, broad spectrum insecticide used in agriculture and urban areas for insect control, is frequently detected in stream systems in the Willamette Valley (Anderson et al., 1996; Anderson et al., 1997). The mode of action and toxicological properties of carbaryl are also well known (Kuhr & Dorough, 1976) and it was selected as a toxic standard to validate the test methodology.

MATERIALS AND METHODS

The test organisms were field-collected macroinvertebrate larvae and nymphs, that were representative of invertebrate communities in Pacific Northwest streams (Table 2.1). Organisms were selected using the following criteria:

Distribution–Test organisms should be widely distributed through different stream habitats. If organisms are only present in isolated areas, their representativeness and ease of use in biomonitoring decreases.

Abundance– Test organisms needed to be available in high enough numbers to enable bioassays at a range of doses.

Season present–Spray events mostly occur during the spring and autumn. Organisms present during each of these seasons were therefore selected.

Life history– Organisms with different life cycle characteristics and lengths were selected in order to capture susceptibility across a wide range of life history strategies.

Trophic position, food source and functional feeding strategy – Trophic and ecological relationships (filter feeders, shredders, predators) were also considered. Detritivores, predators and herbivores may differ in their physiological susceptibility to pesticides. Aquatic macroinvertebrates are

divided into functional feeding groups, based on how they obtain food (Merritt & Cummins, 1996), and this classification was used in the selection of organisms.

Table 2.1: Life history, functional feeding, food source, and body length characteristics of the representative aquatic insects selected for testing. Collection dates and sites are included.

Order / Family	Genus / Species	Voltinism [a]	Emergence	Food Source [a]	Functional Feeding Group[a]	Collection Dates	Mean Body Length (mm)
Hemimetabolous Species							
Plecoptera Perlidae	*Calineuria californica* (Banks 1905)	Mero-	May-August[b]	Other animals, detritus	Predator	9-30-98 to 10-19-98[f]	8.4
Ephemeroptera Heptageniidae	*Cinygma* sp. (Eaton 1885)	Uni-	June-August[c]	Algae / detritus	Scraper/ Collector-gatherer	6-18-98 to 9-21-98[g]	8.8
Ephemeroptera Ameletidae	*Ameletus* sp. (Eaton)	Uni-	April-October[b]	Detritus /Diatoms	Scraper/ collector-gatherer	4-16-99[h] 5-17-99[h] 6-2-99[h]	12.0
Holometabolous Species							
Trichoptera Brachycentridae	*Brachycentrus americanus* (Banks 1899)	Uni-	July-October[d]	Diatoms	Filter Feeder	4-2-99[h] 5-17-99[h] 6-2-99[h] 6-15-99[h]	8.3
Trichoptera Limnephilidae	*Psychoglypha* sp. (Ross 1941)	Uni-	June-Nov.[e]	Detritus	Shredder	4-2-99[h] 4-16-99[h] 5-17-99[h] 6-15-99[h]	10.0 and 16.3
Trichoptera Lepidostomatidae	*Lepidostoma unicolor* (Banks 1899)	Uni-	April-Sept.[c]	Detritus	Shredder (chewer)	4-16-99[h] 5-17-99[h] 6-2-99[h] 6-15-99[h]	8.7

[a] Merritt & Cummins, 1996; [b] Schollmeyer, 1997; [c] Edmunds et al., 1976; [d] Anderson, 1976; [e] Anderson, 1967; [f] Alsea River, OR, [g] Gleason Creek, Muddy Creek Watershed, OR, [h] Metolius River at Sherman Camp, OR

BIOASSAY METHOD

Organisms were transported from field sites to the laboratory in chilled stream water. In the laboratory, the organisms were allowed to acclimate for a period of 24-48 hours in holding tanks that provided re-circulating, chilled (to 10°C), filtered and aerated well water. The filter system contained a pre-filter followed by a microbially active post-filter of shredded paper. Water then entered a sump containing a thermostatically controlled, stainless steel cooler. The different species were held in separate mesh cages in the holding tanks to prevent predation. No food was provided to the organisms prior to testing, although some species may have obtained food from algae and microbes in the tank. The acclimation period allowed for the exclusion of individuals that may have been injured in the collection and transport process. Healthy individuals of similar size were used for testing.

The flask bioassay system was developed to provide well oxygenated, cold water in which native macroinvertebrates could be exposed to pesticides. The tests were conducted in 250 ml Erlenmeyer flasks, containing 200 ml of pesticide solution. During testing, the flask system was placed in a water bath maintained at $10°C \pm 0.5°C$. The flasks were held in place by a fixed grid of shaker flask clips (figure 2.1). A total of 16 flasks could be accommodated at any time. Each flask contained a stainless steel mesh skirt fixed to the aeration tube and a small amount of quartz rock, and both of these provided substrates for animals to cling to (figure 2.2). The test set-up was designed to expose the organisms in optimal conditions while containing vapor contamination within the test system. Each flask was aerated via a plastic tube, inserted through a stopper. Excess air from the aeration exited from the side arms of each flask, which were connected by rubber tubing to a central discharge tube. One-way valves between flasks prevented the flow of air and contaminants from one flask to another. The air was then forced through an activated charcoal filter.
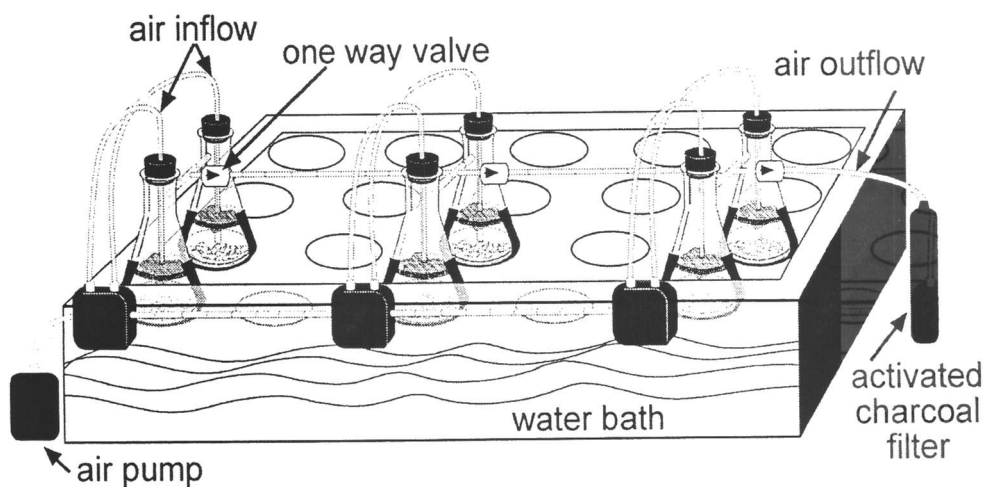
Figure 2.1: Bioassay set-up designed for testing native macroinvertebrates. Air inflow provided through airstones in each flask, contaminated air outflow contained by tubing and treated with an activated charcoal filter. Back contamination prevented by the use of one way valves between flasks. System maintained at 10 °C in a water bath.
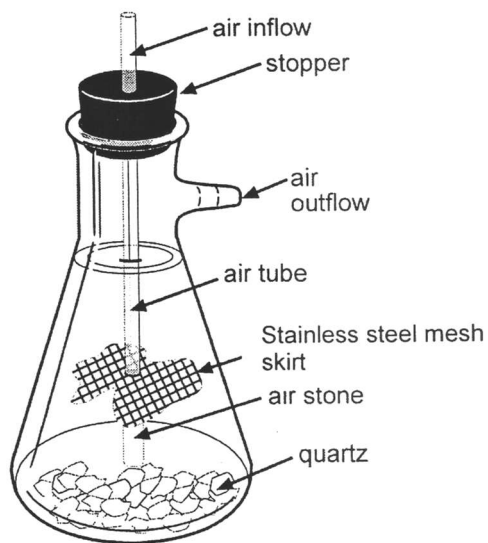
Figure 2.2: Individual flask set-up. Each flask contained a stainless steel mesh skirt, and a thin layer of Quartz rock to minimize organism stress.

Water used throughout testing was obtained from a well, located at the U.S. Environmental Protection Agency's Western Research Station (WRS) in Corvallis, OR. Hardness of the test water ranged from 30 to 40 mg/L, and pH from 7.0-7.5 throughout testing. The water was tested for temperature, pH and hardness at both the beginning and end of testing, according to ASTM standards (ASTM, 1993).

At the start of testing, the organisms were transferred from the holding tanks to the exposure flasks, which contained chilled pesticide dilutions in well water. Depending on organism availability and size, up to 20 organisms were placed in each flask. Organisms of the same species were tested individually, and were randomized between flask treatments. Detailed information on the number of organisms per species tested for each pesticide is provided in Table 2.2.

Table 2.2. Detailed testing information including numbers of organisms tested and concentration ranges for 6 species used to establish lethal concentration estimates.

Organism	Chemical	Total N Test Species	N Concentrations (range)	N Controls	N Organisms Per Test Concentration
Calineuria californica	Carbaryl	64	7 (1-30 µg/L)	1	8
	Triclopyr	49	6 (5-35 mg/L)	1	7-10
Cinygma sp.	Carbaryl	208	21 (4-100,000 µg/L)	4	5-16
	Triclopyr	207	25 (0.9375-1000 mg/L)	4	5-12
Ameletus sp.	Carbaryl	78	6 (10-28 µg/L)	2	4-16
	Triclopyr	30	5 (5-25 mg/L)	1	5
Brachycentrus americanus	Carbaryl	210	15 (5-55 µg/L)	4	9-17
	Triclopyr	115	9 (5-45 mg/L)	2	8-13
Psychoglypa sp.	Carbaryl	164	13 (5-80 µg/L)	3	8-12
	Triclopyr	161	14 (5-45 mg/L)	2	8-16
Lepidostoma sp.	Carbaryl	174	11 (5-80 µg/L)	3	9-15
	Triclopyr	313	18 (5-80 mg/L)	4	9-19

Measurements of numbers knocked down, moribund and dead were recorded at 24-hour intervals throughout the 96-h test period, except for the caddisfly test species, where this was not possible. The flasks were shaken lightly in order to elicit responses from the organisms. This slight manipulation was usually sufficient to determine effects. Cased caddisflies were however difficult to evaluate because of a tendency to retreat into their cases. This made it impossible to determine mortality until the end of the experiment (96 h). End-of-test evaluation often involved dipping the organisms into 80% ethanol in order to elicit a response from retreated individuals. All organisms were preserved in 80% ethanol for confirmation of identification.

DOSING REGIME

Carbaryl (Clean Crop®, Platte Chemical Company, Fremont, NE, USA, EC, 43% AI, w/v) and triclopyr butoxyethyl ester (Garlon 4®, Dow AgroSciences, Indianapolis, IN, USA, EC, 61.1% AI, w/v) were obtained and stored at 2 to 6 °C in glass bottles in sealed tin cans.

Pesticide stock solutions were prepared with chilled well water immediately before each test for triclopyr and within one week of testing for carbaryl. Stock solutions were refrigerated between 2 and 6 °C and stored in 1000 ml sealed, volumetric flasks. Subsequent dilutions using the stock solution were made up on the day of testing. Range finding concentrations were first evaluated, spanning an order of magnitude range around LC_{50} values found in the literature for similar organisms or chemicals. Subsequent tests were then carried out at a narrower range of concentrations to generate data sufficient for statistical analysis. Each test batch consisted of six to seven concentrations and one to two untreated controls (Table 2.2). Concentrations were not replicated.

The chemical concentrations of triclopyr and carbaryl were not held constant throughout testing. Changes in chemical concentration may have occurred

as a result of degradation, sorption and volatilization. This is not however, predicted to have been significant, based on the chemical properties of carbaryl and triclopyr. Carbaryl undergoes hydrolysis in water to 1-naphthol, the rate of which accelerates with increasing pH and water temperature. An aqueous half-life at 25 °C of 14 days and pH 7 and 1 day at pH 8 has been reported (Chapman & Cole, 1992; Wolf et al., 1976). In the current research, the water temperature was held at 10 °C and the pH was less than 7.5. Correcting for pH and temperature differences, the estimated half-life for carbaryl in the test system was 16 days, resulting in an estimated 16% loss due to hydrolysis over 96 hours. Triclopyr butoxyethyl ester hydrolyses to triclopyr acid, which is stable to hydrolysis at pH values of 5, 7 and 9 (Wolf et al., 1976). Calculated Henry's Law constants for carbaryl (2.65×10^{-7}) and triclopyr (9.66×10^{-10}) (Environmental Fate One Line Summaries, 1992) suggest that volatile losses from the test system at 10 °C were insignificant. The increased solubility of the formulated products that were used in the bioassays would have reduced volatile loss further.

ANALYSIS

Lethal concentration estimates to 1% (LC_1) and 50% (LC_{50}) of the test population for the six test organisms were determined for carbaryl and triclopyr by probit analysis (Finney, 1971) using SPSS software (SPSS, 1998). Data from more than one test were often used in the determination of lethal estimates. Concentrations were repeated between experimental runs in order to test for any change in sensitivity over time, and care was taken to ensure that organisms in different batches were of the same stage and size. If the test data were determined to be significantly heterogeneous, a heterogeneity factor was used in the calculation of confidence limits.

Probit analysis (Finney, 1971) was used to determine time to 50% mortality (LT_{50}) for two of the organisms tested, the mayfly *Cinygma* sp. and the stonefly

Calineuria californica, to both carbaryl and triclopyr. In addition to mortality, symptomology including knockdown and the moribund state was examined throughout the test period in order to gain further insight into the poisoning process, and to determine the rates at which symptoms developed as a function of dose.

A distribution based extrapolation method was used for the calculation of hazardous concentrations that theoretically protect significant proportions of the invertebrate community (Kooijman, 1987; Van Straalen & Denneman, 1989). These methods are based upon the assumption of a log-normal distribution of susceptibilities within a community, such that the profile of susceptibility for a complete community can be estimated from limited toxicological data sets. In equation 1, x_m and S_m, are the mean and standard deviation of a sample of $\ln(LC_1)$ values, of size m. K_L is an extrapolation constant that is used for the calculation of one-sided left confidence limits for the logarithmic hazardous concentration for 5% of the species (HC_5). The HC_5 value calculated by equation 1, therefore takes into account uncertainty associated with estimation of the 5th percentile in the logarithmic sensitivity distribution based upon a limited sample of species from the theoretical population. In the present study, the more conservative value of k_L associated with 95% confidence was taken from the tabulated values in Aldenberg and Slob (HC_5 / 95) (Aldenberg & Slob, 1993).

$$HC_5 = \exp\left(x_m - k_L S_m\right) \quad \text{equation 1}$$

RESULTS

Probit statistics (LC_1 and LC_{50} concentrations) calculated for all organisms are presented in Table 2.3 for carbaryl and Table 2.4 for triclopyr. The acute tests show carbaryl to be 1000 times more toxic than triclopyr for all organisms tested, with 96-h LC_{50} values ranging from 11.1 to 61.0 μg/L for carbaryl, compared with 8.55 to 45.0 mg/L for triclopyr. Control mortality was 0% throughout the 96-h test

period in all test batches, with the exception of four tests that had control mortality ranging from 2 to 6%. For carbaryl, comparative toxicity showed *Cinygma* sp. to be the most sensitive, followed by *C. californica*, *Ameletus* sp., *Lepidostoma unicolor*, early instar *Psychoglypha* sp., *Brachycentrus americanus*, and late instar *Psychoglypha* sp. For triclopyr, *C. californica* was the most sensitive, followed by *Ameletus* sp., *B. americanus*, *Cinygma* sp., early instar *Psychoglypha* sp., and *L. unicolor*.

Table 2.3. 96-h lethal concentrations to 1% (LC_1) and 50% (LC_{50}) of the test population and associated toxicological statistics for aquatic macroinvertebrates exposed to carbaryl.

Organism	LC_1 Estimate (+/- 95% CL)[a] ($\mu g/L$)	LC_{50} Estimate (+/- 95% CL)[a] ($\mu g/L$)	Slope	Pearson Chi Square (*df*)
Cinygma sp.	3.0 (0.6-5.1)	11.1 (7.7-13.9)	4.1	41.5 (19)*
Ameletus sp.	7.5	20.4	5.34	15 (4)*
Calineuria californica	9.0 (3.8-12.0)	17.3 (14.06-20.2)	8.24	2.9 (5)
Lepidostoma unicolor	9.5 (2.2-15.5)	29.0 (19.5-37.0)	4.8	21.2 (9)*
Psychoglypha sp. Early Instar (10 mm)	14.8 (2.2-20.2)	30.3 (25.0-40.4)	9.1	4.0 (3)
Brachycentrus americanus	28.8 (14.2-33.1)	41.2 (37.6-50.5)	15.0	14.2 (7)
Psychoglypha sp. Late Instar (16.3 mm)	33.8	61.0 (55.6-68.54)	7.5	8.7 (6)

[a]Confidence limits included where calculable.

χ^2 test for heterogeneity. * indicates significant heterogeneity at $p<0.05$.

Table 2.4. 96-h lethal concentrations to 1% (LC_1) and 50% (LC_{50}) of the test population and associated toxicological statistics for aquatic macroinvertebrates exposed to triclopyr.

Organism	LC_1 Estimate (+/- 95% CL) (mg/L)	LC_{50} Estimate (+/- 95% CL) (mg/L)	Slope	Pearson Chi Square (df)
Ameletus sp.	1.8 (0.02-4.1)	8.55 (3.4-13.0)	3.5	5.2 (3)
Brachycentrus americanus	3.9 (2.0-5.5)	11.3 (9.1-13.4)	5.0	7.6 (7)
Calineuria californica	4.0 (2.3-5.1)	8.1 (7.01-9.06)	7.62	7.14 (9)
Cinygma sp.	4.2 (0.2-8.2)	20.21 (13.5-27.33)	3.4	33.6 (23)
Psychoglypha sp. Early Instar (10 mm)	16.1 (5.4-20.5)	28.34 (24.6-31.9)	9.5	26.5 (12)*
Lepidostoma unicolor	29.0 (19.6-33.3)	45 (42.0-49.7)	5.7	45.6 (16)*

[a]Confidence limits included where calculable.

χ^2 test for heterogeneity; *indicates significant heterogeneity at $p<0.05$.

TIME TO EFFECTS AND SYMPTOMOLOGY ANALYSIS

Symptomology analysis was undertaken for the mayfly *Cinygma* sp. and the stonefly *C. californica* to determine the progression of symptomology as a function of concentration and time of exposure. For carbaryl, organism symptomology included three stages of poisoning; knockdown, moribund and death. However, organisms exposed to triclopyr showed limited symptomology before death, restricting analysis to time-to-death only.

Time to 50% mortality (LT_{50}) values decreased with increasing concentration for both organisms exposed to carbaryl. For *Cinygma* sp., the relationship between LT_{50} and concentration was explained by a power curve of $y = 94.3 \, x^{-2.5}$ (R^2 of 0.93) (figure 2.3), where y is LT_{50} (hrs) and x is log concentration in $\mu g/L$. *C. californica* LT_{50} values appear to follow the same trend, although further testing is needed to verify the relationship.

Knockdown and moribund symptoms increased in a time and dose-dependent manner. At the lowest concentrations tested (8 to 15.6 $\mu g/L$), *Cinygma* sp. showed significant effects approximately 72 h after exposure (e.g. 10 $\mu g/L$, figure 2.4 A; appendix 2.1). LT_{50} values for these concentrations ranged from 80 to 100 h (figure 2.3). At intermediate concentrations (18 to 35 $\mu g/L$), the majority of the population showed effects at 48 h (e.g. 20 and 30 $\mu g/L$, figures 2.4 B and 2.4 C). Mortality occurred earlier in the assessment period, with LT_{50} values ranging from 34.1 to 100.5 hours. At the highest exposure concentrations, 67.5 to 100,000 $\mu g/L$, the progression from knockdown to the moribund state to death occurred in the first 24 hours of exposure (e.g. 125 $\mu g/L$, 500 $\mu g/L$ and 2,200 $\mu g/L$; figures 2.4 D - F). Symptomology progressed more quickly at the higher concentrations requiring that observations were conducted more frequently. LT_{50} values ranged from 2.1 to 11.6 h.
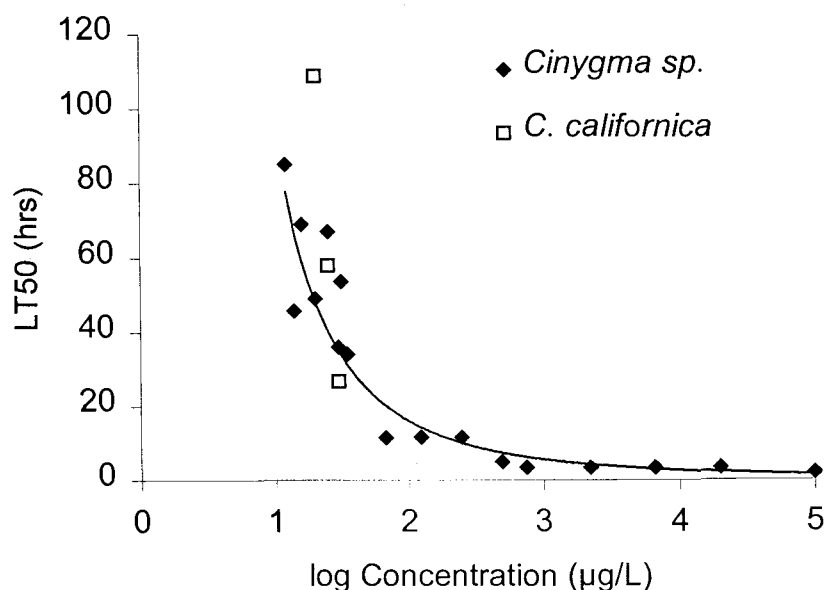
Figure 2.3: Time to 50% mortality (LT$_{50}$) values for *Cinygma* sp. and *C. californica* exposed to carbaryl in 96-hour laboratory bioassays. Data did not allow for the calculation of confidence limits. Equation of data fit: y = 94.293 x - 2.5568, R^2 = 0.93.

Lethal time (LT$_{50}$) values for *C. californica* exposed to carbaryl were calculated as 108.7 h at 20 µg/L, 57.8 h at 25 µg/L and 26.7 h at 30 µg/L (figure 2.3). Concentrations tested at 15 µg/L and below failed to elicit 50% mortality. Comparisons between the two organisms show a similar progression of symptoms. However, a comparison of effects occurring at similar concentrations (e.g. 20 and 30 µg/L; figures 2.4 B and 2.5 D) shows a higher proportion of *Cinygma* sp. responding than *C. californica* at any given time. At concentrations of 15 and 20 µg/L some organisms were exhibiting knockdown symptoms at 96 h (figures 2.5 A-B). At 25 µg/L (figure 2.5 C), the knockdown state was only present until 72 h,

and at 30 µg/L (figure 2.5 D), no organisms exhibited knockdown and then were either moribund or dead at the earliest assessment time (24 h).

Neither organism recovered from the knockdown or moribund states with carbaryl over the 96-h assessment period at any concentration tested. Organisms exhibiting moribund effects survived several days in this state before eventually dying. Variation in the pattern of the moribund state observed in the graphs may be attributed in part to the difficulty in distinguishing moribund symptoms from death during the assessment period. However, at the end of the 96-h test period we found little evidence that moribund organisms recovered when transferred to freshwater.

Figures 2.4 A – F: Symptomology for *Cinygma* sp. exposed to carbaryl concentrations of 10, 20, 30, 125, 500 and 2,200 µg/L, respectively, over the 96-hour test period.

A) 10 µg/L

B) 20 µg/L

C) 30 µg/L
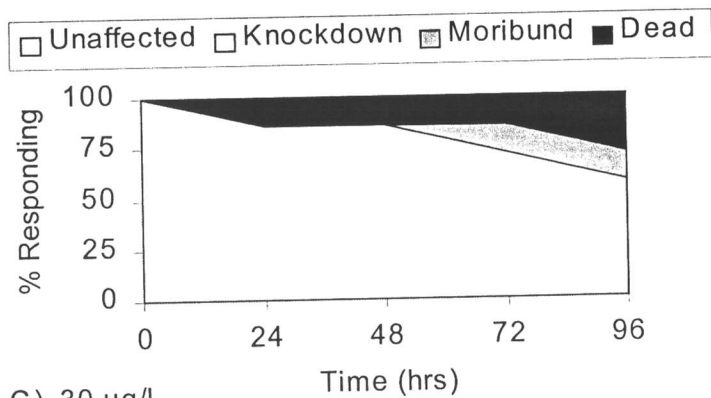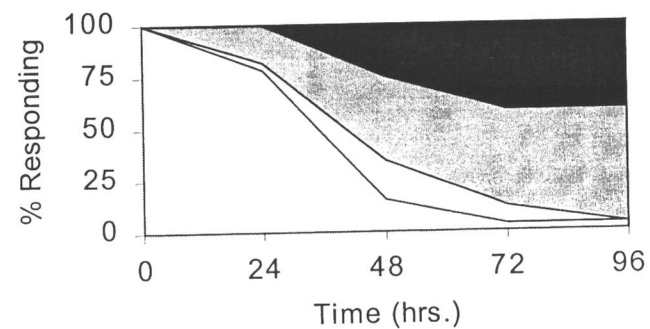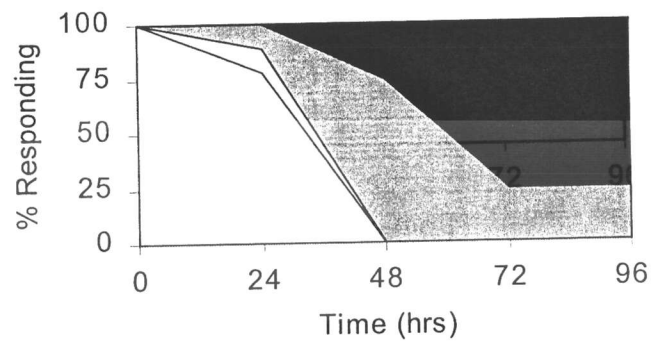
D) 125 µg/L
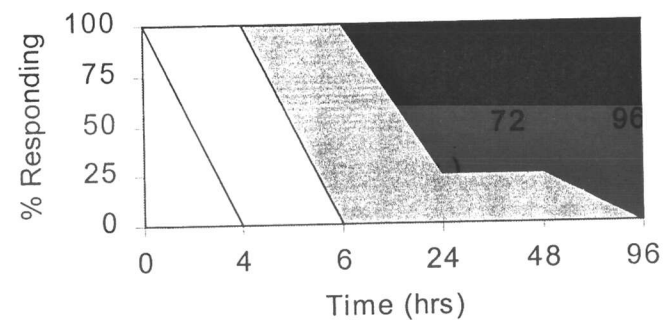
Figure 2.4 Continued

E) 500 µg/L
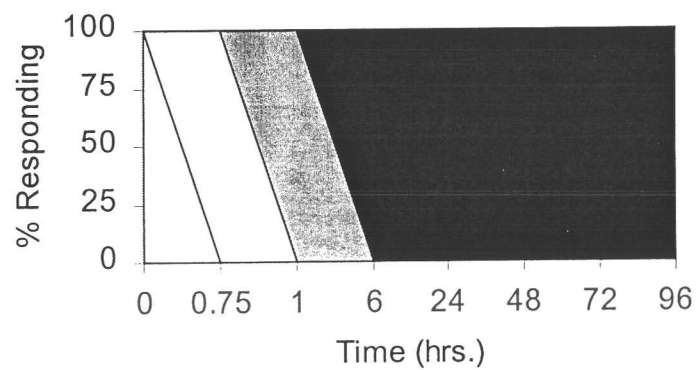
F) 2,200 µg/L
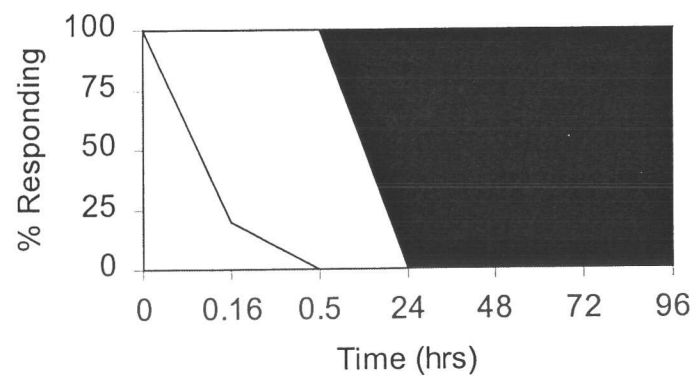
Figures 2.5 A – D. Symptomology progression for *C. californica* exposed to carbaryl at concentrations of 15, 20, 25 and 30 µg/L.

A) 15 µg/L

B) 20 µg/L

C) 25 µg/L
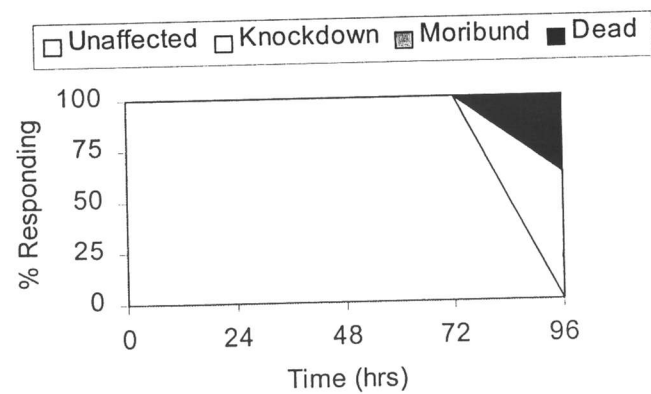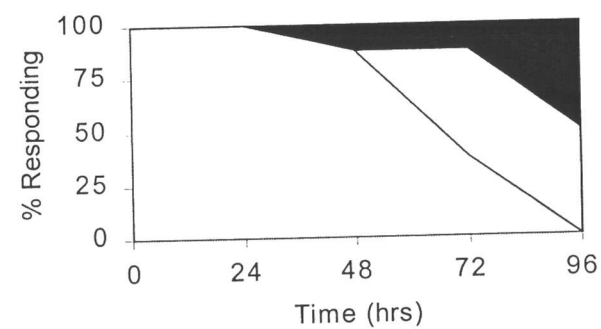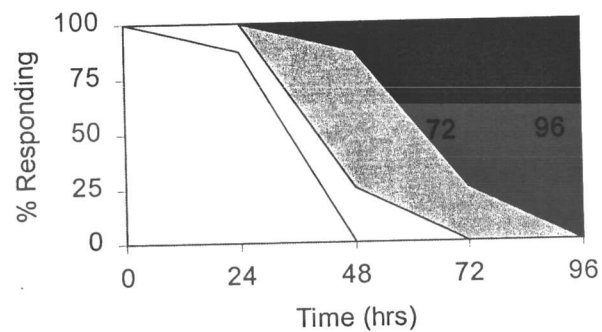
D) 30 µg/L
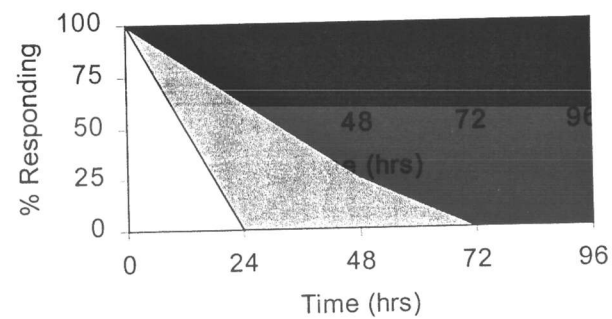
Triclopyr effects did not include significant symptoms of knockdown or the moribund state for either organism (figures 2.6 A - D *Cinygma* sp., and figures 2.7 A – C *C. californica*). Survival was possible at concentrations at or below 22 mg/L for *Cinygma* sp. (e.g. 16 mg/L; figure 2.6 A), and 10 mg/L for *C. californica* (figure 2.7 A) over 96-hour exposures. Time to 50% mortality values decreased as concentration increased, but not all concentrations tested elicited 50% mortality. A comparison of the two organisms at similar concentrations shows *C. californica* to have a higher proportion responding at any given time (figure 2.8).

Figure 2.6 A – D. Symptomology progression for *Cinygma* sp. exposed to triclopyr at concentrations of 16, 30, 125 and 500 mg/L, respectively.

A) 16 mg/L
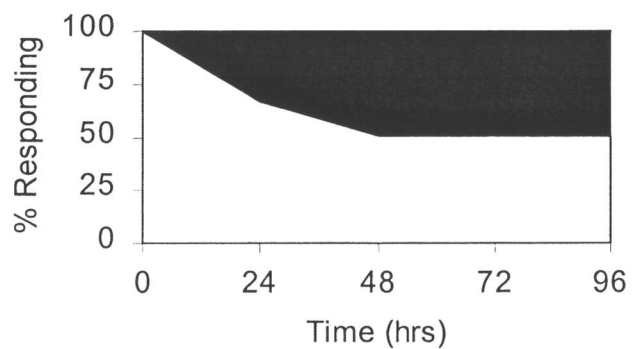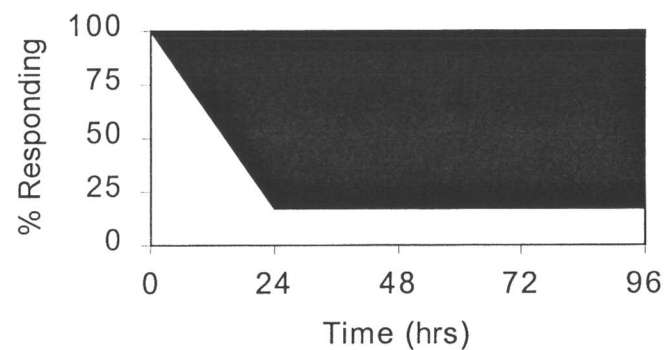
B) 30 mg/L

C) 125 mg/L
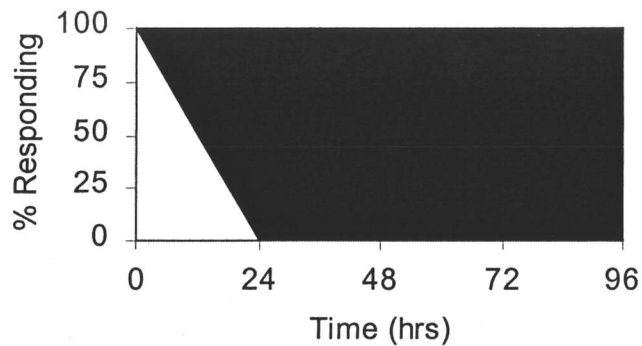
D) 500 mg/L
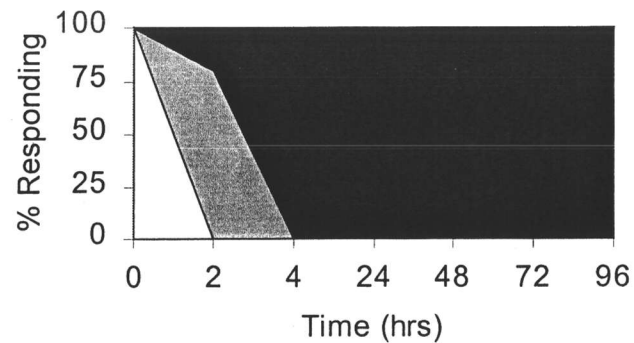
Figures 2.7 A – C: Symptomology progression for *C. californica* exposed to triclopyr at concentrations of 10, 20 and 30 mg/L, respectively.

A) 10 mg/L
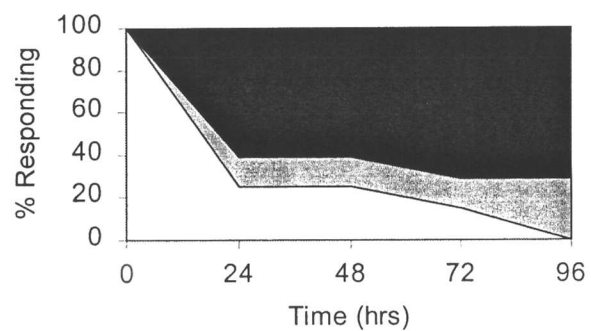
B) 20 mg/L
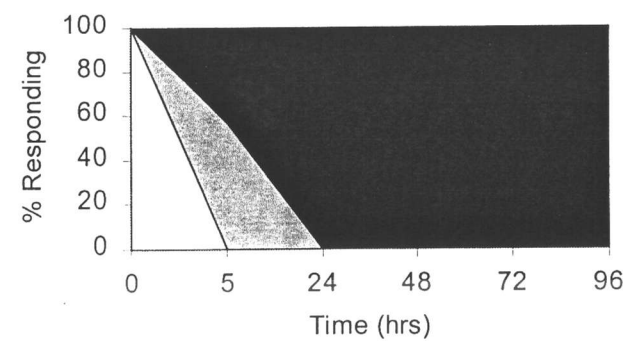
C) 30 mg/L
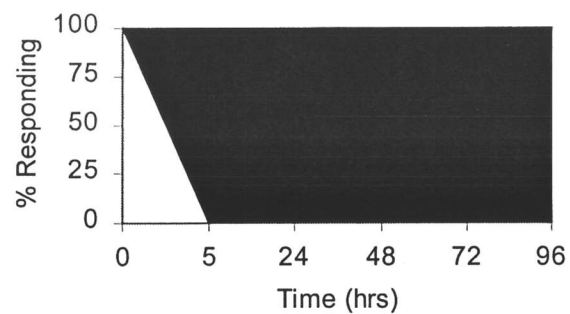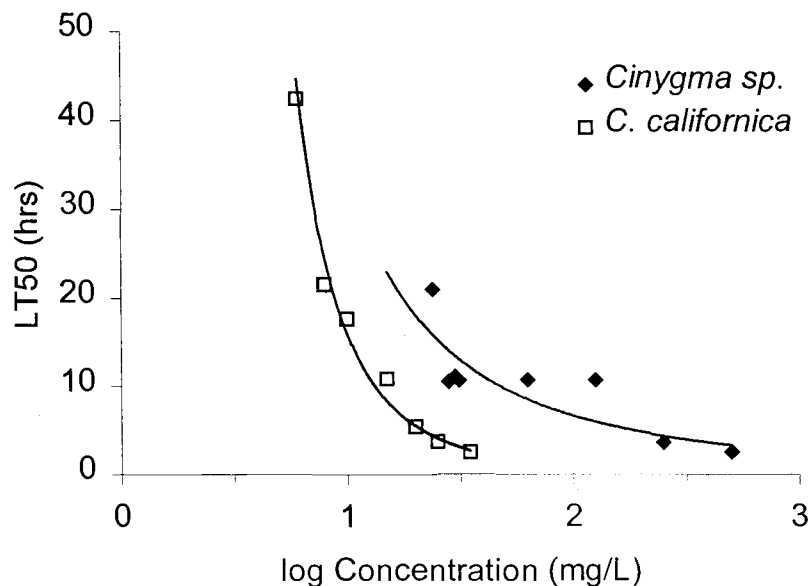
Figure 2.8: Time to 50% mortality (LT_{50}) values for *Cinygma* sp. and *C. californica* exposed to triclopyr in 96-h laboratory bioassays. Data did not allow for the calculation of confidence limits. Equation of data fit: *C. californica* y = 16.073 x - 4.0758, R^2 = 0.9811; *Cinygma* sp. y = 33.54 x - 2.3383, R^2 = 0.7742.

ESTIMATION OF HAZARDOUS CONCENTRATION

Using the LC_1 values in Table 2.3 for carbaryl and Table 2.4 for triclopyr, $HC_5 / 95$ values were calculated (equation 1). For both chemicals, six species were used in the $HC_5 / 95$ calculation. In the case of carbaryl, an additional life stage was added for *Psychoglypha* sp., and two separate $HC_5 / 95$ values were calculated based on the different LC_1 values calculated for this species.. The hazardous concentrations to 5% of the theoretical community ($HC_5 / 95$ values) were 0.11 mg/L for triclopyr and ranged from 0.43 (*Psychoglypha* sp. late instar) to 0.66 µg/L (*Psychoglypha* sp. early instar) for carbaryl.

CONCLUSIONS / DISCUSSION

The goals of this research were to determine the intrinsic susceptibilities of field-collected aquatic invertebrates to forest pesticides and to provide estimates of community sensitivity to those compounds. It is impractical to test all stream organisms in a given habitat, necessitating a selection process that provides representative organisms for testing. A method was outlined for the selection of appropriate organisms that would maximize diversity in functional feeding strategy, life history and taxonomy. The selection of a diverse range of test organisms was an important part of the experimental design that was intended to maximize the probability of detecting effects with a limited number of test species.

Community sensitivity statistics (e.g. HC_5) have been utilized in the determination of protective concentrations for soil (Van Straalen & Denneman, 1989) and aquatic organisms (Wagner & Lokke, 1991). The HC_5 is a community sensitivity assessment based the results of single-species laboratory tests, and has therefore has been criticized for failure to take into account the multiple factors that limit our ability to extrapolate from laboratory data to the field (Forbes & Forbes, 1993; Hopkin, 1993). The method can however provide a valuable statistical basis for the establishment of protective environmental concentrations from laboratory test data (Okkerman et al., 1993; Versteeg et al., 1999). The uncertainty associated with the HC_5 calculation was minimized by taking a number of steps. Firstly, an ecologically based selection process was developed to identify representative test organisms (Van Straalen & Denneman, 1989). Secondly, extreme physiological variation was reduced by not including organisms that encompassed large taxonomic distances (i.e. fish or algae) (Wagner & Lokke, 1991; Lokke, 1994), and restricting tests to representatives of the Insecta. Thirdly, variation in test endpoints (i.e. lethality, growth rate, reproduction) which can result in extrapolation errors (Wagner & Lokke, 1991) was avoided by the use of a single endpoint, mortality (96 h).

Species selection procedures should ideally be free of biases. However, constraints of laboratory testing did introduce certain biases into the organism selection process. In order to obtain the large number of organisms required in toxicity testing, local abundance was an important criterion. This resulted in the exclusion of rare organisms, even though these may be of significance ecologically. In addition, the physiological requirements for some organisms prohibited their use in toxicity testing because these requirements could not be met within the test system. For example, organisms that require high velocity, well oxygenated, currents (e.g. blackfly larvae, Simulidae: Diptera) were not considered for testing.

Carbaryl toxicity is a result of inhibition of the enzyme acetylcholinesterase in synaptic junctions of the nervous system. The appearance, intensity, and duration of symptoms are a function of dose and length of exposure (Kuhr & Dorough, 1976). Reported LC_{50} values in the literature range from 1.7 to 29 µg/L for mayflies, stoneflies and caddisflies (Chapter 1), and from 11.1 to 61.0 µg/L in this research. Variation in organism sensitivity within a given test procedure could be a result of differences in uptake rates, detoxification abilities and metabolism. Insect metabolism of carbaryl occurs through hydrolysis to 1-naphthol, and metabolism rates have been found to increase as larvae proceed from early to late instars (Kuhr & Dorough, 1976). Chemical sensitivity has been found to vary by insect order, and may be linked to the activity of detoxification enzymes (Siegfried, 1993; Siegfried & Young, 1993). Blackflies (Simulidae) have been found to have the highest level of detoxification enzymes, followed by caddisflies, mayflies, and damselflies (Siegfried & Young, 1993).

Analysis of the rate of appearance of poisoning identifies thresholds for sub-lethal effects and may be more protective of stream organisms than analysis that rely solely on mortality assessment. Drift is a behavioral mechanism by which organisms can escape unfavorable conditions (Smock, 1996). Organisms exhibiting symptoms of early poisoning, such as knockdown, may have an impaired ability to maintain a foothold in stream habitats, resulting in loss of biota

to drift (Scherer & McNicol, 1986). Impaired organisms may be unable to return to favorable stream habitats, and may be effectively lost to the ecosystem.

Carbaryl has been detected in stream systems at or near concentrations that have been shown to elicit effects in aquatic organisms. Sampling in the Willamette Valley, Oregon detected carbaryl in 22% of the samples taken in small streams from 1992 to 1994, and 13% of samples taken in 1996 (Anderson et al., 1996; Anderson et al., 1997). Maximum concentrations ranged from 0.11 μg/L to 2.0 μg/L. Another study found maximum stream levels to range from 0.93 to 7.8 μg/L in brooks, and 0.44 to 2.0 μg/L in rivers after an application at 0.84 kg/ha (Stanley & Trial, 1980). These detectable environmental concentrations are close to the HC_5 / 95 values calculated in this research of 0.43 and 0.66 μg/L.

The expected environmental concentration (EEC), calculated as the concentration in a 15 –cm deep body of water directly over-sprayed at the maximum application rate of 4 kg/ha, has been reported as 2.7 mg/L for triclopyr (Kreutzweiser et al., 1994). Maximum stream water residues after a forest aerial application have been reported as 230 to 350 μg/L after a direct over-spray applied at a rate of 3.84 kg/ha (Thompson & Staznik, 1991). Maximum levels detected in the Willamette River Basin ranged from 0.72 μg/L to 6.0 μg/L, with a detection rate of 8% in samples taken from 1992 to 1994, and 23% in 1996 (Anderson et al., 1996; Anderson et al., 1997).

There is no known mode of action for triclopyr. Previous studies evaluating the effects of triclopyr on stream insects found very little potential for adverse effects at environmental concentrations (Kreutzweizer et al., 1994; Kreutzweiser et al., 1992; Kreutzweiser et al., 1998). The HC_5 / 95 value of 0.11 mg/L calculated in this research supports these findings, with an approximately 1000-fold margin of exposure between expected effects and environmental concentration. However, triclopyr residues have been found to accumulate in submerged leaf-pack material at up to 20 times the stream water concentration (Thompson et al., 1995), and 80 times the water concentration in semi-static laboratory microcosms (Kreutzweiser et al., 1994). These residues may present a risk to organisms that inhabit or feed on

leaf packs in stream systems, such as the caddisflies *L. unicolor* (LC_1 = 29.0 mg/L) and *Psychoglypha* sp. (LC_1 = 16.1 mg/L). One experimental study failed, however to detect toxic effects at high triclopyr concentrations in leaf packs (Kreutzweiser et al., 1998).

These methods constitute a first tier of testing in the evaluation of pesticide risk to aquatic macroinvertebrates. We recommend that if effects are detected which fall within expected environmental concentrations, then additional testing should be undertaken to assess chemical effects under environmentally realistic exposure conditions. For streams, this testing should first evaluate the interaction between duration of exposure and concentration in the laboratory, taking into account the nature of pesticide pulses in stream systems. An investigation of the toxicological consequences of pulsed exposures for *Cinygma* sp. and *C. Californica* is presented in (Chapter 3; Peterson et al., *in press* b).

ACKNOWLEDGEMENT

Chapter 2 Appendices

Appendix 2.1. Symptomology graphs for *Cinygma* sp. exposed to carbaryl at concentrations of 8 µg/L (A), 10 µg/L (B), 12 µg/L (C), 14 µg/L (D), 15 µg/L (E), 15.6 µg/L (F), 18 µg/L (G), 20 µg/L (H), 25 µg/L (I), 30 µg/L (J), 31.25 µg/L (K), 35 µg/L (L), 67.5 µg/L (M), 125 µg/L (N), 250 µg/L (O), 500 µg/L (P), 750 µg/L (Q), 2,200 µg/L (R), 6,600 µg/L (S), 20,000 µg/L (T), and 100,000 µg/L (U). Black = Mortality; Dark Gray = Moribund; Light Gray = Knockdown and White = Unaffected.

A) 8 ug/L
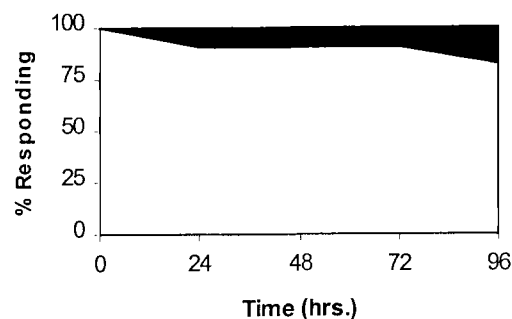
B) 10 ug/L
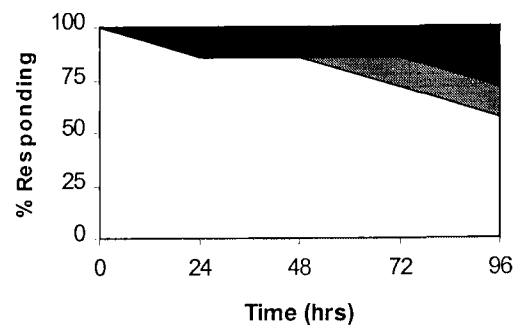
B) 12 ug/L
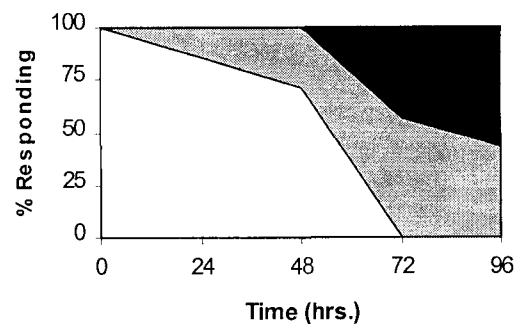
D) 14 ug/L
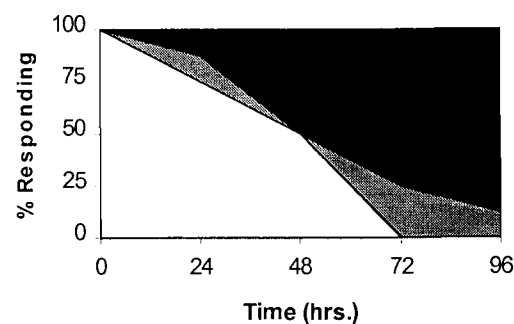
Appendix 2.1 Cont.

E) 15 ug/L
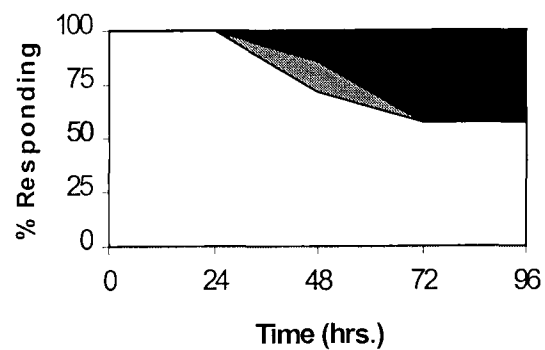
F) 15.6 ug/L
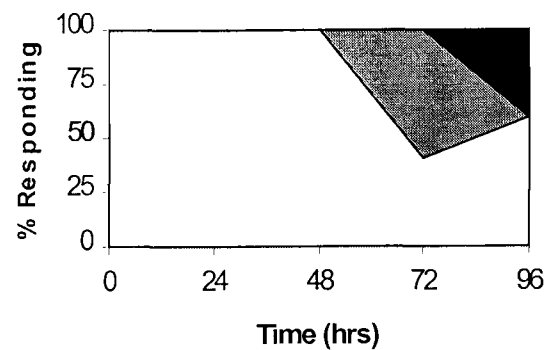
G) 18 ug/L
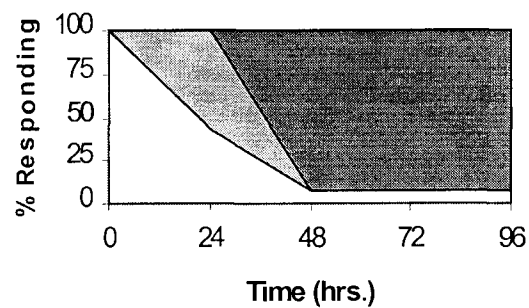
H) 20 ug/L
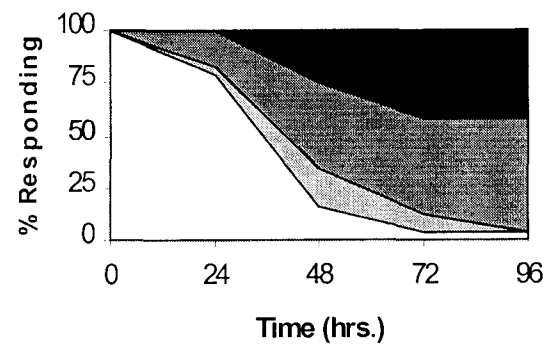
Appendix 4.1 Cont.

I) 25 ug/L
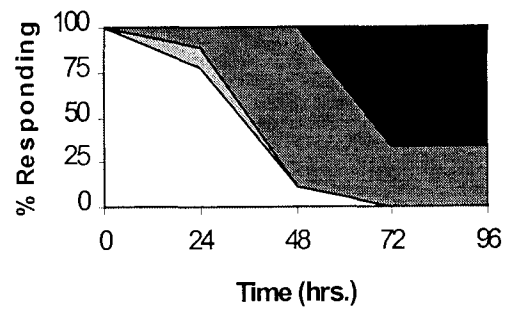
J) 30 ug/L
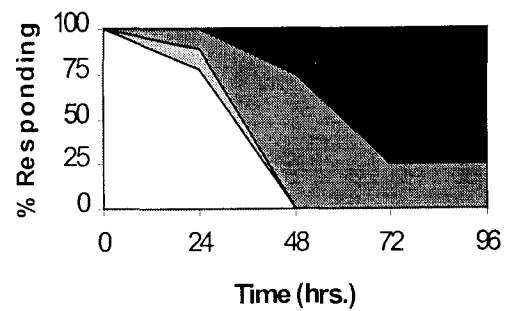
K) 31.25 ug/L
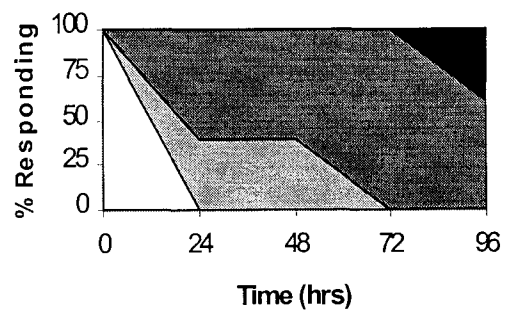
L) 35 ug/L
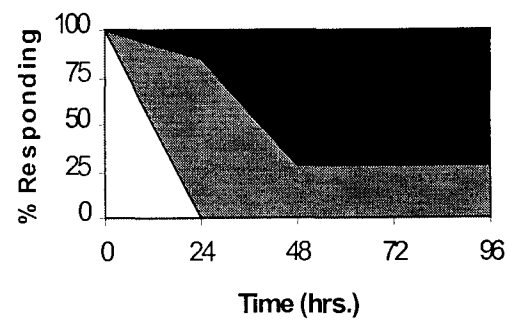
Appendix 2.1 Cont.

M) 67.5 ug/L
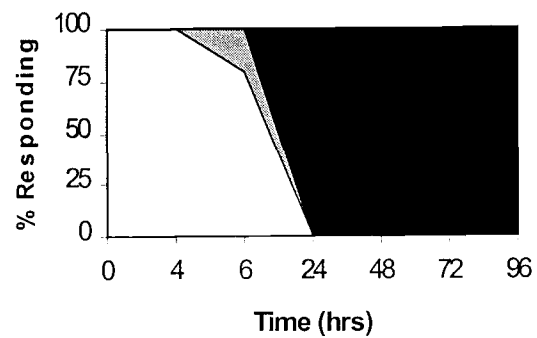
N) 125 ug/L
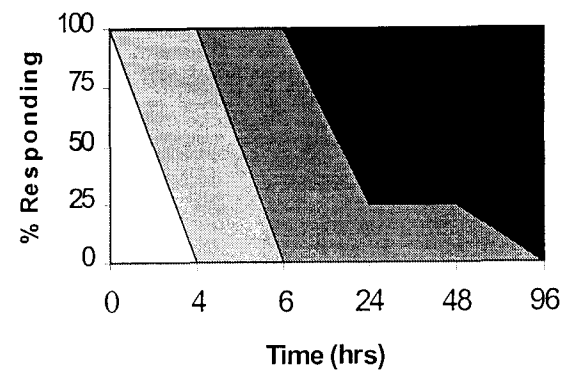
O) 250 ug/L
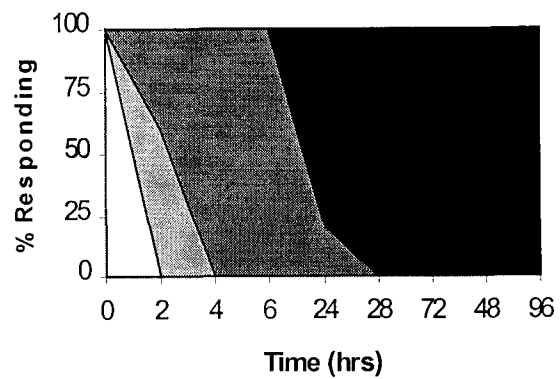
P) 500 ug/L
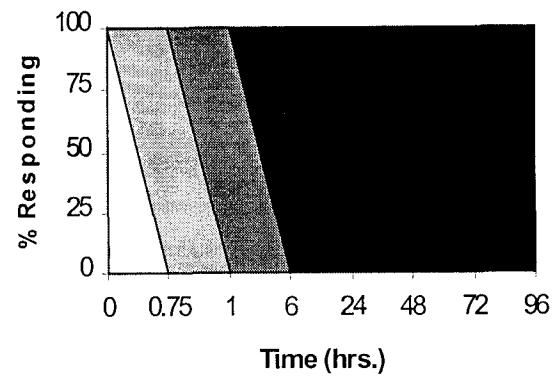
Appendix 2.1 Cont.

Q) 750 ug/L
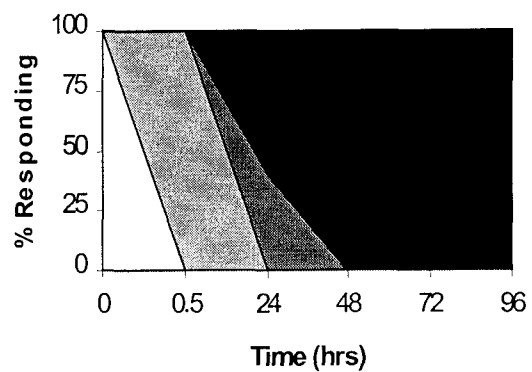
R) 2,200 ug/L
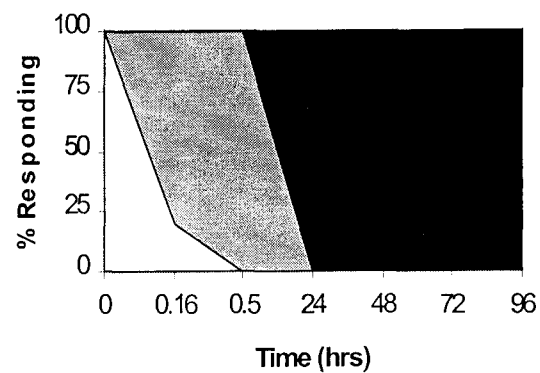
S) 6,600 ug/L
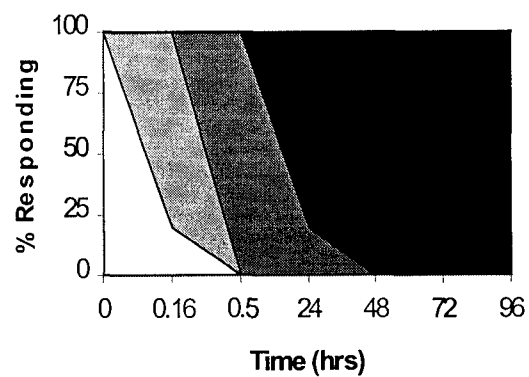
T) 20,000 ug/L
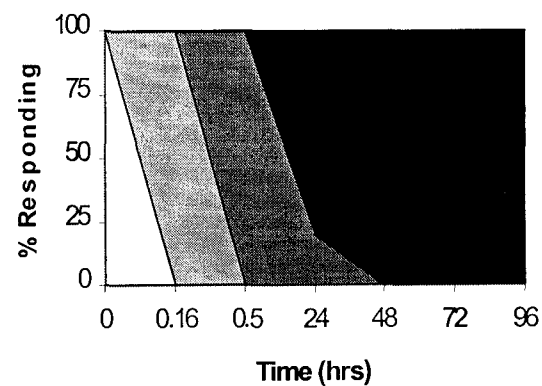
Appendix 2.1 Cont.

U) 100,000 ug/L
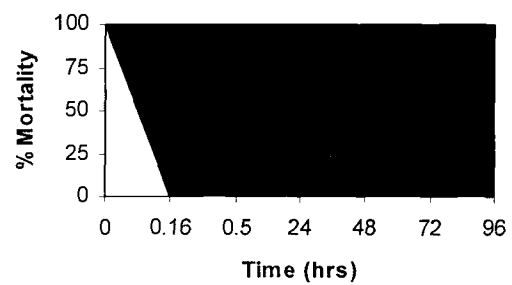
Appendix 2.2. Symptomology graphs for *Calineuria californica* exposed to carbaryl at concentrations of 1 μg/L (A), 5 μg/L (B), 10 μg/L (C), 15 μg/L (D), 20 μg/L (E), 25 μg/L (F), and 30 μg/L (G). Black = Mortality; Dark Gray = Moribund; Light Gray = Knockdown and White = Unaffected.

A) 1 ug/L

B) 5 ug/L

C) 10 ug/L
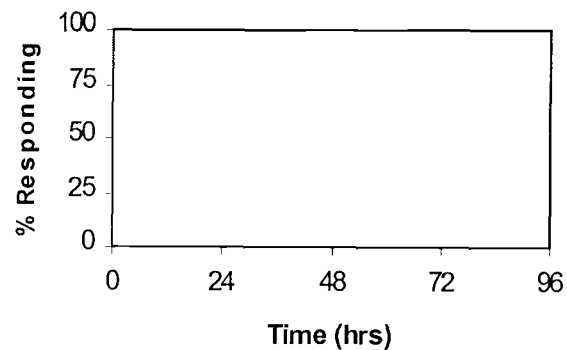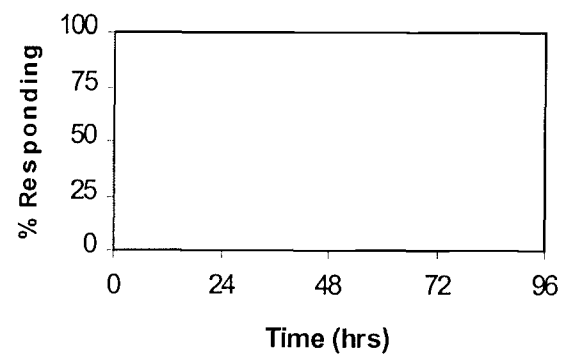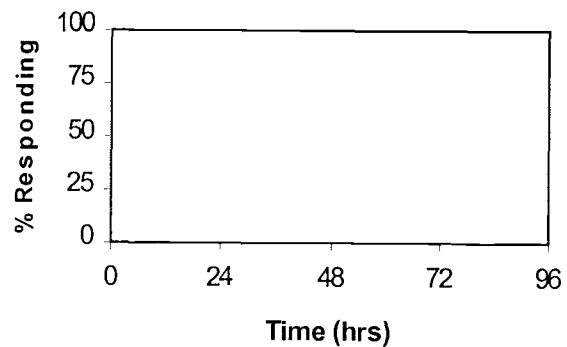
D) 15 ug/L
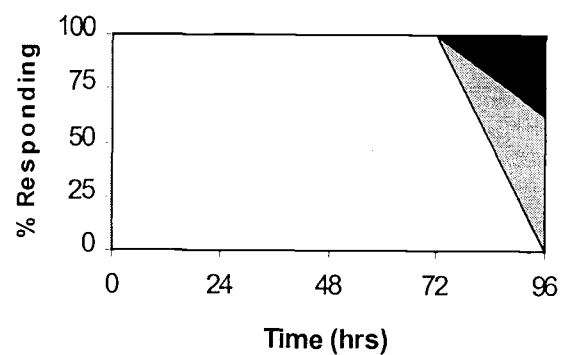
Appendix 2.2 Cont.

E) 20 ug/L
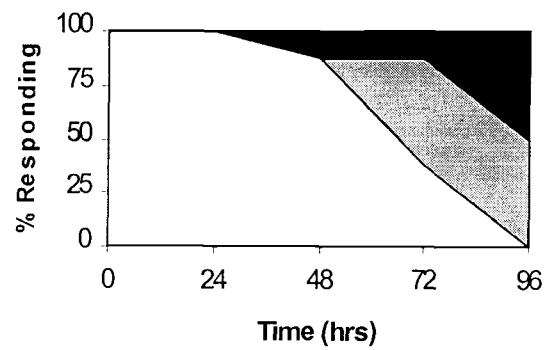
F) 25 ug/L
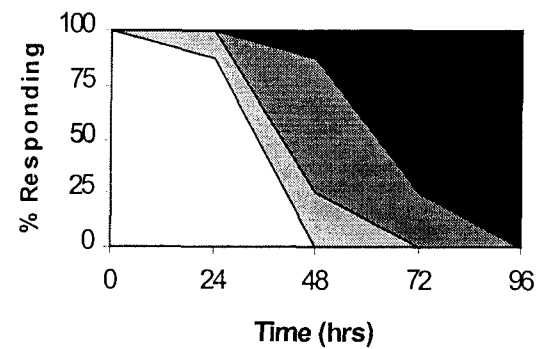
G) 30 ug/L
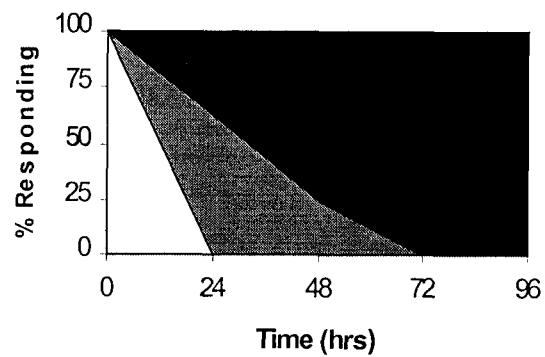
Appendix 2.3. Symptomology graphs for *Cinygma* sp. exposed to triclopyr at concentrations of 15 mg/L (A), 16 mg/L (B), 18 mg/L (C), 20 mg/L (D), 22 mg/L (E), 24 mg/L (F), 26 mg/L (G), 28 mg/L (H), 30 mg/L (I), 31.25 mg/L (J), 62.50 mg/L (K), 125 mg/L (L), 250 mg/L (M), 500 mg/L (N), and 1000 mg/L (O). Black = Mortality; Dark Gray = Moribund; Light Gray = Knockdown and White = Unaffected.

A) 15 mg/L
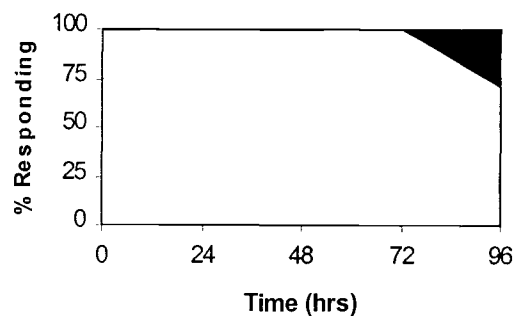
B) 16 mg/L
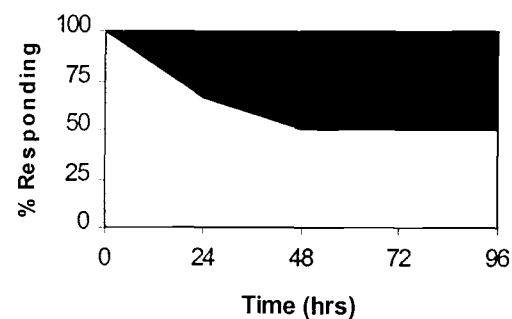
B) 18 mg/L
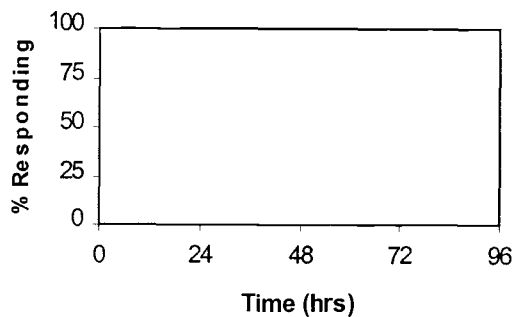
D) 20 mg/L
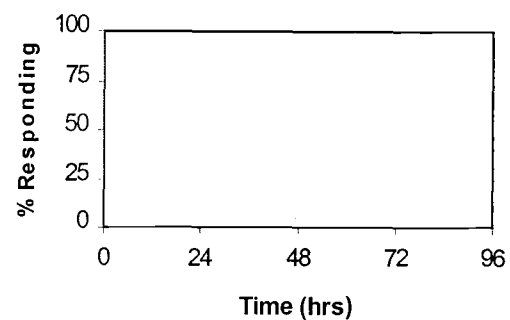
Appendix 2.3 Cont.

E) 22 mg/L
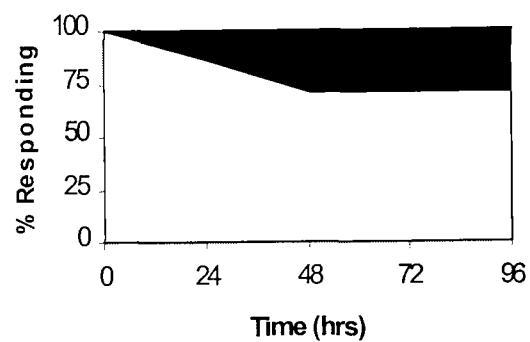
F) 24 mg/L
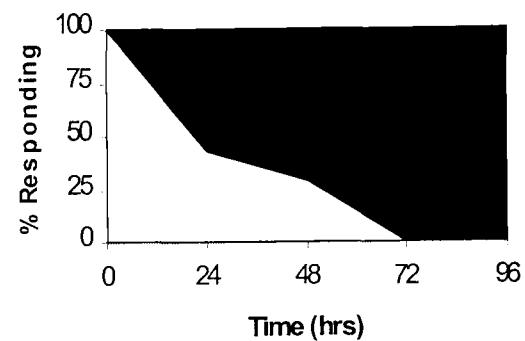
G) 26 mg/L
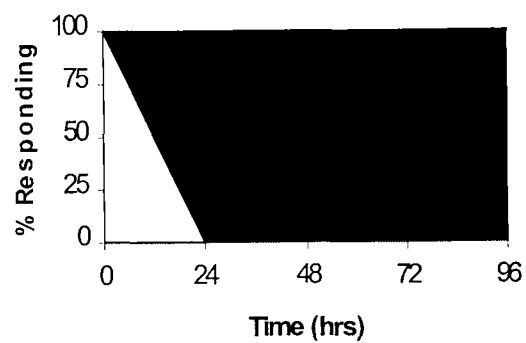
H) 28 mg/L
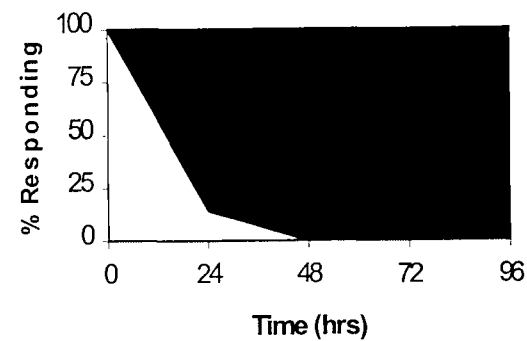
Appendix 2.3 cont.

I) 30 mg/L
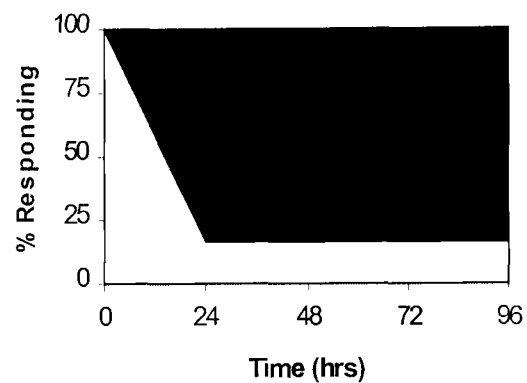
J) 31.25 mg/L
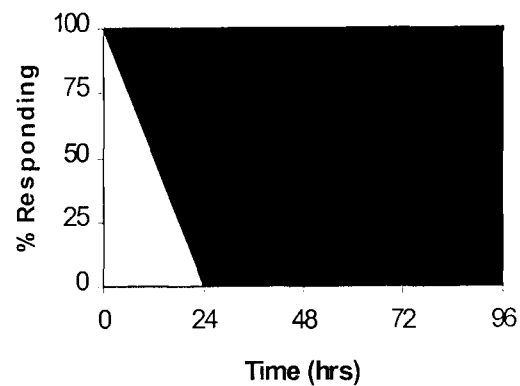
K) 62.50 mg/L
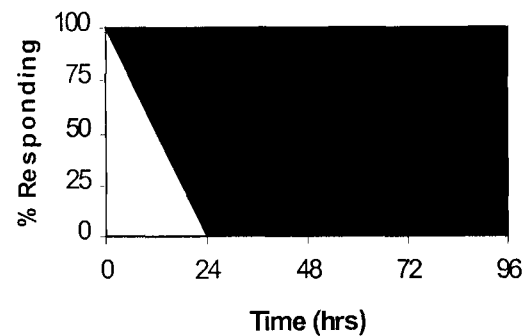
L) 125 mg/L
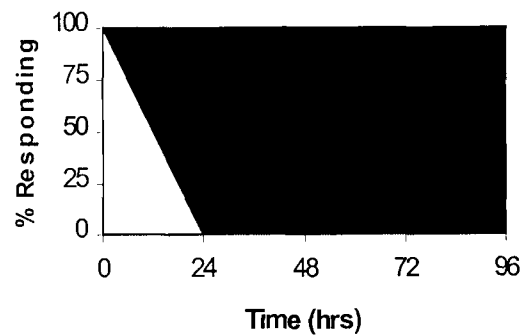
Appendix 2.3 Cont.

M) 250 mg/L
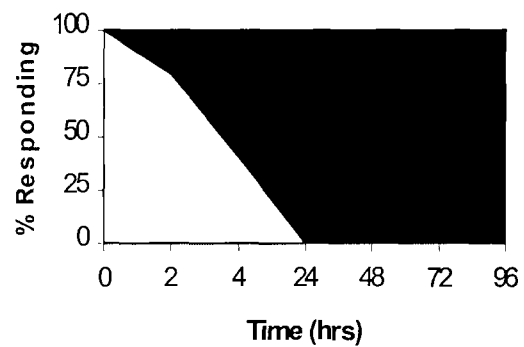
N) 500 mg/L
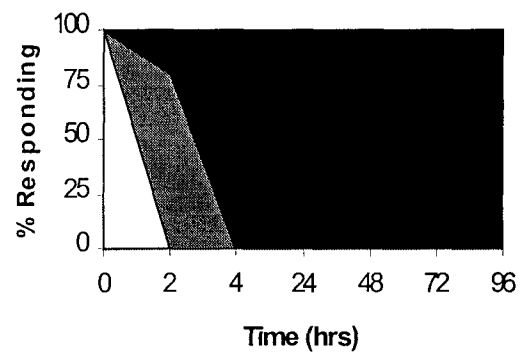
O) 1000 mg/L
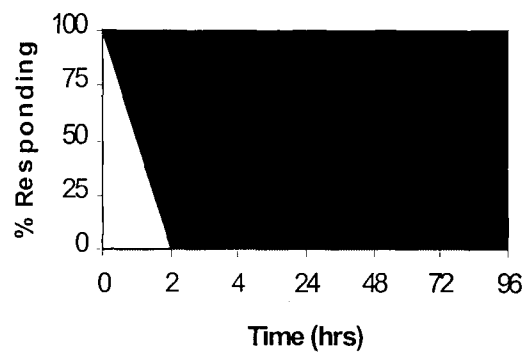
Appendix 2.4. Symptomology graphs for *Calineuria californica* exposed to triclopyr at concentrations of 5 mg/L (A), 6 mg/L (B), 8 mg/L (C), 10 mg/L (D), 12 mg/L (E), 14 mg/L (F), 15 mg/L (G), 20 mg/L (H), 25 mg/L (I), 30 mg/L (J), and 35 mg/L (K).
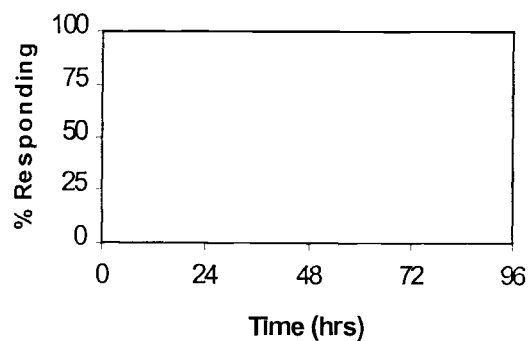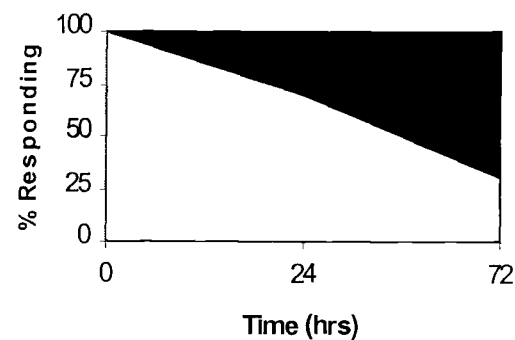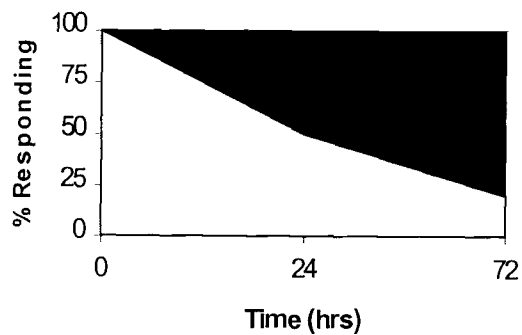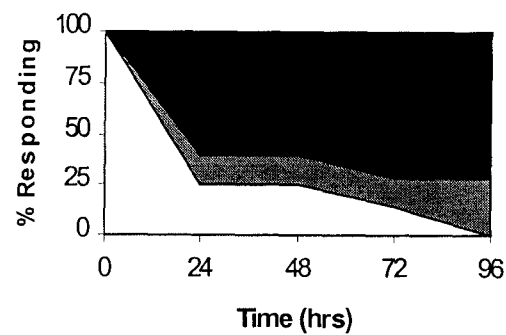
E) 12 mg/L

F) 14 mg/L

G) 15 mg/L

H) 20 mg/L
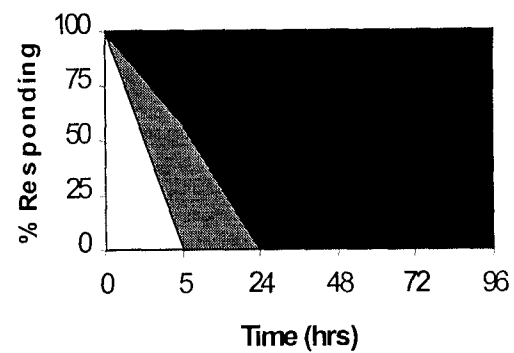
Appendix 2.4 Cont.

I) 25 mg/L
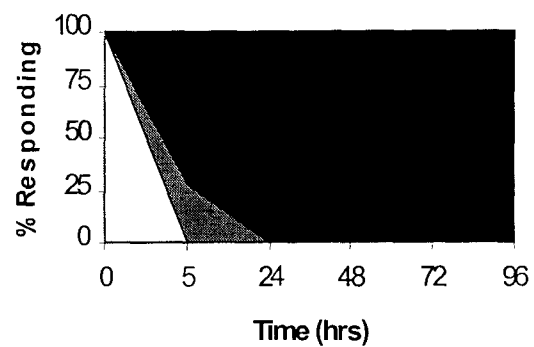
J) 30 mg/L
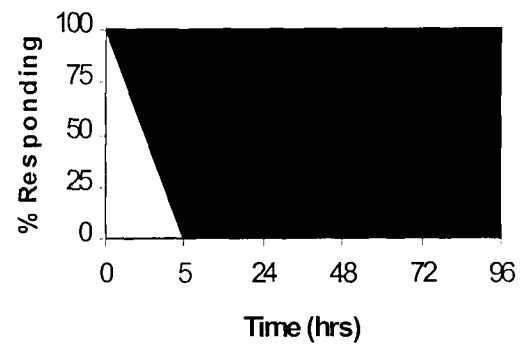
K) 35 mg/L
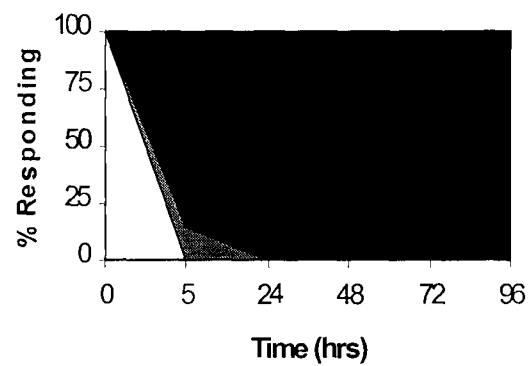
Chapter 3

Effect of Varying Pesticide Exposure Duration and Concentration on the Toxicity of Carbaryl to Two Field-Collected Stream Invertebrates, *Calineuria californica* (Plecoptera: Perlidae) and *Cinygma* sp. (Ephemeroptera: Heptageniidae)

Jennifer L. Peterson, Paul C. Jepson and Jeffrey J. Jenkins

ABSTRACT

The effect of exposure duration on the toxicity of a forest insecticide carbaryl, was assessed under environmentally realistic exposure regimes against two stream invertebrates indigenous to the Pacific Northwest, *Calineuria californica* (Plecoptera: Perlidae) and *Cinygma* sp. (Ephemeroptera: Heptageniidae). Laboratory bioassays were conducted to evaluate the relationship between pulsed exposures of 15, 30 and 60 min and toxicity, for a range of chemical concentrations (10.2 µg/L to 1730 µg/L). *Cinygma* sp. LC_{50} values were calculated as 848 µg/L (15 min), 220 µg/L (30 min) and 165 µg/L (60 min). *C. californica* consistently had lower mortality at a given concentration compared to *Cinygma* sp. Fifteen and 30-min exposures did not elicit 50% mortality with *C. californica* and it had a 60 min LC_{50} of 1139 µg/L.

Time to 50% mortality over 96 h after a 15, 30 or 60-min exposure, with the rest of the test period in freshwater (PLT_{50}), was a function of exposure duration and concentration. Analysis of symptomology throughout the test period for *C. californica* gave evidence of recovery from the knockdown and moribund states, but not for *Cinygma* sp. The pulse duration resulting in 50% mortality was calculated as 43 min for *Cinygma* sp. exposed at 204 µg/L, and 16 min at 408 µg/L. A three dimensional probit plane model [$Y = -10.86 + 4.83 \, (\ln C) + 3.0 \, (\ln T)$], where Y is probit mortality, C is concentration in µg/L and T is time in hours, was used to explain the interaction between concentration (µg/L), and duration of exposure (hrs) for *Cinygma* sp.

INTRODUCTION

Pesticide spray drift or runoff from agriculture or forestry may result in pulsed inputs to waterways (Richards & Baker, 1993). Stream organisms may be

exposed to pulses of pesticides that cause toxic effects despite the fact that maximum concentrations are present for only a short time before attenuation (Crossland et al., 1982). Gradual dissipation of the pesticide pulse occurs as a result of stream flow, hydrological dilution, and habitat and streambed characteristics that determine the degree of partitioning from water to air or sediments (Bath et al., 1970; Wanner et al., 1989). As the chemical moves downstream, organisms may be exposed to progressively lower pesticide concentrations but for longer periods of time (Richards & Baker, 1993; Bath et al., 1970). Pulses of widely used pesticides may however, combine as stream channels merge, thus extending the duration of exposure of organisms in higher order streams, downstream from the spray application.

Exposure assessment is a component of ecological risk assessment in which the contact between the pollutant and organisms in the environment is described and quantified (Suter, 1993). Regulatory testing procedures that are designed to estimate the risks associated with exposure to pesticides in stream habitats should take into account the temporal dynamics of exposure that are unique to stream systems. The majority of standardized laboratory tests for aquatic macroinvertebrates, developed for regulatory purposes, use either a constant chemical concentration for a preset exposure duration, or allow the pesticide to dissipate within a closed system over the duration of the bioassay (i.e. 24, 48 or 96 h) (ASTM, 1993; Webber, 1993). Although these tests form the basis of tier one testing, in which the primary goal is to determine if effects are possible, additional testing may be needed to evaluate whether effects could occur under more realistic conditions of exposure.

Tests with more realistic exposure regimes provide a more accurate exposure : response model, where the pattern of exposure (concentration versus time profile) is as similar as possible to that encountered in the field (Clark et al., 1987; Poirier & Surgeoner, 1988). Pulse exposure tests have been advocated for use in risk assessment, in order to more accurately evaluate effects that may occur under natural exposure conditions, while retaining the advantages of simplicity and

repeatability associated with single species laboratory tests (Abel, 1980; Pascoe & Shazili, 1986; Holdway & Dixon, 1986; Parsons & Surgeoner, 1991; Brent & Herricks, 1998; Naddy et al., 2000).

The purpose of this study was to determine the relationship between exposure time, concentration and toxicity using carbaryl, a carbamate insecticide, and two aquatic insects native to the Pacific Northwest, *Calineuria californica* (Banks, 1905) (Plecoptera: Perlidae) and *Cinygma* sp. (Eaton, 1885) (Ephemeroptera: Heptageniidae). These macroinvertebrates were selected as representatives of the Oregon stream fauna (Chapter 2; Peterson et al., *in press* a). Analysis of community sensitivity statistics based on 96 h LC_1 values for six indigenous macroinvertebrate species including these two (Chapter 2; Peterson et al., *in press* a), revealed that the 95% protection level (HC_5 - hazardous concentration to 5 % of the theoretical community based on the lower 95% confidence limit (HC_5 / 95)) (Van Straalen & Denneman, 1989), fell within the range of carbaryl concentrations that are detected in Oregon streams (Anderson et al., 1996; Anderson et al., 1997). This study aimed to evaluate toxic effects under more realistic exposure regimes to provide insight into the level of uncertainty that is associated with risk assessments based upon 96 h, continuous exposure tests. The objective of this research was to vary both pesticide exposure time and concentration in order to investigate the toxicity of brief exposure events more characteristic of pesticide exposure in forest stream systems. To enable the data to be comparable with previous continuous exposure tests (Chapter 2; Peterson et al., *in press* a) the experiments used the same flask bioassay system, running under identical conditions, with the same basic regime of observations and a 96-h endpoint.

MATERIALS AND METHODS

TEST ORGANISMS

The test species were field-collected stonefly and mayfly nymphs, *C. californica* and *Cinygma* sp. These species were selected because they are representative of Oregon stream communities during the application season for pesticides in forestry. They are also highly abundant, facilitating their use in toxicity tests (Chapter 2; Peterson et al., *in press* a). The organisms were collected in the autumn of 1998 from two different stream sites in western Oregon, USA. *Cinygma* sp. was collected from Gleason Creek, a first order headwater stream, and *C. californica* was collected from the Alsea River. Every effort was undertaken to ensure the correct species was used, however, confirmation of identification was made for all organisms at the end of the test period.

Organisms were transported in chilled and aerated water to the laboratory, where they were transferred to holding tanks. The tank system provided the chilled (10°C), oxygenated ground water required to maintain stream insects in the laboratory (Chapter 2). Organisms acclimated for at least 24 h prior to testing in order to eliminate individuals injured during collection and transport. No food was provided over the 24 h prior to testing.

CHEMICALS USED

Formulated carbaryl (Clean Crop®, Platte Chemical Company, Fremont, NE, USA, EC, 43% AI, w/v) was obtained for this study. Stock solutions were prepared and stored according to a previously described methodology (Chapter 2; Peterson et al., *in press* a). Stock solutions were subsequently diluted to the appropriate test concentrations (Table 3.1). Carbaryl has been found to be

moderately toxic to aquatic organisms in acute tests (Kamrin, 1997), and has predictable dose-dependent symptomology and toxic effects, including hyperactivity, incoordination, convulsions, paralysis and death (Kuhr & Dorough, 1976). It is also detected in stream waters in Oregon, and this investigation provided further quantitative analysis of the risks that it might pose to aquatic macroinvertebrates.

Table 3.1: Treatment concentrations and durations for *Calineuria californica* and *Cinygma* sp., including tolerance values that set the dose range.

Test Organism	Body Size (mm)	96 h LC$_{50}$ μg/L (+/- SD)[a]	Test concentrations (μg/L)	Exposure Times (min)
Plecoptera: Perlidae *Calineuria californica* (Banks, 1905)	8.4	17.3 (14.06-20.2)	17.3, 173, and 1730	15, 30, 60
Ephemeroptera: Heptageniidae *Cinygma* sp (Eaton, 1885)	8.8	11.1 (7.7-13.9)	10.2, 102, 204, 408, and 1020	15, 30 60

[a] LC$_{50}$ value calculated in Chapter 2; Peterson et al., *in press* a.

TEST METHODOLOGY

The water in laboratory holding tanks and test systems (stock and test solutions) was obtained from a groundwater source located at the Environmental Protection Agency's Western Research Station (WRS) in Corvallis, OR. Water hardness, pH, and temperature were measured before and after testing according to ASTM standards (ASTM, 1993). Hardness of the test water ranged from 30 to 40 mg/L, and pH from 7.37 to 7.87.

A flask bioassay system (Chapter 2; Peterson et al., in press a) was used to expose organisms for a range of fixed times to carbaryl. The system consisted of a series of 250 ml Erlenmeyer flasks, chilled to 10°C ± 0.5°C within a water bath. Water within each flask was oxygenated using airstones (inflow), and the air outflow was routed through an activated charcoal filter to absorb pesticide vapor in the exhaust gases. Loss of carbaryl was unlikely to have been significant in the low water temperature and pH of the test system. This conclusion is supported by the Henry's Law constant (2.65×10^{-7}) (Environmental Fate One Line Summaries, 1992) which falls in the mid-range for pesticides, and particularly by the short durations used in this study.

The test concentrations were the statistically derived LC_{50}, 10 times the LC_{50}, and 100 times the LC_{50} (Table 3.1) for each organism obtained from a 96-h continuous exposure study (Chapter 2; Peterson et al., *in press* a). For *Cinygma* sp., two additional intermediate concentrations (204 μg/L and 408 μg/L) were tested because organism availability was not limiting. Availability was limited for *C. californica* and numbers of test concentrations were therefore smaller. The test organisms were exposed for 15, 30 and 60 min at each concentration. Test concentrations were not replicated.

At the start of testing, organisms were removed from the holding tanks and distributed randomly into mesh exposure cages. These were designed to transfer stream organisms from exposure flasks containing insecticide to observation flasks containing fresh water without damaging or stressing the organisms excessively (figure 3.1). Up to 10 organisms were placed in each cage, depending upon organism availability. The cages were then lowered into exposure flasks, where they remained for the appropriate time period (15, 30 or 60 min). At the end of the exposure period, the cages were removed, rinsed three times in fresh water and placed in flasks containing fresh water for the remainder of the 96-h test period. Control organisms for each test period were subjected to the same transfer process in order to evaluate handling effects. Assessments of knockdown, the moribund state and mortality were made throughout the test observation period. Knockdown

was defined as the inability of the insect to hold on to and maintain position within the test cages. The moribund state was characterized by a lack of significant movement with the exception of characteristic twitching of the legs and mouth-parts. Mortality was determined by absence of movement of the body, mouth-parts or gills after stimulation. At 96 h, the organisms were removed from the cages, evaluated and preserved in 80% ethanol for confirmation of identification.
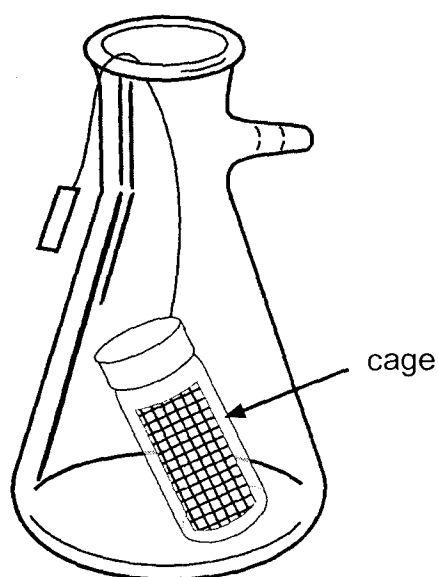
Figure 3.1: Exposure cage used to expose aquatic organisms to brief pesticide exposures. Mesh cages allowed a transfer from a flask containing pesticide to one containing freshwater without damaging the organisms.

STATISTICAL TREATMENT

Five analytical methods were used to explore the data. Method 1 (calculation of lethal estimates) enabled quantitative comparisons between the results of continuous exposure bioassays and the pulsed exposure assays in the present research. Lethal concentration estimates to 1% (LC_1) and 50% (LC_{50}) of

the test population for the two test organisms were determined by probit analysis (Finney, 1971) using SPSS software (SPSS, 1998). Method 2 (analysis of symptomology) enabled trends in recovery as a function of exposure duration to be calculated. Method 3 (analysis of lethal times in each dose/duration treatment: PLT_{50}) determined the time for lethal effects to take place in each treatment. Method 4 (duration of pulsed exposure to give 50% mortality at 96 h) enabled estimation of the time it would take for lethal doses to be accumulated. Finally, method 5 (probit plane analysis) was used to generate a model that predicted mortality at 96 h for different combinations of dose and exposure. This final analytical step enables tentative extrapolation to field conditions.

RESULTS

LC_{50} ANALYSIS

Lethal concentrations (LC_{50}) for exposure durations of 15, 30 and 60 min at 96 h were determined by probit regression analysis (Finney, 1971) using SPSS software (SPSS, 1998). Percent mortality values at 96 h after 15, 30, or 60 min exposures increased as exposure time increased for both organisms (Table 3.2). The proportion dead for *Cinygma* sp. was consistently greater than *C. californica,* exposed for the same amount of time and at similar concentrations. This was despite the fact that LC_{50} values for 96-h continuous exposure were similar for both species (LC_{50}+/-95% confidence limits: *Cinygma*, 11.1 µg/L (7.7 – 13.9) & *C. californica*, 17.3 µg/L (14.1-20.2)) (Chapter 2; Peterson et al., *in press* a). Control mortality was 0% in all test runs for both organisms.

Table 3.2. Percentage mortality and estimates of lethality ($LC_1{}^a$, $LC_{50}{}^b$) at 96 h for *Calineuria californica* and *Cinygma* sp. exposed to carbaryl for periods of 15, 30, or 60 minutes, and then transferred to clean water.

Concentration (μg/L)	96-h % Mortality and LC_1 and LC_{50} estimates after the exposure period (+/- 95% CL)[c]		
	15 min	30 min	60 min
Calineuria californica			
17.3	30.0	0	0
173	0	14.3	12.5
1730	22.2	30	60.0
LC_1 Estimates (μg/L)	-	-	31.1 (0-152.3)
LC_{50} Estimates (μg/L)	-	-	1139.4 (370.0-15410.0)
Slope	-	-	2.48
Cinygma sp.			
10.2	0	10.0	0
102	0	10.0	10.0
204	0	22.2	77.8
408	33.3	100	100
1020	100	100	100
LC_1 Estimates (μg/L)	14.7[d,*]	13.0[d,*]	61.0 (15.6-91.0)
LC_{50} Estimate (μg/L)	848[d,*]	220[d,*]	165 (124-232)
Slope	2.89	3.8	3.36

[a] Lethal concentration to 1% of the test population.
[b] Lethal concentration to 50% of the test population.
[c] Confidence limits included where calculable.
[d] Insufficient data for the calculation of confidence limits.
Dashes indicate 50% mortality was not reached during test period; LC_{50} and slope values could not be calculated.
χ^2 test for heterogeneity; * indicates significant heterogeneity at p<0.05.

For *Cinygma* sp., the relationship between LC_{50} and the duration of exposure was curvilinear (figure 3.2). *C. californica* LC_{50} values could not be calculated for 15 and 30 min exposures because 50% mortality was not reached in any test concentration (figure 3.3). We assume the 15 min 17.3 μg/L mortality of

30% was an anomaly because no mortality occurred at the 30 and 60 min exposure times at the same concentration. Sixty minute LC_{50} values were however significantly different between species (p<0.05), with *Cinygma* sp. being more sensitive than *C. californica* (Table 3.2).
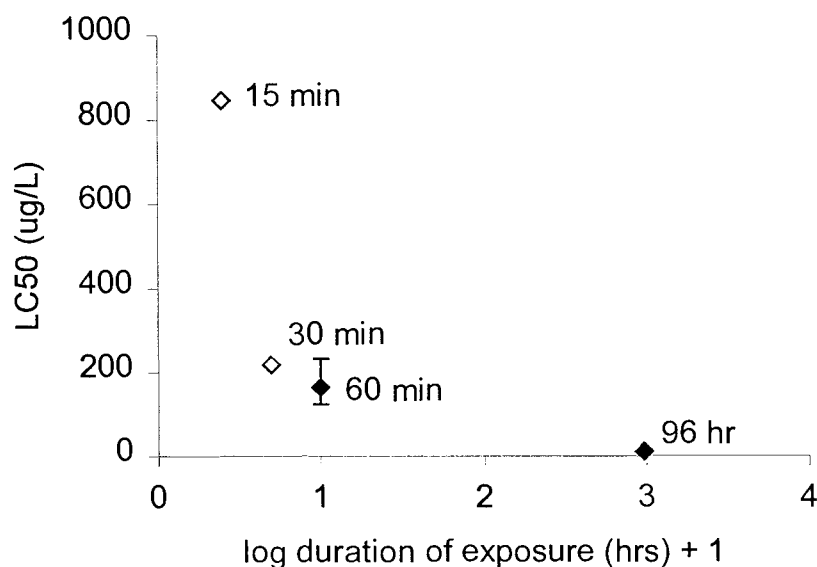
Figure 3.2. *Cinygma* sp. lethal concentrations to 50% of the test population (LC_{50}) for a 15, 30 and 60 min pulsed exposure to carbaryl. Open symbols indicate where confidence intervals could not be calculated. 96-h value from Chapter 2; Peterson et al., *in press* a.
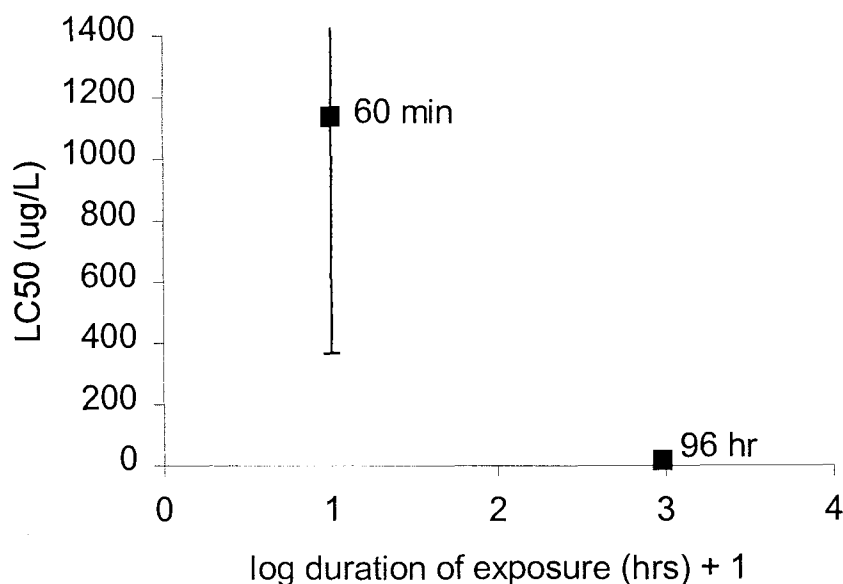
Figure 3.3. *Calineuria californica* lethal concentrations to 50% of the test population (LC$_{50}$) for a 15, 30 and 60 min pulsed exposure to carbaryl. 96-h value from Chapter 2; Peterson et al., *in press* a.

ANALYSIS OF RECOVERY

Analysis of the symptomology of carbaryl intoxication showed that significant recovery did not occur after the appearance of effects for *Cinygma* sp. The majority of organisms exhibiting knockdown or the moribund state at the first assessment after exposure eventually died during the recovery period in fresh water (appendices 3.1-3.3). Symptomology analysis for *C. californica* however, provided evidence for recovery 5 h after 15, 30 or 60 min pulsed exposures to high concentrations (for example: for 1730 µg/L, figure 3.4 A-C and appendices 3.4–3.7.

Figure 3.4. *Calineuria californica* symptomology over the 96-h test period after a 15 (A), 30 (B) or 60 (C) min exposure to a carbaryl concentration of 1730 µg/L.

A)
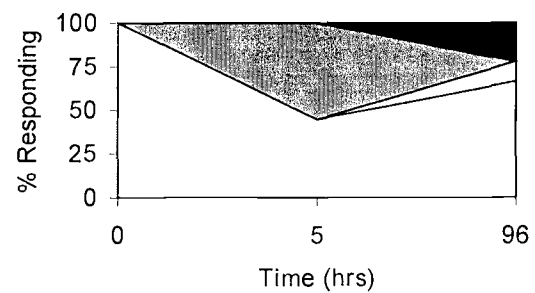
B)
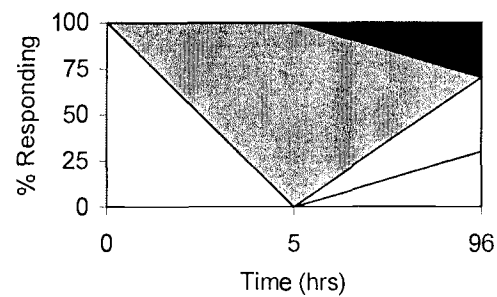
C)
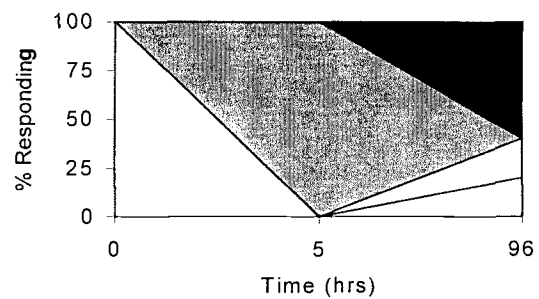
LETHAL TIMES FOR PULSED EXPOSURES: PLT_{50}

Probit analysis was used to determine LT_{50} values (lethal time to 50% mortality over the 96 h test period) for all combinations of dose and duration of exposure that gave graded lethal responses spanning 50% mortality over the 96-h assessment period for each assay. The data for mortality at each assessment time over 96 h were analyzed by probit regression. This was termed the "PLT_{50}" to indicate pulsed exposure followed by a recovery period in uncontaminated water. For *Cinygma* sp., up to seven mortality values could be included in the analysis (i.e. data for assessments at 0.5, 1.0, 5, 24, 48, 72 and 96 h) (Table 3.3). For *C. californica*, there were only two assessment times (0.5 h and 96 h), and PLT_{50} values were estimated graphically.

Table 3.3. Time (hrs) to 50% mortality for *Cinygma* sp. and *Calineuria californica* after an exposure duration of 15, 30 or 60 min with the remainder of the 96-h test period in clean water (PLT_{50}).

Concentration	PLT_{50} (hrs) after the given exposure time (minutes) (+/- 95% CL)[a]		
(µg/L)	15	30	60
Calineuria californica			
17.3	-	-	-
173	-	-	-
1730	-	-	81.0[b]
Cinygma sp.			
10.2	-	-	-
102	-	-	-
204	-	-	18.0 (6.1-30.2)
408	-	20.4 (9.0-29.7)	20 (8.5-28.6)
1020	0.56[c]	1.62 (0.003-4.2)	1.62 (0.003-4.2)

[a] Confidence limits included where calculable.
[b] Value estimated graphically.
[c] Insufficient data for the calculation of confidence limits.
Dashes indicate values not calculated; organisms did not reach 50% mortality during test period.
χ^2 test for heterogeneity; * indicates significant heterogeneity at $p<0.05$.

Lethal time (PLT_{50}) values decreased with increasing concentration for both species tested. Values for *Cinygma* sp. were lower than for *C. californica* exposed at similar concentrations. For example, the 60 min, 1020 µg/L PLT_{50} for *Cinygma* was 1.62 h (97 min), while the 60 min, 1730 µg/L PLT_{50} for *C. californica* was 81 h (figures 3.5 C and 3.6 C, respectively). PLT_{50} values for *Cinygma* sp. at 1020 µg/L and both 30 and 60 min pulses, were significantly lower than the 408 µg/L PLT_{50} values at 30 and 60 min and the 204 µg/L PLT_{50} at 60 min.

Graphical analysis was used to compare trends in PLT_{50} values from the present study, and LT_{50} values from 96-h continuous exposure bioassays reported in Chapter 2; Peterson et al., *in press* a (figures 3.5 A-C *Cinygma* sp. and figures 3.6 A-C *C. californica*). For both species, mortality values did not exceed 50% across a wide range of concentrations that yielded LT_{50} values in the 96-h continuous exposure investigation. For *Cinygma* sp. 15 minute pulses, exposure was non-lethal at 10.2, 102 and 204 µg/L (figure 3.5 A), and effects were limited at 408 µg/L. Lethal time (PLT_{50}) values at 1020 µg/L for 30 and 60 min exposures fell below the LT_{50} value from continuous exposure; however, this difference was not statistically significant based upon the regression model reported in Chapter 2; Peterson et al., *in press* a (p>0.05) (i.e. the LT_{50} at 1020 µg/L, predicted from the regression model for LT_{50} (h) vs log concentration (µg/L) in Chapter 2 (Peterson et al., *in press* a) was 0.6 h (30 min) and the PLT_{50} at 1020 µg/L (+/- 95% confidence limits) for both 30 and 60 min pulses was 1.62 h (0.003-4.2)).

Over 30 and 60 min pulsed exposures, the trends were similar, with a number of doses failing to elicit 50% mortality in pulsed tests within the range that would be lethal and generate an LT_{50} value over continuous exposure. Intermediate concentrations gave PLT_{50} values that exceeded the 96-h continuous values, and PLT_{50} values at higher concentrations were closer to the continuous exposure LT_{50} values.

For *C. californica*, all combinations of dose (17.3, 173 and 1730 µg/L) and exposure time (15, 30 and 60 min) were non-lethal or did not reach 50% mortality, with the exception of the 60 min 1730 µg/L (figure 3.6 C). The PLT_{50} value in this

treatment was greater than the LT_{50} value from continuous testing. Exposure over shorter periods, even at these high concentrations, failed to elicit significantly toxic effects, suggesting again, that rates of uptake may be low for this species over short, pulsed exposures.

Figure 3.5 A – C. Lethal time to 50% mortality after a pulsed exposure with the remainder of the test period in freshwater (PLT_{50}) for *Cinygma* sp. after a 15 (A) , 30 (B) or 60 min (C) exposure to carbaryl. As a basis for comparison with the trends observed in continuous testing, the solid line denotes lethal time to 50% mortality (LT_{50}) values calculated in 96-h tests reported in [16]. Solid diamonds represent PLT_{50} values obtained in pulsed exposure tests, solid squares indicate tests where 50% mortality was not reached, and open squares indicate tests where 0% mortality resulted during the 96-h test period. Symbols that occur within the range of the curve, but that fall below it, indicate effects that are lower than would have been found under continuous exposure at the same concentration.

A) 15 min exposure
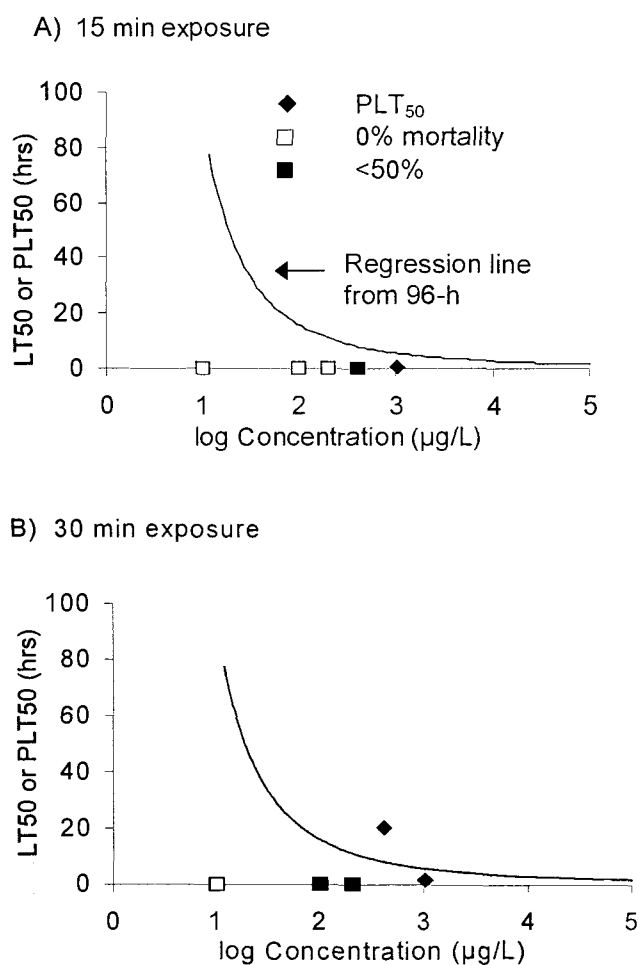
B) 30 min exposure

(Continued)

Figure 3.5 (Continued)

C) 60 min exposure
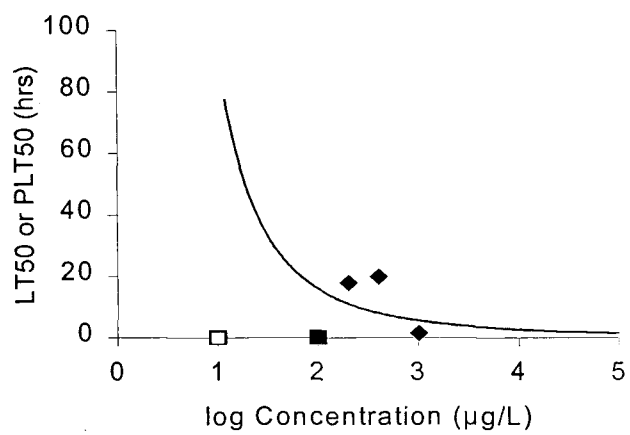
Figure 3.6 A – C. Lethal time to 50% mortality after a pulsed exposure with the remainder of the test period in freshwater (PLT_{50}) for Calineuria californica after a 15 (A), 30 (B) or 60 min (C) exposure to carbaryl. Symbols are the same as figure 3.5.

A) 15 min exposure
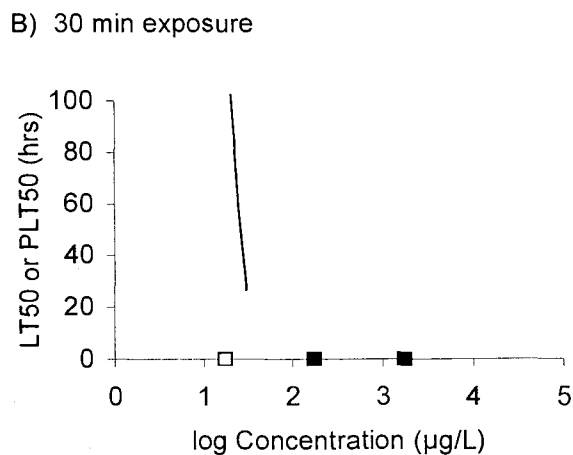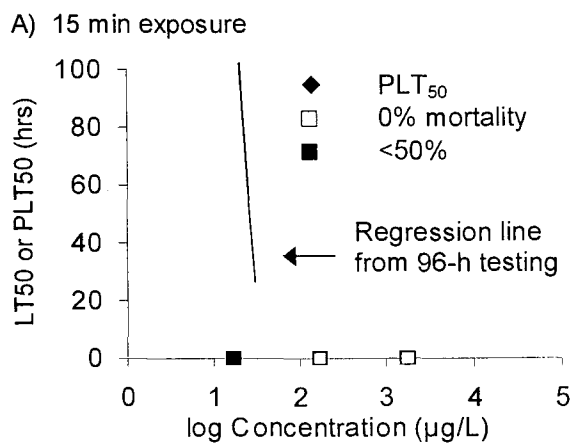
B) 30 min exposure

(Continued)

Figure 3.6 (Continued)

C) 60 min exposure
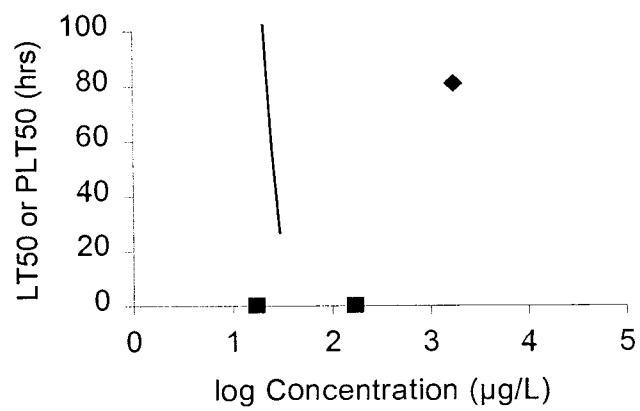
DURATION OF EXPOSURE TO 50% MORTALITY

Lethal response data for organisms exposed to specific concentrations for different periods allowed for calculation of the exposure time needed to elicit 50% mortality at a given concentration. The 96-h mortality data, and pulse durations at each concentration were analyzed by probit analysis. This analysis was only possible for *Cinygma* sp. exposed at concentrations of 204 µg/L and 408 µg/L, where responses spanned mortality ranges that could be analyzed over different exposure times. Concentrations higher than 408 µg/L elicited 100% mortality at all exposure times, while some concentrations lower than 204 µg/L failed to elicit 50% mortality. This is indicative of a steep dose response curve.

In order to elicit a 50% response over 96 h at 204 µg/L, *Cinygma* sp. would have to be exposed to carbaryl for 43 min. Increasing the concentration to 408 µg/L results in this exposure time falling to 16 min. The exposure duration required to elicit 50% mortality over 96 h at concentrations tested above 408 µg/L was estimated to be less than 15 min. These estimates were consistent with measurements derived from sequential assessments during continuous 96-h exposure at these concentrations (Chapter 2).

It is logical that the values for time to 50% effect are shorter in duration than the calculated PLT_{50} values. The times estimated by method 4 provide an estimate of how long it takes for a sufficient dose of pesticide to be accumulated for 50% mortality to be recorded at 96 h. The PLT_{50} values report the time for lethal effects to evolve within the 96-h assessment period, based upon direct observations during the period in fresh water. The time to appearance of symptoms tends to be considerably longer than the time it takes to accumulate a lethal dose of pesticide.

PROBIT PLANE ANALYSIS

A three dimensional probit plane model (Finney, 1971; Hewlett & Plackett, 1979) was used to explore the interaction between pesticide concentration, duration of exposure and mortality. Each point on this plane represents a particular combination of mortality, concentration and time. The standard equation for the probit plane model is:

$$P = a + b \ln (C) + d \ln (T)$$

Where P is probit mortality, C is pesticide concentration in $\mu g/L$ and T is duration of exposure, in hours.

A model was determined for the mayfly *Cinygma* sp. Concentrations used in the analysis included 10.2, 102, 204, 408 and 1020 $\mu g/L$ with exposure durations of 15, 30 and 60 min. Estimates for mortality over 96 h of continuous exposure were made from the probit analysis reported in Chapter 2; Peterson et al., *in press* a).

The resulting probit plane model for *Cinygma* sp. exposed to carbaryl is:

$Y = -10.86 + 4.83 \ln (C) + 3.0 \ln (T)$ (Chi-squared test for homogeneity not significant, $p>0.05$) (Table 3.4).

Table 3.4: Model parameter estimates for the probit plane model developed to explain the interaction of concentration ($\mu g/L$) and duration of exposure (hrs) on percent mortality.

Parameter	Regression Coefficient	Standard Error	Pearson Chi Square (*df*)
Concentration ($\mu g/L$)	4.83	0.942	6.78 (17)
Duration of Exposure (hrs)	3.0	0.605	

This model permits mortality to be predicted from exposure duration (hours) and carbaryl concentration (μg/L) (figure 3.7). This approach could help to quantify the level of uncertainty associated with predicting impacts in the field, where both parameters may vary. The zones of high risk (combinations of dose and time that would elicit >99% mortality), intermediate risk (combinations that elicit 1-99% mortality) and low risk, where <1% mortality is predicted, can for example be compared with known data for pulse duration and environmental concentration. In the case of Oregon, surface water concentration estimates for carbaryl have not been found to exceed 2 μg/L (Anderson et al., 1996; Anderson et al., 1997), and low risk is predicted over a wide range of exposure durations for *Cinygma* sp.
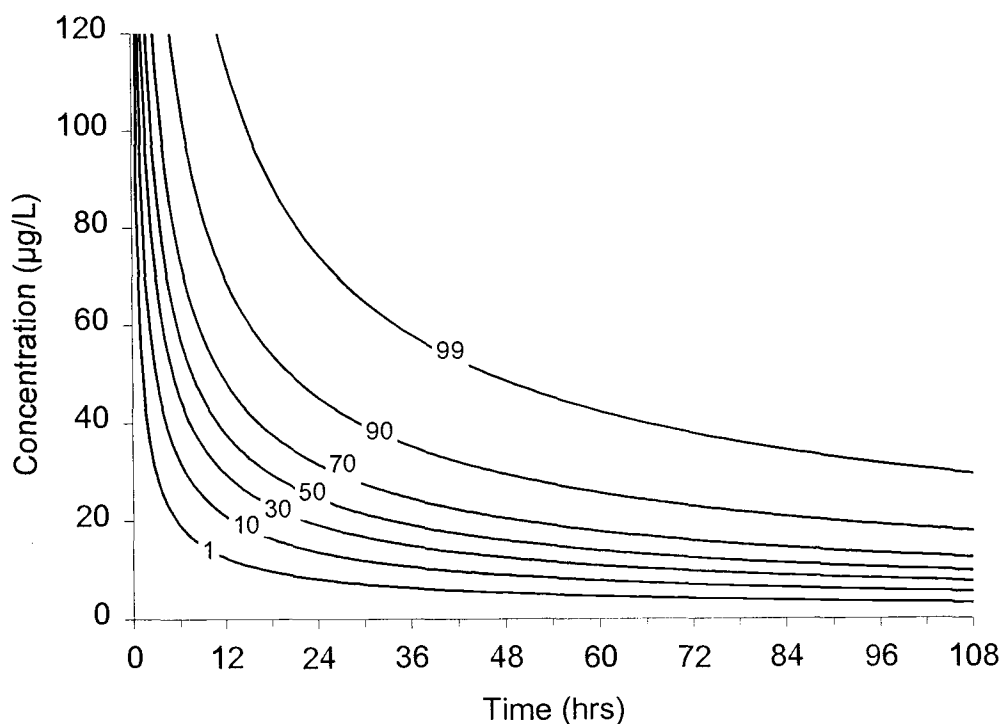
Figure 3.7. Interaction of concentration (μg/L) and time of exposure (hrs) on % mortality as predicted from the model Y = -10.86 + 4.83 ln (C) + 3.0 ln (T). Lines represent % mortality at various combinations of concentration and time.

Model validation was conducted by plotting values predicted by the model against those observed in 96-h continuous exposure, which were not included in the probit plane analysis (figure 3.8). The model overestimates mortality at doses that elicit limited effects (below 60% lethal impacts) and under-estimates mortality at high concentrations. These deviations are likely to be a result of the differing modes of exposure in the data sets used for model generation (pulsed exposures, of 60 min or less in duration), and the data used for validation (obtained during 96-h exposure assays).
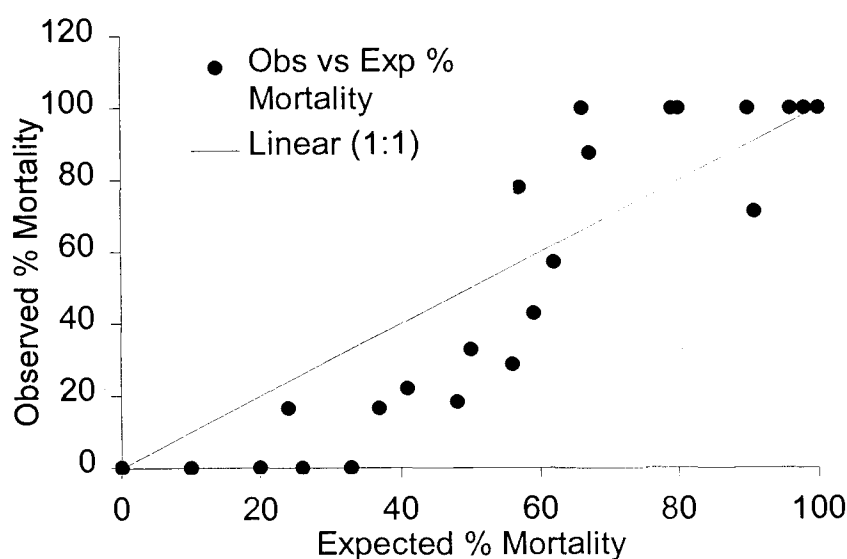
Figure 3.8: Observed % mortality for *Cinygma* sp. exposed to carbaryl from continuous [16] and pulsed exposure plotted against % mortality expected from the model $Y = -10.86 + 4.83 \ln (C) + 3.0 \ln (T)$.

CONCLUSIONS / DISCUSSION

The use of a post-exposure observation period and a 96-h assessment endpoint allowed the toxicity of pulsed exposures to be compared with effects seen in 96-h continuous exposure bioassays. Mortality decreased with decreasing

exposure time for both organisms tested. This is consistent with other studies evaluating effects from brief exposures to carbaryl (Parsons & Surgeoner, 1991; Kallander et al., 1997). The use of test concentrations that were considerably higher than those that commonly occur in the environment enabled effects to be properly resolved, and provided a basis for estimating impacts at the predicted environmental concentration.

Differences in the responses to pulsed exposures were observed in the two test species. *C. californica* was approximately 1000-fold less sensitive to carbaryl than *Cinygma* sp. during short, pulsed exposures, although the relative susceptibilities of these species after 96 h of continuous exposure were similar (Chapter 2; Peterson et al., *in press* a). This may reveal significant differences in rates of uptake and time to equilibration between the two species. Differences of this form, if they occurred widely amongst stream macroinvertebrates, could radically alter rankings of relative susceptibility based on continuous exposure, and alter the findings of risk assessment procedures that were based upon this standard methodology. Morphological differences, including cuticular thickness or gill surface area, could account for the differences detected in the present study. Increased rates of metabolism or excretion by *C. californica* could also explain these differences, although the similarity in 96-h LT_{50} values suggests that the difference between the two species may lie in rates of uptake.

Carbaryl toxicity results from inhibition of acetylcholinesterase in the nervous system. Carbamate insecticides, unlike the organophosphates which share a similar mode of action, have poor complementarity with the active site, and can be displaced after exposure to the chemical ceases (Reiner, 1971). Reactivation of the carbamylated enzyme occurs through hydrolysis. With the half-life of carbamylated enzymes reported as 30 to 40 min, nearly complete recovery of enzyme activity could occur several hours after removal from chemical exposure (Kuhr & Dorough, 1976). Mosquito larvae have been shown to recover from immobilization following short exposures to carbaryl (0.5 to 4 hours) (Parsons & Surgeoner, 1991). Ability to recover was shown to decrease with increasing

exposure time and after 8 to 24-h exposure there was little or no recovery. Mosquito larvae have been shown to recover from 2-h exposures to carbaryl if six hours in clean water is provided (Kallender et al., 1997). Black fly larvae exposed to carbaryl were found to recover from immobilization following a 5 to 20 min exposure to carbaryl (Travis & Wilton, 1965).

There is evidence from this study that duration of exposure and chemical concentration both determine the degree of toxicity. Significant mortality occurred at high concentrations, even if exposure time was short (15 min). At lower concentrations, recovery was more likely, and rates of mortality decreased. For concentrations lower than the apparent lethal threshold for the exposure durations tested (204 and 408 μg/L), reversal of acetylcholinesterase inhibition, in conjunction with metabolism and excretion may have enabled recovery following exposure. The lowest concentrations tested (10.2 and 17.3 μg/L) elicited very little mortality at any exposure duration, indicating that insufficient amount of toxin penetrated to the site of action to elicit lethal effects.

Sub-lethal effects such as knockdown that may have occurred directly after the exposure period (15, 30 and 60 min) were not assessed. The first observations were normally made about an hour following exposure. Some organisms that may have been initially affected by the chemical may have recovered in this time. Further analysis of short-term effects that may cause drift or a failure to locate favorable habitat conditions is required. Pulsed exposure to pesticides in stream systems has been associated with increases in invertebrate drift (Muirhead-Thompson, 1987). In addition to mortality, sub-lethal effects of pesticide poisoning including hyperactivity and knockdown symptomology may result in an impaired ability to recolonize available habitat. For example, an increase in downstream displacement in the form of drifting and crawling organisms was attributed to increased locomotor activity in *Acroneuria lycorias* (Plecoptera) in response to methoxychlor exposure (Scherer & McNicol, 1986). Drift can occur at lower concentrations than those required to elicit mortality, resulting in a loss of

organisms from the system and possible shifts in community structure (Cuffney et al., 1984; Scherer & McNicol, 1986; Wallace et al., 1989).

Lethal time (PLT_{50}) analysis (method 3) determined the amount of time following pulsed exposure for lethal effects to appear. Short exposures can yield effects at 96 h, highlighting the importance of having an extended post-exposure assessment period where effects may accumulate over days. The need for post-exposure observation periods has been reported previously (Pascoe & Shazili, 1986; Brent & Herricks, 1998). The PLT_{50} analyses are distinct from those obtained in the analysis of duration of exposure required to elicit 50% mortality (method 4). Method 4 provides an estimate of exposure time required for the organism to acquire a chemical dose that would to elicit a 50% response at 96 h. Symptoms and lethal effects may occur however, well after the uptake has taken place. The probit plane model analysis (method 5) also used 96 h toxicity data, and therefore incorporated symptoms that were expressed over the full 96-h test period.

The duration of a pulsed exposure event in stream systems will be a function of the physical characteristics of the watershed, the pattern of the pesticide application and the physical and hydrological characteristics of the stream. Peak pesticide concentrations have been found to be higher, but present for shorter durations in small streams compared to larger ones (Richards & Baker, 1993). However, duration : concentration relationships need to be established for stream systems of the Pacific Northwest. The LC_{50} values calculated in this research show that mortality is unlikely to occur as a result of the short, pulsed exposures that may be expected in high order, high gradient streams where the pesticide pulse would be expected to move through the system quickly. Based on the LC_{50} values calculated in 96-hour conventional tests (Chapter 2; Peterson et al., *in press* a) (11.1 for µg/L *Cinygma* sp., and 17.3 for µg/L *C. californica*) compared with the LC_{50} values for more realistic exposure regimes of 15 (848 µg/L for *Cinygma* sp.), 30 (220 µg/L for *Cinygma* sp.), and 60 min (165 µg/L and 1139 µg/L for *Cinygma* sp. and *C. californica*, respectively), conventional tests may greatly overestimate the acute toxicity of carbaryl to stream insects exposed to short pesticide pulses.

Invertebrates in lower gradient valley streams that integrate chemical inputs from throughout agricultural watersheds may be exposed for longer periods and LC_{50} values from continuous testing may more closely approximate the risks that these organisms face. These data demonstrate the need to characterize the nature of the chemical pulse in pesticide risk assessment for aquatic macroinvertebrates.

Previous research (Chapter 2; Peterson et al., *in press* a) evaluated uncertainty associated with single species standardized tests by assessing sensitivity across a representative assemblage of native macroinvertebrate species. The resulting statistical model of community sensitivity, which took into account the number of species tested, suggested that a proportion of the macroinvertebrate community could be at risk in Oregon streams contaminated with carbaryl. In the community sensitivity analysis, the main source of uncertainty was that associated with variation in susceptibility across the whole community of macroinvertebrates. The statistical correction used (Aldenberg & Slob, 1993), reduced in direct proportion to the number of test species, and validation of the HC_5 can only be obtained through undertaking a number of further 96-h bioassays and determining the change in HC_5 relative to environmental concentration.

The pulsed exposure analysis for two of the test species explored uncertainty that might derive from variation in the responses of organisms to short pulses, compared with continuous exposure, both of which could occur in the real world. Large differences in the form of the response of the two test species to pesticide pulses were observed, and this provides a strong case for more detailed analysis of pesticide exposure, uptake and symptomology across a wide range of species. The probit plane model provides a statistical tool for the estimation of risk under realistic conditions of exposure. The model must, however, be developed for a number of species before the relative effectiveness of continuous vs pulsed assay regimes in risk assessment can be properly evaluated.

ACKNOWLEDGEMENT

Chapter 3 Appendices

Appendix 3.1. *Cinygma* sp. symptomology over the 96-h test period after a 15 minute exposure to carbaryl, with the rest of the test period in freshwater, to concentrations of 10.2 μg/L (A), 102 μg/L (B), 204 μg/L (C), 408 μg/L (D) and 1020 μg/L (E). Black indicates mortality, dark gray moribund, light gray knockdown and white unaffected.
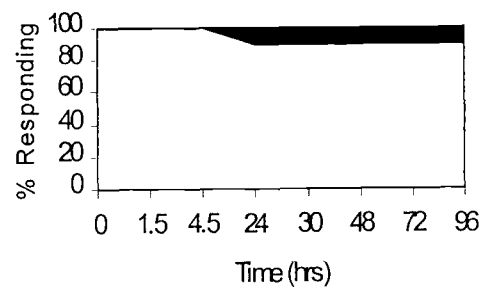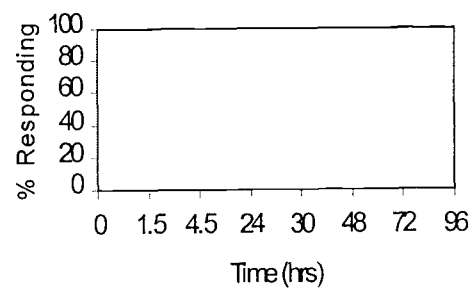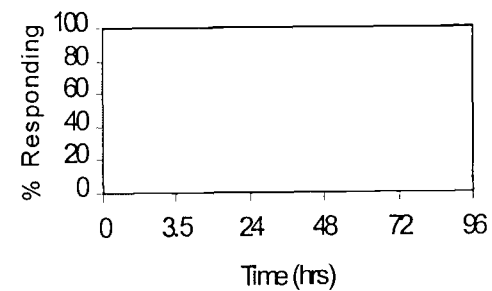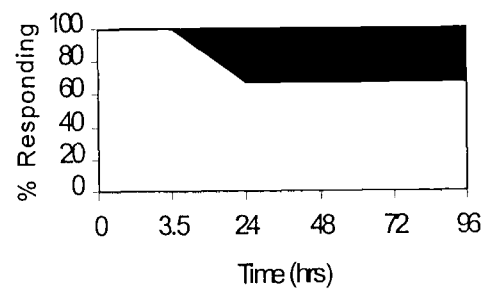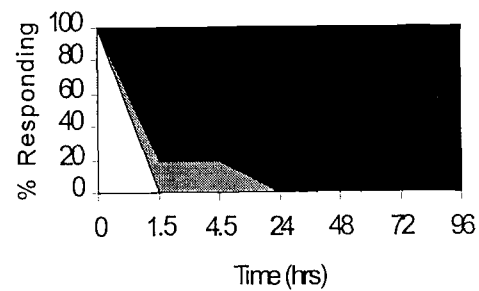
Appendix 3.2. *Cinygma* sp. symptomology over the 96-h test period after a 30 minute exposure to carbaryl, with the rest of the test period in freshwater, to concentrations of 10.2 µg/L (A), 102 µg/L (B), 204 µg/L (C), 408 µg/L (D) and 1020 µg/L (E). Black indicates mortality, dark gray moribund, light gray knockdown and white unaffected.
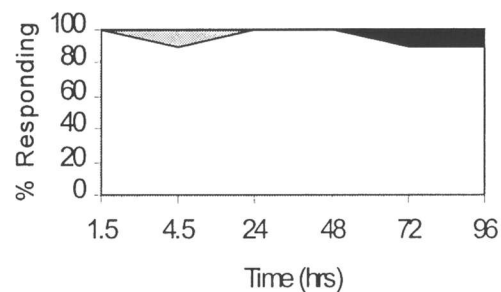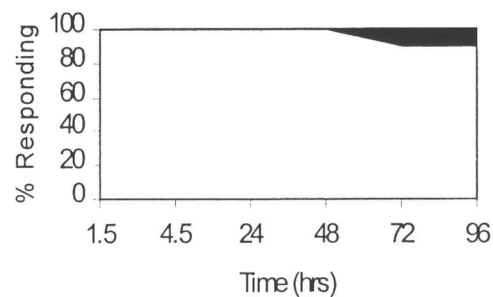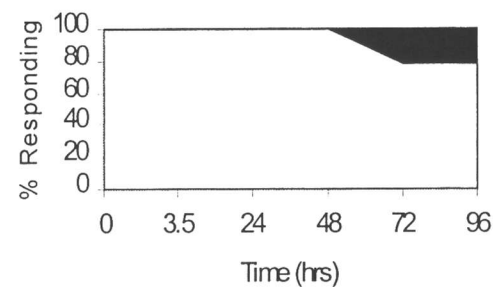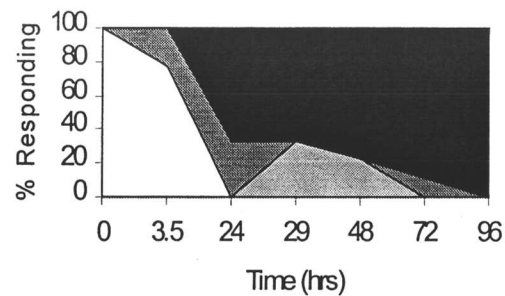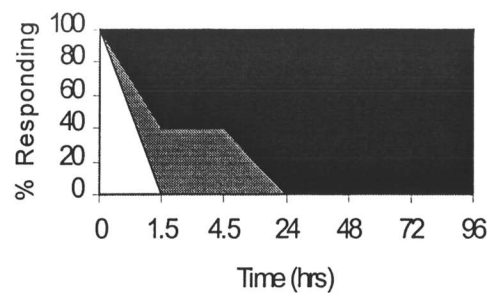
Appendix 3.3. *Cinygma* sp. symptomology after a 60 minute exposure to carbaryl with the rest of the test period in freshwater. over the 96-h test period to a concentration of 10.2 µg/L (A), 102 µg/L (B), 204 µg/L (C), 408 µg/L (D) and 1020 µg/L (E). Black indicates mortality, dark gray moribund, light gray knockdown and white unaffected.

A. 10.2 µg/L

B. 102 µg/L

C. 204 µg/L

D. 408 µg/L
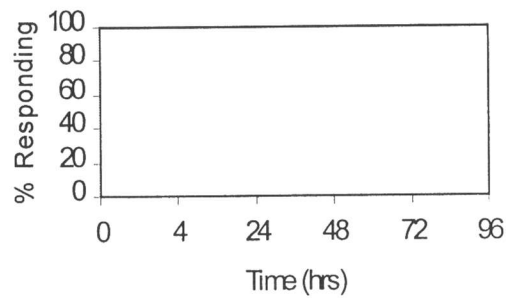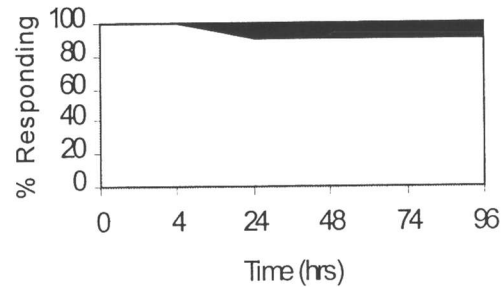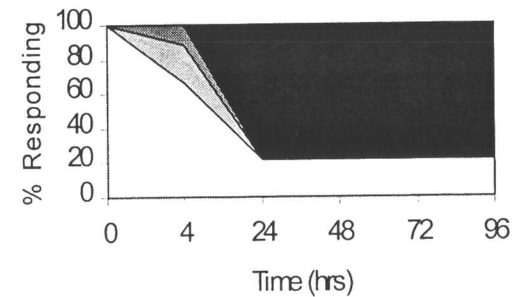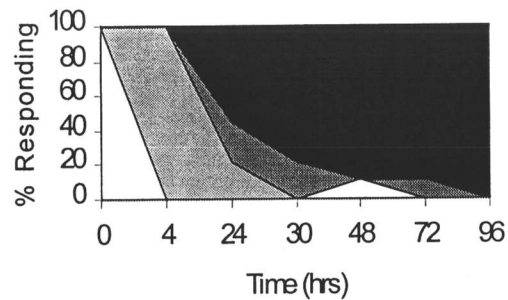
E. 1020 µg/L
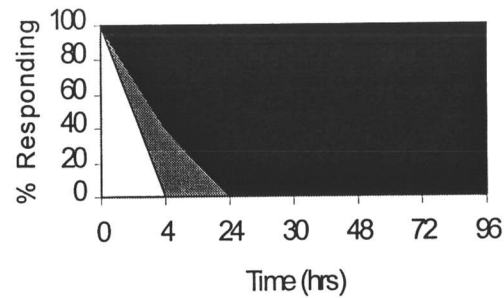
Appendix 3.4. *Calineuria californica.* symptomology over the 96-h test period after a 15 minute exposure to carbaryl, with the rest of the test period in freshwater, at concentrations of 17.3 µg/L (A), 173 µg/L (B) and 1730 µg/L (C). Black indicates mortality, dark gray moribund, light gray knockdown and white unaffected.

A. 17.3 µg/L

B. 173 µg/L
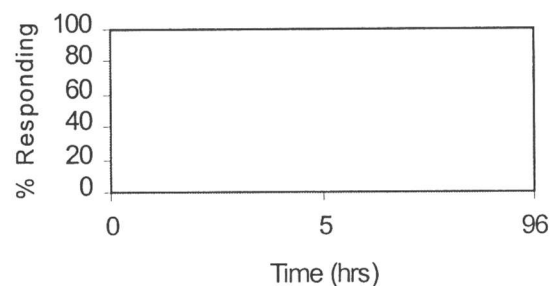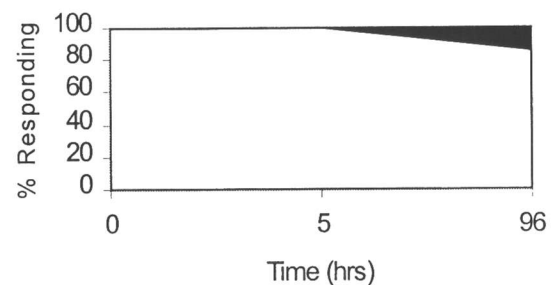
C. 1730 µg/L
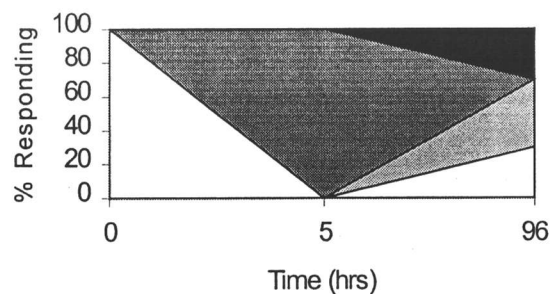
Appendix 3.5. *Calineuria californica.* symptomology over the 96-h test period after a 30 minute exposure to carbaryl, with the rest of the test period in freshwater, at concentrations of 17.3 µg/L (A), 173 µg/L (B) and 1730 µg/L (C). Black indicates mortality, dark gray moribund, light gray knockdown and white unaffected.

A. 17.3 µg/L

B. 173 µg/L
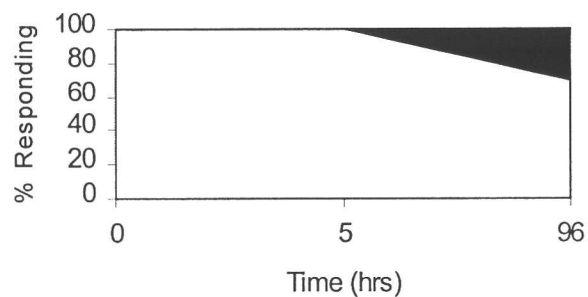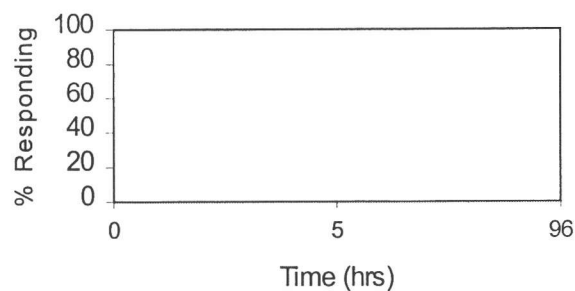
C. 1730 µg/L
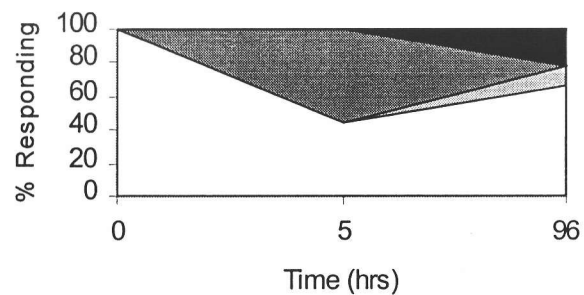
Appendix 3.6. *Calineuria californica.* symptomology over the 96-h test period after a 60 minute exposure to carbaryl, with the rest of the test period in freshwater, at concentrations of 17.3 µg/L (A), 173 µg/L (B) and 1730 µg/L (C). Black indicates mortality, dark gray moribund, light gray knockdown and white unaffected.

A. 17.3 µg/L

B. 173 µg/L
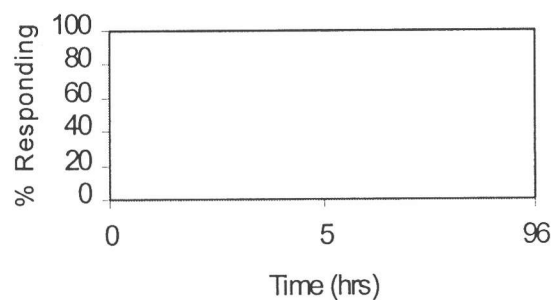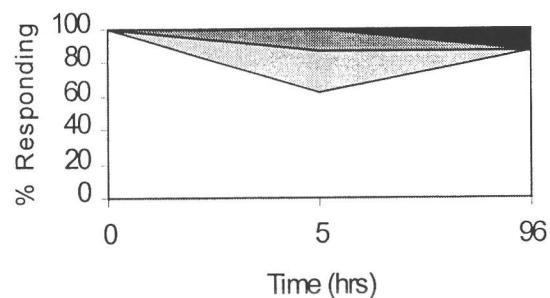
C. 1730 µg/L
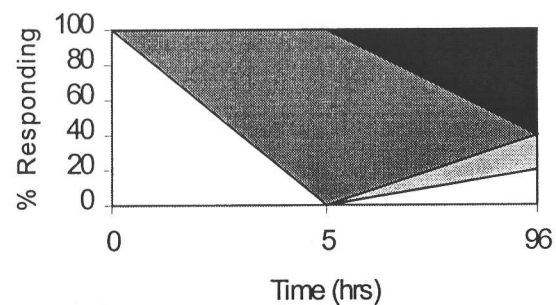
Chapter 4

A Database Analysis of Variation in Invertebrate Characteristics That Determine the Potential for Short-and Long-term Pesticide Effects in the Field

Jennifer L. Peterson, Paul C. Jepson, Phil Heneghan and Jeffrey J. Jenkins

ABSTRACT

Characteristics of stream invertebrates were identified within categories of exposure and uptake (short- term effects) and recovery (long-term effects) that influence the potential for effects in the field. Variation in the range of characteristics that exist in an invertebrate community within parameters of morphology, behavior and life history that may determine the potential for short- and long-term effects were described and ranked to represent possible trends in relative risk. Rankings were assigned using first principles, literature search or expert consensus. Rule-based modeling was used to incorporate weighted parameters of ranked organism characteristics into model formulae designed to calculate individual component relative risk indices for representative genera from a range of aquatic invertebrate families. Variation in component and short-and long-term relative risk indices was evaluated between species, and taxa were identified with different combinations of potential for short- and long-term risk that may alter risk assessments based on susceptibility data alone.

INTRODUCTION

Ecological risk assessment can not rely upon toxicological measurements alone. A wide range of physical and ecological attributes also contribute to organism exposure and the longer term impact of the toxin (Jepson, 1993). Assessment precision could be improved if the range of organism morphology, behavior, habitat association and life history that mediate ecotoxicological impacts could be incorporated into model predictions. The objective of this analysis is to explore the morphological and ecological factors that help determine individual and population level toxic effects. Central to this analysis is the assumption that combinations of these factors operate on two distinct temporal and spatial scales.

Characteristics of the organism that determine initial level of chemical exposure and uptake are important in determining short-term toxicological impacts, and the ecological attributes of the organism are important in determining long-term impacts at the population level. Consideration of both short-term and long-term effects is necessary if we are to accurately assess adverse impacts in the field.

Evaluating variation within components that determine the potential for short-term effects in the field will improve risk assessment. Species could in theory be partitioned into groups subject to differing risks of short-term effects, based on the attributes that determine exposure and uptake. This analysis will provide a basis for evaluating this variation by summarizing the attributes of a given species according to rules developed from first principles or experiments. This approach may lead to a better interpretation of short-term field experimental data, and expand the potential for extrapolating test data for a small number of species to a whole community.

SHORT-TERM EFFECTS

The potential for short-term effects from pesticide exposure is a function of the interaction of: 1) characteristics of the environment where the organism is found (habitat) including hydrology and geology; 2) physical properties of the chemical that determine distribution and persistence; and 3) characteristics of the organism such as physiological and morphology , susceptibility, and behavior (figure 4.1). This analysis will concentrate on the biological attributes of organisms that determine exposure, uptake and long-term effects in the field. The importance of habitat and chemical properties will be discussed, but these factors are not incorporated within the analysis.
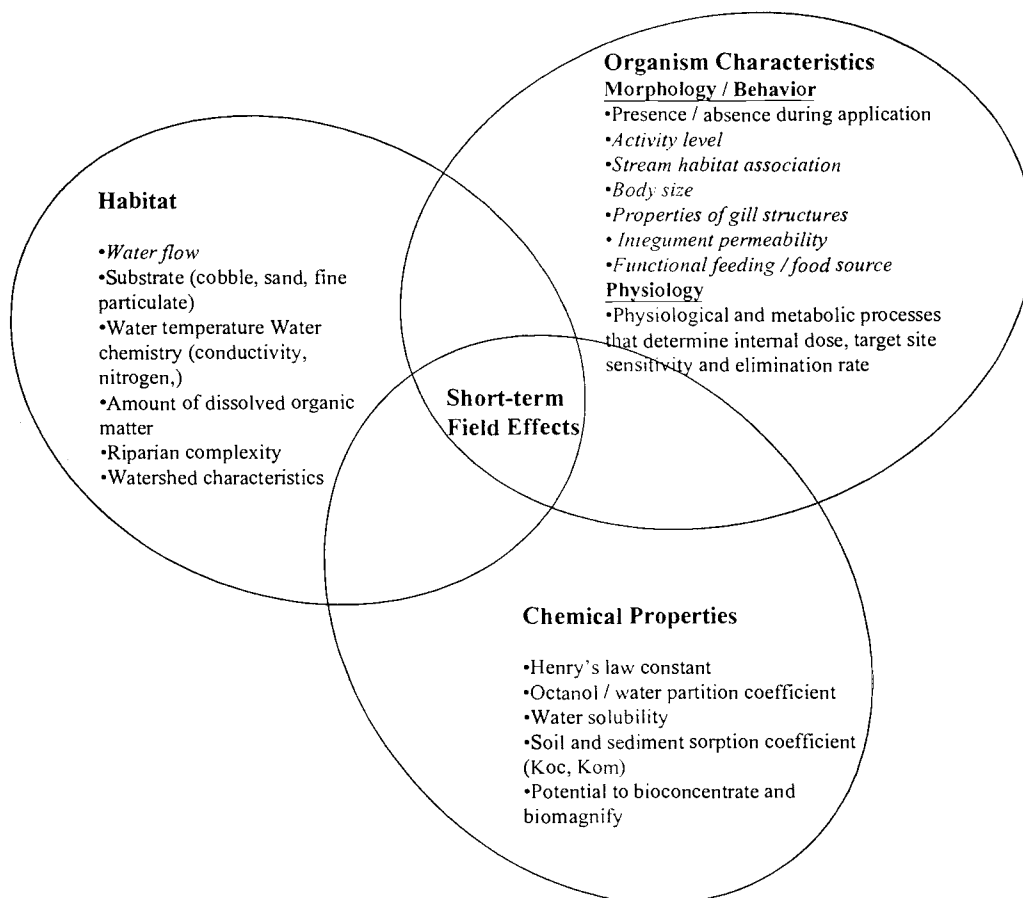
Figure 4.1. The interaction of components of habitat, chemical properties and organisms characteristics which may determine the degree of short-term effects in the field for stream invertebrates. Parameters within each component are important in determining component processes. For example, parameters relating to differences in morphology between organisms may correlate to differences in short-term effects. Highlighted parameters are those that are addressed in the following analysis.

At the individual level, the potential for short-term effects is a function of morphological characteristics and physiological processes that determine internal

dose, target site sensitivity, metabolism, and elimination. Differences between organisms in the levels of detoxification enzymes has been found for different aquatic invertebrates, with blackflies (Diptera, Simulidae) having the highest levels, followed by the caddisflies, mayflies, and damselflies (Siegfried, 1993; Siegfried & Young, 1993). Variation in intrinsic susceptibility differences between organisms, can be determined using laboratory bioassays where concentration and duration of exposure are controlled. For example, variation in susceptibility of six species of aquatic invertebrates to two pesticides, carbaryl and triclopyr, was found in 96-hour bioassays with mayflies (Ephemeroptera) being the most sensitive overall followed by the stoneflies (Plecoptera) and caddisflies (Trichoptera) (Chapter 3). However, in addition to toxicological susceptibility, the potential for short-term effects is also a function of processes that determine degree of exposure, such as the type of habitat association and the behavior of the organism.

Morphological and behavioral differences have been found to contribute significantly to uptake and exposure of pesticides in terrestrial systems and alter susceptibility rankings based on physiological susceptibility data alone (Jepson et al., 1990; Wiles & Jepson, 1993; Wiles et al., 1994). While this approach has provided valuable insights into exposure and uptake for terrestrial organisms (Salt & Ford, 1984), little research has been conducted to determine pesticide interaction with organisms in the stream environment, and criteria are not defined to identify species sensitivity in the field. However, knowledge of species differences in morphology, behavior, and distribution are already exploited to selectively target aquatic invertebrates considered to be pests or health threats. For example, blackfly larvae (Diptera, Simulidae), vectors of human diseases such as onchocerciasis, are controlled worldwide by selectively altering the size of the particulate pesticide Temephos (Abate) to match the fan size of the target pest (Muirhead-Thompson, 1987). The pesticide selectively removes the invertebrate of concern and minimizes the likelihood of effects to non-target invertebrates within the same habitat. The same concept is used to control mosquito larvae (Diptera, Culixidae).

These organisms remain closely associated with the air / water interface of aquatic systems, because of their dependence on atmospheric oxygen. They can then be targeted by pesticides which by their nature partition into this region.

This chapter argues that information on the variation in morphological characteristics, functional feeding group, behavior and habitat association can be used in the risk assessment process to identify those species that may be at an increased risk for pesticide exposure and uptake. This approach may lead improved precision in the prediction of short-term risks, and it may provide a basis for extrapolating test data for a particular species to potential impacts in the field.

Exposure

Exposure is the initial processes by which an organism acquires a dose of a toxin (Suter, 1993). Exposure assessment evaluates how the environmental media that the organism comes into contact with results in chemical exposure, and requires analysis of habitat, chemical properties and organismal characteristics. Although this analysis will concentrate upon biological characteristics of the organism that determine exposure and uptake in the field, properties of the chemical and properties of the habitat that are important in determining a chemical's fate in the environment will be discussed briefly.

We currently exploit our knowledge of chemical properties to help predict the fate and distribution of chemicals in the environment. Chemical properties can estimate a chemical's tendency to partition from the water column to the atmosphere (Henry's law constant) (Mackay et al., 2000), from the water into an organism (octanol / water partition coefficent) (Leo, 2000), and from the water to sediment and organic matter (Koc and Kom) (Doucette, 2000) (figure 4.1). Water solubility is also important, as water-soluble chemicals will tend to dissolve freely into water and remain there until degraded, while relatively insoluble chemicals

will have a greater tendency to partition out of aqueous solution into other phases such as air, soil, sediment and the biota (Mackay, 2000).

Habitat properties include watershed and riparian characteristics that may determine input pathways into the stream system, water flow characteristics that determine chemical distribution and rate of dissipation, stream substrate and dissolved organic matter which influences degree of chemical partitioning, and characteristics of the water such as temperature and chemistry that influence degradation rates (figure 4.1).

An organism's habitat association has been found to be important in determining the degree of contact between the chemical and the organism, and will be the focus of this analysis. For example, in terrestrial systems, the toxicity of pyrethroid deposits to soil organisms was found to be 40 to 50 times lower than the same organisms exposed from the leaves at an equivalent dose rate (Wiles & Jepson, 1994; Jepson et al., 1995). For stream systems, water flow conditions may have the greatest influence on exposure by determining the spatial and temporal distribution of chemical concentrations. For example, organisms in fast water stream habitats, such as riffles and glides, may be exposed to pulsed doses of chemical for shorter amounts of time than organisms that inhabit pools or areas of slow water flow (Richards & Baker, 1993; Bath et al., 1970). For individual contaminates, chemical and physical properties which determine persistence and distribution in the system, i.e., partitioning between air, water, sediment, and biota, may be of equal or greater importance, but are not considered here.

Characteristics of the organisms that influence degree of short-term effects include morphology and behavior that determine exposure and uptake, and physiological and metabolic processes that determine susceptibility. This analysis will concentrate on morphological and behavioral characteristics that influence degree of exposure. These include phenology, which determines whether or not the organism may be present during pesticide application seasons, and behavior, such as activity level, which influences rate of chemical encounter in the environment.

The seasonal change from aquatic larvae to aerial adults varies widely between aquatic insects. Organisms may have seasonal cycles (slow or fast) where emergence occurs about the same time each year many (Plecoptera, Ephemeroptera, Trichoptera, Diptera), or they can have non-seasonal cycles where individuals of several stages are present year round (large Plecoptera and Megaloptera) (Wallace & Anderson, 1996). This type of life history information can be used to determine if, and at what life stage, an organism may be exposed depending on the application season.

Behavioral characteristics of organisms can also play an important role in determining frequency and severity of chemical exposure. For example, organisms actively feeding (i.e. predators), or migrating are likely to be more exposed than those that are less active. In addition, organisms in inactive states, such as the egg stage of development, may be more protected against toxin effects (Ide, 1967).

Uptake

Factors that determine exposure alone do not determine the internal dose the organism will receive. They must be considered in conjunction with morphological, physiological and behavioral factors that determine route and rate of uptake. These include properties of the cuticle, respiratory appendages, and surface area that will determine the rate of absorption of the chemical into the body of the organism, in addition to the diet and mode of feeding that determine probability and rate of ingestion. Differences in these processes that exist between aquatic insects will be used in this analysis to identify organisms that may be at an increased risk for chemical uptake in the stream environment.

Morphological differences including characteristics of the integument which influence permeability, and appendages that increase the degree of exchange with the surrounding environment, such as gills, vary greatly between insects depending

on their habitat and lifestyle (Chapman, 1998). Respiratory surfaces, because of their increased surface area, have been identified as potential target areas of absorption and accumulation of contaminants in aquatic environments (Boudou et al., 1991; Saouter et al., 1991). In addition thin cuticular surfaces have been identified as one characteristic leading to increased susceptibility to chemicals in the field (Maki & Johnson, 1977; Gilderhus & Johnson, 1980). Variation between these characteristics is likely to influence the degree of uptake. Variation in uptake rates between organisms could lead to variation in actual dose, and subsequent effects between organisms. For example, tissue residues have been found to be variable between species exposed to identical aqueous concentrations of a variety of contaminants (Jarvinen & Ankley, 1998). Significant differences in susceptibility between a mayfly *Cinygma* sp. (Ephemeroptera, Heptageniidae) and a stonefly *Calineuria californica* (Plectoptera, Perlidae) to identical exposure durations was established in previous research (Chapter 3).

An organism's food source represents an uptake pathway, because ingested food can be contaminated with the chemical. Aquatic insects vary widely in both their food source (i.e. CPOM, FPOM, algae, other animals) and their mode of feeding (filter feeder, scraper, predator, collector-gatherer) (Merritt & Cummins, 1996). Each food source may vary in how likely it is to be contaminated with the chemical. For example, fine particulate organic matter has more binding sites for chemicals in the environment relative to larger food particles, such as coarse particulate organic matter (CPOM; $>10^3$ microns). As a result there is a higher probability that organisms feeding on fine particulate organic matter (FPOM; $<10^3$ microns) will be ingesting higher chemical concentrations per food weight (Schwarzenbach, 1993). In addition, some modes of feeding may result in a higher rate of uptake than others. For example, predators may feed sporadically, while filter feeding organisms may be constantly filtering food particles from the environment.

LONG-TERM EFFECTS

In contrast to short-term effects, long-term effects are measured at the population level, and are driven by ecological processes. Assessments which evaluate effects that occur over limited spatial scales, such as standardized laboratory bioassays, only identify those species that may be likely to experience effects in the short-term. Physiologically similar organisms may, however, be affected to the same degree in the short-term, but differ in long-term responses because of differences in life history that affect the persistence of populations of individual species (Jepson, 1989; Sherratt & Jepson, 1993).

Aquatic insects vary widely in characteristics that determine a population's ability to recover after a chemical disturbance. For example, the duration of an aquatic insect's life cycles can range from less than two weeks (e.g. some Baetidae and Tricorythidae (Ephemeroptera) and Culicidae and Chironomidae (Diptera)) to several years (e.g. some Elmidae (Coleoptera) and Odonata) (Wallace & Anderson, 1996). Voltinism can also vary within the same species depending on the geographic location and climatic conditions. In addition to differences in life history, organisms vary in their dispersive abilities, as larvae (i.e. propensity to drift) and adults (vagility, fecundity), resulting in a spectrum of recolonization potential for affected stream reaches after disturbance. For example, affected headwater streams will receive less immigration from drifting organisms than would be seen in higher order streams, which may significantly delay recolonization (Cuffney et al., 1984; Wallace et al., 1986; Whiles & Wallace, 1992; Hutchens et al, 1998).

Although not considered here, habitat characteristics (figure 4.2) and chemical properties are also important in determining the potential for long-term effects. Local stream habitat, such as riparian zone integrity, characteristics of the landscape including proximity to nearby recolonizing stream populations, and degree of habitat fragmentation from roads and development will influence

recolonization and recovery rates of affected stream communities (Gore & Milner, 1990; Wallace, 1990; Yount & Niemi, 1990; Chung, 1993; Milner, 1996). In addition, chemical properties determine the persistence and potential for re-distribution to additional habitats. For example, a very persistent chemical which is not degraded readily in the environment, such as DDT, may present a long-term risk to populations for many generations.

Organism Characteristics
<u>Morphology / Behavior</u>
•*Number of Generations per year*
•Fecundity
•*Dispersive ability of larvae (drift) and adult (flight)*
<u>Physiology</u>
•Endocrine disruption
•Genetic alterations

Habitat

•Proximity to nearby streams
•Degree of habitat fragmentation
•Riparian complexity
•Watershed characteristics

Long-term Effects

Chemical Properties

•Persistence
•Probability of redistribution new habitats

Figure 4.2. Three interacting components that determine the potential for long-term effects for stream insects. Parameters addressed in this research are italicized.

This analysis will examine variation in the characteristics of the organisms that determine how species differ in their potential to sustain long-term effects from pesticide exposure. Currently, there is no basis for identifying organisms at risk for long-term effects from laboratory obtained susceptibility data. However, taxa can be identified using ecological criteria. Those at the highest risk for long-term effects may have characteristics leading to limited recolonization of affected habitat, such as low dispersive abilities in either the larval or adult stages, or characteristics resulting in a limited potential for population increase such as long generation time or low fecundity.

MODEL APPROACH

Described here is a quantitative rule-based model which determines and catalogs indices of short-term and long-term effects of chemical exposure to stream invertebrates. Comparison between indices provides a useful first approximation of the relative risks of adverse outcomes among stream invertebrates, and allows for the complexity of contributing factors to be analyzed separately.

Addressing variation in organism characteristics that determine short-term and long-term effects may also lead to a more quantitative basis for risk assessment at the community level, and help form a basis for identifying groups of organisms at an increased risk for the effects of pesticides. This more comprehensive approach further refines aquatic risk assessment as it allows for a more robust analysis of outcomes which considers information not attainable from laboratory assays and provides a mechanism for the evaluation of organisms for which adverse impacts based on laboratory and field data are contraindicated. For example, a physiologically susceptible organism may compensate for the initial effects of high exposure to toxins in the field by possessing life history characteristics that promote

recovery. Even if effects are predicted to be low in the short-term, a low potential for recovery can lead to high effects in the long-term.

This analysis describes the methodology through which critical components of short-and long-term risk are identified and values assigned to them, such that the distribution of properties of a given community can be identified. To evaluate the potential for short-term effects, individuals are ranked based on scores of relative exposure and uptake characteristics. These scores are derived from characteristics of the organisms relating to habitat, morphology and behavior. For a stream invertebrate these characteristics include those presented in figure 1, including stream habitat association and related water flow, behavior / activity, morphology, and dietary differences by examining food source and functional feeding group. Relative weightings of these characteristics related to short and long-term effects are used to describe variation that exists in these characteristics between organisms, and the subsequent variation in associated risk. The actual values require experimental verification but the example below provides a tentative analysis with values defined from first principles. These rankings are used to generate frequencies of key characteristics, within communities of organisms, that will further evaluate the relative risk of adverse outcomes among species within components of exposure, uptake and recovery.

The use of qualitative, rule-based modeling will be used to permit the simultaneous evaluation of complex processes of exposure, uptake, and recovery pertaining to an assessment of potential effects based on a series of distinguishing attributes. This approach has been used in other studies to construct a dynamic ecological model that incorporates the complexity of both biotic (abundance of functional groups) and abiotic characteristics (water salinity levels) which regulate the productivity of an estuarine lake (Starfield et al., 1989). It has also been used to describe the combined effects of climate and human disturbance on the structure of grassland vegetation (Campbell et al., 1999), and to identify erosional problem

areas for a hilly catchment in order to determine proper conservation planning and sustainable development (Adinarayana et. al., 1999).

The assumptions used in this model are described in Box 4.1. The primary focus of this modeling effort is evaluating variation that exists between organisms that determine the potential for short-term and long-term effects of chemical exposure to stream insects, rather than all invertebrates, in the field. The importance of the chemical properties is acknowledged, but not addressed in this assessment. It is assumed that the chemical is a relatively short-lived organic pesticide distributed evenly in the water column. Likewise, it is noted that physical habitat characteristics will vary between streams and between regions, and is likely to have a strong influence on the potential for field effects in the short and long-term. However, properties of the habitat will only be included here as they relate specifically to organism characteristics of exposure and uptake.

Data for some organism characteristics important in determining the potential for short-and long-term field effects were not included because of their availability for only a few species of stream invertebrates. For example, fecundity is an important parameter for assessing long-term effects, but data is not available for many species of aquatic invertebrates. Therefore, this model concentrated on areas where information was available and in a form that could be used for analysis.

Box 4.1: Model Assumptions

- Organism: Stream insects will be the primary focus of this model rather than all stream invertebrates.
- Chemical: A non-persistent organic that does not have a high tendency to partition into organic matter or sediments. Chemical is assumed to be dissolved in the water column.
- Type of waterbody: Streams with a mixture of stream habitat and water flow conditions; not the steepest Cascade stream with predominately fast moving water and not exclusively valley streams with predominately slow moving water.

MATERIALS AND METHODS

The model consists of a series of components that describe variation in the potential for short-and long-term effects between stream invertebrates. The component for short-term effects includes exposure and uptake, and the long-term effects component includes the potential for recovery. For each short-term and long-term effects component, contributing factors are identified and weighted. Parameters within components include behavioral, physiological and morphological characteristics of organisms that impact component processes. For each parameter there is a range of options that represent expected variation between stream invertebrates. An outline of model components and parameters is provided in Table 1.

Model description, formulae calculations and analysis will be described in the following steps:

1. Definitions and descriptions of each model components for short- (exposure and uptake) and long- term (recovery) effects will be outlined, including the following parts:

   a) Description, selection and justification for each component parameter used in the model.

   b) The range of options that exist within a insect community for each parameter are described. Rank values are assigned to parameter options which may follow a simple sequence (1,2,3) or a more complex series (0,3,4,5,9) to represent possible trends in relative risk of impact that each option confers. Rankings were assigned using first principles, literature search or expert consensus. In this analysis, high numbers contributed significantly to short-term risk. A rank of 0 represented a case of no additional risk. In the case of long-term impacts, high values indicated a large positive contribution to population level recovery.

   c) Description of weighting values assigned to each parameter within a component to allow for scaling and adjustment of individual

parameters within a component. Values were again assigned in the following analysis based upon first principles.

2. Organisms selected for model analysis and the methods for the collection of the data required by the model for each species.

3. The incorporation of parameter option ranks and weights into model formulae designed to calculate individual component (exposure, uptake and recovery) relative risk indices for each species tested.

4. Model formulae used to calculate overall relative risk indices relating the potential for short- and long-term effects for each species using the individual component values calculated in (3).

5. Methods used to undertake initial analysis of variation in component and short- and long-term relative risk indices between species.

1). SHORT-TERM EFFECTS COMPONENTS AND PARAMETERS

Exposure Component

Exposure is the process by which an organism acquires a dose (Suter, 1993). Chemical exposure will be evaluated by characterizing water flow by distinguishing habitats that influence the amount, duration, and frequency of exposure to stream insects. In addition to water flow, organism activity level (resting versus active) can be used to characterize the within species variation in chemical exposure associated with life stages that determine activity. The tables that follow will rank each option within parameters of habitat and life-stage by the potential for exposure associated with each option. For example, a rank of 1 would indicate a low potential for exposure compared to a rank of 10 (i.e. Table 4.1).

Exposure Parameter Descriptions

Habitat

Stream habitat was characterized as erosional or depositional. Habitat classification was conducted following the convention of Merritt & Cummins (1996). This reference provides information pertaining to habitat for each insect order, by categorizing the organisms as lotic erosional or depositional. Erosional areas are defined as areas of riffle (areas of high turbulence where the water surface is broken), or glide (fast moving water without the surface broken). Depositional areas are areas of slow or standing water that is found in pools and backwaters. A third form of stream habitat, the hyporheic zone, is a subsurface region of stream flow where surface water and ground water can mix. This region can be extremely important to the stream system, providing habitat for numerous aquatic organisms at various stages of their lives or throughout their life histories (Stanford & Ward, 1988; Williams, 1989; Smock et al., 1992; Stanley & Boulton, 1993).

Erosional, depositional and hyporheic stream habitat were assigned rank values based on the amount and duration of potential for exposure to chemicals in the water column (Table 4.1). For example, because the hyporheic zone is a region separated from the surface flow, there is a lower probability of exposure for hyporheic inhabitants of stream systems exposed to non-persistent organic chemicals. Erosional and depositional habitats were assigned values based on likely residence time of the chemical. For example, exposure time is likely to be longer in areas of slow water flow compared to fast water flow (Bath et al., 1970). A laboratory exposure rating was used to represent the highest possible relative exposure risk, and exemplifies a habitat lacking complexity or refugia that may modify toxicity (Table 4.1).

For this analysis, the hyporheic zone classification was rarely used, since organisms occupy this zone at different stages of their life cycle. However, if the literature described the genus as conducting the majority of its life cycle in this zone it was assigned the hyporheic risk value. If the literature listed an organism as predominately occupying two habitats, such as erosional and depositional, the organism was assigned the average of the two rank values.

Table 4.1. Options, definitions and assigned rank values for the exposure parameters related to habitat. Rank value represents possible trends in relative potential for exposure that each option confers, with a low value relating low potential for exposure, and a high value relating a high potential.

Habitat Options	Definition	Relative Risk	Rank Value
Hyporheic Zone	The area below the bed of the stream where interstitial water moves by percolation[1]	Concentration lower compared to surface waters	1
Erosional	Turbulent (riffle) and non-turbulent (glide) of fast water.	Low retention of chemical - passes through quickly	4
Depositional	Slow moving water and backwaters	Chemical retained for the longest period of time	7
Laboratory	Represents a scenario of the highest possible exposure – no refuge or other behavioral or physical processes to decrease exposure	Organism exposed for duration of experiment	10

[1] Merritt & Cummins, 1996

Life Stage

The life stage parameter assigns risk associated with organism activity. The model assumes that an organism in an active life stage has a higher probability of chemical exposure than an organism in a resting life stage. Accordingly, organism life stages were assigned relative risk values (Table 4.2). Assignment of these values captures the occurrence and seasonality of inactive stages as determined by the life history strategies of stream insects.

The organisms used in this analysis were assumed to be active in order to for comparisons to be made between organisms in short- and long-term effects regardless of the season of the chemical input. However, this parameter of the model could be used to compare differences in exposure between species or instars that would occur at different seasons of the year. For example, comparisons could be made between fall and spring communities, with organisms known to be in diapause or in the egg stage conferring a lower exposure rank than those active during a particular season.

Table 4.2. Options, definitions and assigned rank values for the exposure parameter related to life stage.

Life Stage Options	Definition	Relative Risk	Rank Value
Resting	Organism in diapause or egg stage	Low - Organism is inactive; low encounter rate	1

Table 4.2 (Cont.)

| Active | Not in a diapause or egg stage | High – Organism may accumulate a dose through respiring, feeding and physical substrate. Organism will exploit more habitats and increase the probability of encountering the pesticide. | 2 |

Exposure Parameter Weighting

Weighting values were used to allow for scaling of the parameters within a component. Habitat, considered the primary factor, was assigned a weight of 0.8, and life-stage a weight of 0.2, to reflect the relative contribution of each.

Uptake Component

The uptake component represents variation in intrinsic susceptibility to chemical exposure as a function of physiological and morphological characteristics that determine route and rate of uptake. In defining uptake, the model considers the surface area of the organism (body size), physical properties of the cuticle related to permeability, and dietary intake. Accordingly, four parameters are identified to assess the variation in potential for uptake; body size, respiratory exchange mechanism (i.e. atmospheric, cuticular, gills), integument permeability (sclerotization of the cuticle), and food source.

Uptake Parameter Descriptions

Respiratory Exchange Mechanism

Respiratory surfaces are highly permeable, and often have increased number of sites for ion exchange (Komnick, 1977). Respiratory surfaces have been shown to be a sensitive biological barrier in the accumulation of toxins from the water (Boudou et al., 1991). Chemical uptake may therefore vary between organisms as a function of type and degree of respiratory surface area, which is the focus of continued research at Oregon State University (Buchwalter, pers com). Variation will be evaluated according to different respiratory strategies (defined by Eriksen et al., 1996), and how each varies in the potential for chemical uptake.

Respiratory exchange mechanisms vary considerably among insects (Chapman, 1998), and include those that extract dissolved oxygen from the water, and those that rely on maintaining contact with atmospheric oxygen. Aquatic insects which utilize atmospheric oxygen may use appendages to maintain contact with the surface, such as the siphons seen in some families of Diptera (e.g. Culicidae, Chaoboridae and Dixidae). Others collect air bubbles at the surface and utilize these under water (e.g. aquatic Coleoptera). Organisms that utilize dissolved oxygen may do so through diffusion across the cuticle or the organism may possess tracheal gills which increase the area of respiratory exchange. Size and location of gills varies between organisms. For example, damselflies (Zygoptera) have caudal gills, caddisfly (Trichoptera) larvae have filamentous abdominal gills, and the position of stonefly (Plecoptera) gills tend to vary from species to species (Chapman, 1998).

Organisms were assigned rank values based upon permeable respiratory surface area (Table 4.3). Organisms classified as incurring no risk from respiratory uptake were those that rely on atmospheric oxygen sources (e.g. breath through the

use of a siphon or other appendage, or obtain air at water surface); these included plant breathers (piercers), and those that utilize air stores underwater temporarily (physical gill) or permanently (plastron respiration). In addition, those organisms that utilize spiracular gills (some Coleoptera and Diptera) also were considered atmospheric air breathers, since these gills are associated with an atmospheric air source (Eriksen et al., 1996). Atmospheric respiration was assigned a relative risk value of 0 (no additional risk) because this mechanism for obtaining oxygen does not require intimate contact with the water.

Organisms utilizing cuticular respiration are those that have a closed tracheal system and no gills; they obtain oxygen by diffusion. These include most small, worm-shaped larvae such as chironomids, some tipulids, simuliids, and gill-less plecopterans and trichopterans (Erikson et al., 1996). Organisms with tracheal gills, which increase the surface area of the organisms for obtaining dissolved oxygen from the water, include many ephemeropterans, plectopterans, trichopterans and odonates as well as some dipteran and coleopteran families (Merritt & Cummins, 1996). The rank risk value for cuticular respirators was given a value of one, and those that use additional respiratory gill surfaces a value of two and three, indicating whether or not the gills were directly exposed to the water or cased, respectively. Cased gills may have less contact with the surrounding water column than those that are uncased.

Table 4.3. Options, definitions and assigned rank values for the uptake parameter related to respiratory exchange mechanism. Rank values are graded evenly to reflect the degree of surface area the organism has for respiratory exchange, and increase as respiratory surface area increases. Based on first principles, the higher the surface area to volume ratio the greater the degree of uptake.

Respiratory Exchange Options	Definition	Relative Risk	Rank Value

Table 4.3 (Cont.)   0

Atmospheric	Oxygen source is atmospheric – do not obtain dissolved oxygen from the aquatic environment	No respiratory uptake risk	
Cuticle	Oxygen is obtained by diffusion through the cuticle. Surface to volume ratio is high in order to facilitate the process	Low. Chemical can cross cuticle through respiratory processes, but no specialized respiratory structures (gills)	1
Cased Gills	Gill surfaces are not in direct contact with the water flow, but are instead within a case constructed of various materials.	High to Intermediate. Increased respiratory tissue and subsequent surface area to volume; however gills are not exposed to surface flow directly	2
Uncased Gills	Gill surfaces are in direct contact with the water flow.	High. Increased respiratory surface and gills are exposed to water flow	3

Integument Permeability

The integument permeability component addressed the likelihood that the chemical could enter the organism by simple diffusion across its body surface, which may be a function of the properties of the cuticle. Based on first principles, the rate of diffusion is a function of the permeability and surface area of the substrate, which in this case is the tissue of the organism. Cuticular permeability varies greatly between insects, depending on their habitat and lifestyle. For example, the cuticle of adult water beetles, aquatic Heteroptera and larval *Sialis* sp. (Megaloptera) is relatively impermeable, in contrast to the majority of larval forms that have highly permeable cuticles, or at least some areas of the cuticle which are

permeable (Chapman, 1998). Insects with more impermeable cuticles generally have greater degree of sclerotization and wax thickness per unit area than species with more permeable cuticles (Chapman, 1998).

For this model parameter, it is therefore assumed that an insect with a hardened (sclerotized) cuticle will have less potential for uptake one with a membranous cuticle. For this model, it is assumed that an organism with a harder integument (i.e. stoneflies) may be less susceptible to chemical uptake across the body surface than soft-bodied organisms (i.e. most Diptera). Organisms classified with a hard integument were those with the majority of the body sclerotized, such as representatives from the Coleoptera, Hemiptera, Plecoptera and some Ephemeroptera. The majority of the mayflies were considered mixed because they possess membranous and sclerotized areas. A mostly membranous cuticle was defined as soft, as represented by most of the Diptera and the Trichoptera. Although caddisflies may have a sclerotized pronotum and mesonotum, the majority of the integument is membranous. Table 4.4 summarizes the integument permeability definitions, consequence for risk and rank values.

Table 4.4. Options, definitions and assigned rank values for the uptake parameter related to integument permeability. Because integument properties govern the degree of diffusion, risk rankings are a graded scale from 1 to 3 based on amount permeable integument.

Integument Permeability Options	Definition	Relative Risk	Rank Value
Hard	The majority of the body is sclerotized and encased in a thickened, rigid cuticle.	Relatively impermeable to exchange with water; low uptake risk	1
Mixture of	Hardened areas of	Intermediate potential for	2

Table 4.4 (Cont)

Hard and Soft	the cuticle mixed with unsclerotized (soft-bodied) areas, e.g. caddisflies.	uptake	
Soft	The majority of the cuticle is unsclerotized and the body is soft, e.g. some Diptera	Based on first principles of diffusion, an organism with a thinner cuticle will have more potential for exchange with the surrounding medium; uptake will be the most rapid	3

Food Source

An organism's food source represents an exposure pathway, because ingested food can be contaminated with the chemical. For some life stages that do not feed (e.g. egg stages; diapausing organisms), therefore this exposure pathway contributes nothing to the potential for uptake. Others are predators or feed on algae, or detritus in different forms (CPOM and FPOM) (Cummins & Merritt, 1996). Food source risk was assigned based on how likely the food item is to be contaminated with the chemical (Table 4.5). Although potential for contamination may depend on the properties of the chemical, some general assumptions can be made.

Fine particulate organic matter has more chemical binding sites relative to larger food particles, such as course particulate organic matter (CPOM; $>10^3$ microns). As a result there is a higher probability that organisms feeding on fine particulate organic matter (FPOM; $<10^3$ microns) will be ingesting higher chemical concentrations per unit mass of food (Schwarzenbach, 1993). In addition, some organisms feeding on FPOM utilize fan-feeding structures which pulls food

particles directly into the body of the organism (*Cummins & Merritt*, 1996). This appendage places organisms at an increased risk for direct ingestion and therefore is assigned the highest relative risk ranking in this model.

Food source was determined by using summaries of ecological and distributional data for the appropriate insect order in Merritt & Cummins (1996). This provided information on functional feeding (collector-gatherers, predators, shredders, scrapers) and the food source (other animals, herbivores, detritivores, algae, diatoms). If the genus used in the model could not be found here, supplemental references appropriate to the order were used.

Table 4.5. Options, definitions and assigned rank values for the uptake parameter related to food source. The rank values for these parameter options were not graded, but jumped from 0 to 3, and then 6 to 9. This was to incorporate the perceived greater jump in risk from an organism feeding on FPOM as a collector-gatherer to an organism that uses a fan for feeding.

Food Source Options	Definition	Relative Risk (Chemical Association with Food)	Rank Value
Nothing	The organism does not feed.	No Risk	0
Other Animals	The organisms main food source is other organisms (predator)	Bioaccumulation low; risk low	3
Algae	The organism feeds on algae – includes most scrapers	Chemical can accumulate in algae; low to intermediate risk	4
Detritus / CPOM	Detritus and course organic matter – most shredders, collector gatherers	Chemical can bind to CPOM; intermediate risk	5
FPOM (No fan)	Feeds on fine particulate organic matter, but no fan is used in food capture. Includes collector gatherers.	Chemical can bind to FPOM to a higher degree than CPOM; intermediate to high	6

Table 4.5 (Cont.)

| FPOM (Fan used) | Uses a fan appendage to trap fine particulate organic matter. | Chemical binding is high, and mode of feeding concentrates particles rapidly from water column; combination results in extremely high risk | 9 |

Body Size

Rate of diffusion of a chemical across an insect's cuticle is a function of the surface area of the cuticle (Chapman, 1998). Many studies have shown smaller individuals to be more sensitive to contaminants than larger individuals of the same species (Club, 1975; Powlesland & George, 1986; McCahon et al., 1989; Diamond et al. 1992, Kiffney & Clements, 1994; Kiffney & Clements, 1996). Increased sensitivity of small individuals may result from larger surface area : volume ratios, higher initial lipid content, or a greater mass-specific metabolism that would facilitate uptake of toxicants. First instars of the trichopteran larvae *Agapetus fuscipes* were found to be more sensitive to cadmium than third and fourth instars (McCahon et al., 1989). First instars were also found to be more sensitive than last instars for the caddisfly *Hydropsyche angustipennis* and the midge *Chironomus riparius* exposed to diazinon (Stuijfzand et al., 2000). Small class sizes of the mayfly *Drunella grandis* have been shown to be more sensitive to metals than larger ones within the same population (Kiffney & Clements, 1994). A strong relationship between body size and sensitivity to metals was found for *Baetis tricaudatus* (Baetidae), *Ephemerella infrequens* (Ephemerellidae), *Rhithrogena hageni* (Heptageniidae), and *Pteronarcella badia* (Pteronarcyidae); an inverse relationship was determined for body size and survivorship (Kiffney & Clements, 1996).

For this model, body size is distributed into three size classes of small (0 - 5 mm), medium (5.1 – 20 mm) and large (20.1 mm and higher) based on the size range of insects found in Pacific Northwest streams. Within this range, risk ranking was assigned based on surface area to volume ratio (Table 4.6).

Table 4.6. Options, definitions and assigned rank values for the uptake parameter related to body size. Values are assigned on a graded scale from 1 to 3 based on the surface area to volume ratio. Body size ranges are based on the range of sizes that are found in stream systems, and not on a known relationship between size and susceptibility.

Body Size Options	Definition	Relative Risk	Rank Value
Large	20.1 mm and up	Surface area to volume ratio is low; low uptake potential	1
Medium	5.1 to 20 mm	Intermediate surface area to volume ratio	2
Small	0 to 5.0 mm	Surface area to volume ratio is high; high potential for uptake	3

Uptake Parameter Weighting

This component has four parameters, gas exchange mechanism, integument permeability, food source and body size. Each parameter is assigned approximately an equal weight, with the exception of gas exchange, which was assigned a higher weight of 0.3. This is because gills may constitute a particularly sensitive cuticular surface for those organisms that posses them. Respiratory surfaces have thin cuticular coverings, thereby increasing the potential for uptake of substances in the surrounding medium. Integument permeability and body size are given equal weights (0.25) because they are processes, based on first principles, that determine the amount of chemical that will enter an organism. An organism is constantly exposed to a chemical in the water column through its integument or respiratory surface, but feeding is usually not a constant process and potential for uptake through this pathway may be lower. Therefore, the food source parameter was assigned the lowest weight (0.2).

LONG-TERM EFFECTS RECOVERY COMPONENT AND PARAMETERS

The recovery component analyzes variation between organisms in the potential for long-term effects through an index describing the likelihood of recolonizing to pre-exposure population density within a year following exposure. Options within these parameters examine recolonization potential by examining propensity to drift (none, low and high), the range of flight of the adults (none, weak or strong flyer), and the longevity of the adult (hours, days and weeks). An additional parameter, number of generations per year, was selected to estimate potential reproduction rates. A low parameter option for recovery signifies a low contribution to recovery, while a high number represents a large contribution.

Parameters were selected, in part, from a literature search to determine migration sources and mechanisms for recolonization for stream benthos. These were downstream migration or drift, upstream migration, vertical upward migration from within the substrate, and aerial sources (Williams & Hynes, 1976; Williams, 1981). One study found the order of importance for contribution to recolonization as drift > aerial sources > movement from the hyporheic zone > upstream movement (Williams & Hynes, 1976). Upstream migration has been reported, but the numbers of individuals involved has been found to be far less than numbers drifting (Williams, 1977). Movement of organisms from the hyporheic zone also provides colonists (Williams & Hynes, 1974), but based on the literature may not contribute as much to recolonization as drift and aerial sources. The two mechanisms of recolonization considered to be primary to most stream systems, drift and adult flight, were considered for parameters in this model.

Recovery Parameter Descriptions

Generation Time

The recovery time of a particular species is dependent on its generation time. The number of generations per year affects the population growth rate (Begon et al., 1996). A population that reproduces several times a year (multivoltine) will have a higher probability of returning to pre-treatment levels sooner than one that reproduces once every two to three years. Recovery has been found to be rapid for taxa with short generation times such as larval Chironomidae (Pusey et al., 1994). For example, a study of the recovery of a headwater stream following insecticide disturbance found multivoltine insect taxa, such as some Diptera, reached pre-disturbance levels rapidly within the first year, which was followed by univoltine taxa. However, semivoltine organisms (e.g. Plecoptera: Peltoperlidae) colonized at low densities, or did not colonize within the first year (Whiles & Wallace, 1992). Recovery after an exposure of methoxychlor was faster for organisms with relatively short life cycles such as chironomids (Huryn, 1990), as compared to those with long life cycles, such as Plecopterans (e.g. *Beloneuria* sp., Peltoperlidae) and large bodied trichopterans (e.g. *Fattigia* sp. and *Pycnopsyche* sp.) (Chung et al., 1993).

For this model, an organism that reproduces once every three years was assigned a risk rank value of 1, which indicates a low contribution to recovery within a year. Conversely, an organism that reproduces at a rate of more than two generations per year is assigned the high contribution to recovery relative to the first species (Table 4.7).

Table 4.7. Options, definitions and assigned risk values for the recovery parameter related to generation time. A high rank value indicates a high potential for recovery, while a low rank value indicates a low potential.

Generation Time Options[1]	Definition	Recovery	Rank Value
Multivoltine	More than two generations per year	High probability of repopulating impacted area within 1 year based on reproductive potential	7
Bivoltine	Two generations every year	High – Intermediate probability	5
Univoltine	One generation every year	Intermediate probability	4
Semivoltine	One generation every two years	Intermediate – Low probability	3
Merovoltine	Takes longer than two years to complete a generation	Low probability	1

[1]Wallace and Anderson, 1996

Propensity to Drift

Downstream drift is a major pathway for the recolonization of streams affected by natural or pollution events (Williams & Hynes, 1976; 1977; Gore, 1982; Sheldon 1984; Brittain & Eckeland, 1988). Recolonization of denuded stream reaches has been found to occur primarily by drift dispersal, comprising 80 to 90% of all colonizers (Townsend & Hildrew, 1976). Species with a high propensity to enter the drift can re-colonize disturbed areas rapidly (e.g. Gray & Fisher, 1981, Fisher et al., 1982, Wallace et al., 1986, Whiles & Wallace, 1992). Mayflies (Ephemeroptera) and caddisflies (Trichoptera) have been reported to be

found most often in the drift (Waters, 1972), as well as Chironomids (Williams & Hynes, 1976).

Recovery times for streams after pesticide are longer for those that lacked upstream sources for re-colonization (Wallace et al., 1986; Chung et al., 1993; Hutchens et al., 1998). Organisms with a high tendency to drift have a higher probability of re-colonizing impacted reaches downstream (i.e. chironomid larvae) (Williams & Hynes, 1976) (Table 4.8).

Table 4.8. Options, definitions and assigned rank values for the recovery parameter related to drift behavior. A high rank value indicates a high potential for recovery, while a low rank value indicates low potential (zero indicates no potential).

Propensity to Drift	Definition	Relative Contribution	Rank Value
High	Drifting is a regular behavioral component of the insect's life cycle; the organism is often found in the drift	High – drifting organisms increased recolonization rates	5
Low	The organism is found in the drift, but in low numbers.	Low – organisms are present in the drift, but recolonization rates may be slower than above	3
None	The organism does not drift	No contribution to recovery via drift	0

Adult Flight

Adult flight is an important mechanism of recovery for impacted streams. Recolonization of impacted stream reaches by adult flight has been found to be especially important for streams quite a distance away from unperturbed sites

(Niemi et al., 1990). Aerial colonization can be sufficient to rapidly establish depleted stream reaches in the absence of drift, upstream migration, delayed hatching, or hyporheic sources (Ladle et al., 1980). For example, oviposition by adults from neighboring streams was found to be the most important factor governing recolonization in a stream severely impacted by a gasoline spill (Pontasch & Brusven, 1988), and following insecticide treatments, taxa having vagile aerial adults were found to be the most abundant in litterbag communities under observation (Chung et at., 1993). Options related to adult flight were ranked as either strong or weak, or none (wingless adult) (Table 4.9).

Table 4.9. Options, definitions and assigned rank values for the recovery parameter related to adult flight. High rank values indicate a high potential for recovery, while low values indicate a low potential. A value of zero indicates no contribution.

Adult Flight Options	Definition	Relative Contribution	Rank Value
Strong Flyer	The adult is a strong flyer, and has a high potential to recolonize impacted streams	High	5
Weak Flyer	The adult is a weak flyer, has the potential to recolonize streams in close proximity.	Intermediate	3
No Flight	The adult stage is apterous (wingless)	None	0

Adult Life-span

How long the adult lives can influence the probability of recolonization. An adult that only lives a few hours is less likely to disperse far enough to

repopulate reaches within the same stream, or those in adjacent stream systems. Those that live longer may be able to recolonize adjacent streams affected by pesticide inputs. For example, the recolonization of desert streams disturbed by floods has been attributed to aerial colonization of long-lived adults (Fisher et al., 1982; Gray & Fisher, 1981). Rankings were subsequently assigned based on the life-span of the adult stage, ranging from hours to weeks (Table 4.10).

Table 4.10. Options, definitions and assigned rank values for the recovery parameter related to adult flight.

Adult Life-span Options	Definition	Relative Contribution	Rank Value
Weeks	The adult stage lives more than one week	Potential to recolonize adjacent streams high	3
Days	The adult stage lives 1 to 7 days	Potential to recolonize adjacent streams is intermediate	2
Hours	The adult stage lives up to 24 hours.	Potential to recolonize adjacent streams low	1

Recovery Parameter Weighting

The recovery component has four parameters; generation time, drift behavior, adult flight and adult life-span. The emigration of species from undisturbed areas has been found to be an important factor influencing recovery and a return to pre-treatment numbers (Wallace, 1990). In stream systems, drifting animals from upstream of the disturbed area has been found to be the primary mechanism (Gore, 1982). Therefore, the drift parameter was assigned the highest proportional weight (0.40). Emigration of flying adults has also been found to be

important in this process, and this was assigned a weight of 0.25. Generation time, also considered to be important determining the rate of population increase, was assigned a weight of 0.25. Life-span was assigned a weight of 0.10, to indicate its lower perceived contribution to recovery relative to the other three.

2). ORGANISMS SELECTION AND DATA COLLECTION

In order to explore the variation that exists between groups of aquatic insects, single genera were selected from each family within the Orders Plecoptera, Trichoptera, Ephemeroptera, Diptera, Coleoptera, Odonata, Hemiptera and Megaloptera. These orders were chosen because they are well represented by or are entirely aquatics. Selection at this taxonomic level allowed for the use of genus-specific traits which are required at the parameter level of the model. This means that the attributes of the selected genus, rather than the mean attributes for a family, were incorporated within the model. It was assumed that the selected genera were representative of families in aquatic orders, but care was taken not to extrapolate or generalize findings, assuming that they would apply to all genera within a family. In addition, an effort was made to select organisms that can be found in the Pacific Northwest. However, if a Pacific Northwest representative could not be found in a particular aquatic family, genera considered by the literature to be widespread within North America were selected.

The primary reference used in the classification of organism characteristics was the Aquatic Insects of North America (Merritt & Cummins, 1996). If detailed information at the genus level was not available in Merritt & Cummins, it was obtained from literature more specific to each Order (Plecoptera, Stewart & Stark, 1998; Trichoptera, Wiggins, 1977; Ephemeroptera, Edmunds et al., 1976; Diptera, Coleoptera, Odonata, Hemiptera and Megaloptera, Stehr, 1993).

Taxon specific data for the recovery component parameters were obtained from two different sources. Data on number of generations per year was obtained at the genera level from literature. For parameters of adult flight, propensity to drift and adult life-span data was obtained at the family level through expert opinion, due a lack of this kind of information in the literature. A summary of expert option obtained on these characteristics is presented in appendix 4.1 for each family.

Organism data in the components of exposure and uptake (short-term) and recovery (long-term) were collected from the literature and assimilated in Excel (Microsoft®, 1997) spreadsheets by Order (appendices 4.2 – 4.9 A). Organism characteristics were listed and referenced for each genus by model component.

3) CALCULATION OF PARAMETER AND COMPONENT RISK INDICES

Component relative risk indices are calculated by adding the values assigned to parameter options, and the weighting values assigned to each parameter within a component (Table 4.11). This enables scaling and adjustment of the contributions that individual parameters make to the final risk index for each taxon.

Table 4.11: Summary of model components and parameters. Each parameter has several options which represents the range of potential attributes that exist within a insect community. These options are ranked based on first principles, literature search, or expert consensus, to represent possible trends in relative risk of impact that each option confers. For components of short-term effects, exposure and uptake, a high rank value contributes significantly to short-term risk. For the long-term effect component of recovery, a high value indicates a large positive contribution to recovery. Weight values were assigned to each parameter to allow for scaling and adjustment of individual parameters.

Table 4.11
(Continued)

COMPONENT	Parameter	Weight	Parameter Options	Option Rank Value
EXPOSURE				
	Habitat	0.8		
			Hyporheic Zone	1
			Erosional	4
			Depositional	7
			Laboratory	10
	Life Stage	0.2		
			Resting	1
			Active	2
UPTAKE				
	Respiratory Exchange Mechanism	0.3		
			Atmospheric	0
			Cuticle	1
			Cased Gills	2
			Uncased Gills	3
	Integument Permeability	0.25		
			Hard	1
			Mixture	2
			Soft	3
	Food Source	0.2		
			Nothing	0
			Other Animals	3
			Algae	4
			Detritus / CPOM	5
			FPOM (no fan)	6
			FPOM (fan)	9
	Body Size	0.25		
			Large (20.1 mm and up)	1
			Medium (5.1 to 20.0 mm)	2
			Small (0 to 5.0 mm)	3

Table 4.11
(Continued)
RECOVERY

Generation Time	0.25			
		Multivoltine		7
		Bivoltine		5
		Univoltine		4
		Semivoltine		3
		Merovoltine		1
Drift Behavior	0.4			
		High		5
		Low		3
		None		0
Adult Flight	0.25			
		Strong		5
		Weak		3
		None		0
Adult Life-span	0.1			
		Weeks		3
		Days		2
		Hours		1

Parameter values (equation 1) and component relative risk indices (equation 2) were calculated as follows. Parameter values (P) (converted to a % scale to aid in interpretation) are calculated as:

Equation 1 $P = [R / R_{Max} \times W] \times 100$

Where:

    R = Value assigned to a specific parameter option
    R_{Max} = Maximum value assigned to any option within a specific parameter
    W = Parameter weighting value

A component risk index (C) for each taxon, is then calculated as the addition of the weighted parameter values (1-100) (equation 2).

Equation 2 $C = \Sigma P_{1-n}$

Appropriate option rank values for within each parameter were assigned according to Table 4.11 for each species used in the model, and Excel® spreadsheets were used to calculate parameter values according the formulae in Box 4.2. Parameter values for each species used in the model are can be found, by Order, in appendices 4.2 – 4.9 B. Parameters were weighted (Table 4.11) and component values of exposure, uptake and recovery were calculated according to Box 4.3 (appendices 4.2 – 4.9 C).

Plots of component values of exposure, uptake and recovery distributions for each family were developed from the spreadsheets to explore patterns of effects for the selected genera, within and between orders (appendices 4.2 – 4.9 D).

BOX 4.2: Parameter Calculations

Exposure

$$P_H = (O_H) / (O_{H(MAX)} \times 100$$
$$P_L = (O_L) / (O_{L(MAX)} \times 100$$

Where:

O_H = *Habitat option value*

$O_{H(MAX)}$ = *Habitat option maximum value*

O_L = *Life-stage option value*

$O_{L(MAX)}$ = *Life-stage option maximum value*

Uptake

$$P_R = (O_R) / (O_{R(MAX)} \times 100$$
$$P_I = (O_I) / (O_{I(MAX)} \times 100$$
$$P_F = (O_F) / (O_{F(MAX)} \times 100$$
$$P_B = (O_B) / (O_{B(MAX)} \times 100$$

Where:

O_R = *Respiratory exchange option value*

$O_{R(MAX)}$ = *Respiratory exchange option maximum value*

O_I = *Integument permeability option value*

$O_{I(MAX)}$ = *Integument permeability option maximum value*

O_F = *Food source option value*

$O_{F(MAX)}$ = *Food source option maximum value*

O_B = *Body size option value*

$O_{B(MAX)}$ = *Body size option maximum value*

Recovery

$$P_{LH} = (O_{LH}) / (O_{LH(MAX)}) \times 100$$
$$P_D = (O_D) / (O_{D(MAX)}) \times 100$$
$$P_{AF} = (O_{AF}) / (O_{AF(MAX)}) \times 100$$
$$P_{AL} = (O_{AL}) / (O_{AL(MAX)}) \times 100$$

Where:

O_{LH} = *Life history option value*

$O_{LH(MAX)}$ = *Life history option maximum value*

O_D = *Drift option value*

$O_{D(MAX)}$ = *Drift option maximum value*

O_{AF} = *Adult flight option value*

$O_{AF(MAX)}$ = *Adult flight option maximum value*

O_{AL} = *Adult life-span option value*

$O_{AL(MAX)}$ = *Adult life-span option maximum value*

BOX 4.3. Component Calculations

The parameter values and weighting in Table 4.11 can be integrated to generate risk estimates for components by applying equations 1 and 2, as follows.

Exposure (C_E):

$$C_E = (P_H \times P_H W) + (P_{LS} \times P_{LS} W)$$

Where:
P_H = *Habitat parameter value*
$P_H W$ = *Habitat parameter weight*
P_{LS} = *Life-stage parameter value*
$P_{LS} W$ = *Life-stage parameter weight*

Uptake (C_U):

$$C_U = (P_R \times P_R W) + (P_I \times P_I W) + (P_F \times P_F W) + (P_B \times P_B W)$$

Where:
P_R = *Respiratory exchange parameter value*
$P_R W$ = *Respiratory exchange parameter weight*
P_I = *Integument permeability parameter value*
$P_I W$ = *Integument permeability parameter weight*
P_F = *Food source parameter value*
$P_F W$ = *Food source parameter weight*
P_B = *Body size parameter value*
$P_B W$ = *Body size parameter weight*

Recovery (C_R):

$$C_R = (P_G \times P_G W) + (P_D \times P_D W) + (P_{AF} \times P_{AF} W) + (P_{AL} \times P_{AL} W)$$

Where:
P_G = *Generation time parameter value*
$P_G W$ = *Generation time parameter weight*
P_D = *Drift behavior parameter value*
$P_D W$ = *Drift behavior parameter weight*
P_{AF} = *Adult flight parameter value*
$P_{AF} W$ = *Adult flight parameter weight*
P_{AL} = *Adult life-span parameter value*
$P_{AL} W$ = *Adult life-span parameter weight*

4) CALCULATION OF SHORT-AND LONG-TERM RISK INDICES

Short-term Effect Index Value

In order to compare relative differences between the potential for short-term effects (mortality) between organisms, component risk values were combined in an assessment of effects for a hypothetical community of aquatic insects. In order to develop a prediction of effects, susceptibility would need to be known. However, the lack of susceptibility data for most organisms precludes its use here. The analysis permits the degree of variation in factors that underlie levels of exposure and uptake to be quantified. Relative risk values for exposure and uptake were combined to yield and interpret a value that was taken to represent the contribution that the selected characters would make to short-term effects of the pesticide (equation 3). For the purposes of comparison in this model, the hypothetical initial population was 1000.

Equation 3 Short-Term Effect Value = (Initial Population Size x C_E) x C_U

Long-term Effect Index Value

The potential for long-term effects were estimated through the recovery index component of the model (equation 4). Data were collected to represent families, and therefore may not be tuned to particular species. Most of our analyses are were made at the Order level, using the selected genera as representatives of families from that Order. We do not examine between family differences. In addition, values for the parameters of this component are not considered risk values, but rather contribution values, where a high value represents a high contribution to the recovery process (Table 4.11).

Equation 4 Long-Term Effect Value = C_R

5) ANALYSIS OF VARIATION IN SHORT- AND LONG-TERM INDICES

Components that underlie short-term risk are largely morphological, toxicological and physiological in nature, while the underlying mechanism of long-term impacts are mostly ecological and landscape level processes. Component indices of short and long-term effects were not therefore combined quantitatively. Instead, the relative patterns of short and long-term risk between families of different orders were compared, so that families with sets of attributes that might lead to high levels of risk could be identified.

Short-term effect indices were compared with recovery indices to identify groups of organisms with particular combinations of short and long-term effects. Ranks of 1 to 10 were assigned according to index values for short-term and long-term effects. For short-term effects a 1 indicates a low effect value (i.e. relatively high survival). However, for recovery a value of 1 indicates a low contribution to recovery (i.e. relatively poor recovery). Organisms were identified that fell into combinations of short-and long-term effects according to categories of different combinations of risk defined as: category 1: high short-term risk index, high long-term risk index; category 2: high short-term risk index, low long-term risk index; category 3: low short-term, high long-term risk index; and category 4: low short-term risk index, and low long-term risk index. Category 1 organisms (high short-term and high long-term effects indices), are at the greatest risk for prolonged effects of pesticide exposure. This is because they possess characteristics relating to a high potential for short-term impacts, as well as characteristics of low recovery potential, or a high potential for long-term effects. Category 3 organisms may also show prolonged effects long-term effects, because although effects are low, recovery may be slow. Category 2 organisms (high short-term but low long-term

effects indices) may be highly affected initially following pesticide exposure, but re-colonization is predicted to occur rather quickly. Category 4 organisms would be expected to be affected the least from the short- and long-term effects of pesticide exposure.

RESULTS

SHORT-TERM EFFECTS

Exposure and uptake risk indices calculated for representative genera in each family of the aquatic orders are presented in Table 4.12. The objective of the analysis below is to explore within and between order variation in the ecotoxicological component processes. We assumed that the selected genera were representative of the families that they belong to and we assume that the risk ranking values are weightings represent the underlying processes that determine risks to species in the real world. However, given that only single genera for each family were selected, most of our conclusions are drawn at the order or individual genus level.

Table 4.12. Relative indices for model components of exposure, uptake and recovery for the 8 orders of aquatic insects. Exposure and uptake indices were combined to result in a calculated relative effect out of an initial population of 1000.

Order	Family	Species	Exposure	Uptake	Effect	Recovery
Plecoptera	Nemouridae	*Zapada sp.*	64	74	476	63
	Capniidae	*Eucapnopsis sp.*	28	63	176	52
	Leuctridae	*Paraleuctra sp.*	28	54	152	60
	Taeniopterygidae	*Taenionema sp.*	52	54	283	57
	Peltoperlidae	*Yoraperla sp.*	64	66	423	57
	Pteronarcyidae	*Pteronarcys sp.*	52	58	300	67
	Perlidae	*Calineuria sp.*	52	53	277	67
	Perlodidae	*Isoperla sp.*	52	42	217	57
	Chloroperlidae	*Swelsta sp.*	52	42	217	60
Trichoptera	Rhyacophilidae	*Rhyacophila sp.*	52	70	364	34
	Glossosomatidae	*Glossosma sp.*	52	61	315	14
	Hydroptilidae	*Hydroptila sp.*	52	89	462	53
	Philopotamidae	*Dolophilodes sp.*	52	75	390	30
	Psychomyiidae	*Psychomyia sp.*	52	65	338	34
	Hydropsychidae	*Arctopsyche sp.*	52	70	364	34
	Polycentropodidae	*Polycentropus sp.*	52	50	260	38
	Limnephilidae	*Dicosmoecus sp.*	52	63	329	45
	Uenoidae	*Neothremma sp.*	52	73	378	45
	Lepidostomatidae	*Lepidostoma sp.*	64	73	466	42
	Brachycentridae	*Brachycentrus sp.*	52	71	367	34
	Phyrganeidae	*Agrypnia sp.*	76	63	481	58
	Calamoceratidae	*Heteroplecton sp.*	76	64	490	53

Table 4.12 (Continued)

	Odontoceridae	*Namamyia sp.*	64	73	466	53
	Helicopsychidae	*Helicopsyche sp.*	52	71	367	50
	Leptoceridae	*Oecetis sp.*	64	68	437	50
	Sericostomatidae	*Gumaga sp.*	52	73	378	50
Ephemeroptera	Ameletidae	*Ameletus sp.*	64	73	469	21
	Siphlonuridae	*Siphlonurus sp.*	76	72	549	21
	Ametropodidae	*Ametropus sp.*	64	72	462	60
	Baetidae	*Baetis sp.*	64	73	469	34
	Isonychiidae	*Isonychia sp.*	52	73	381	17
	Heptageniidae	*Cinygma sp.*	52	74	387	60
	Leptophlebiidae	*Leptophlebia sp.*	64	73	469	60
	Ephemeridae	*Hexagenia sp.*	76	68	519	61
	Ephemerellidae	*Ephemerella sp.*	64	65	416	52
	Tricorythidae	*Tricorythodes sp.*	76	66	502	56
	Caenidae	*Caenis sp.*	76	74	566	60
	Baetiscidae	*Baetisca sp.*	76	66	502	76
Diptera	Tipulidae	*Dicranotoa sp.*	64	40	256	57
	Psychodidae	*Pericoma sp.*	76	55	418	57
	Ptychopteridae	*Bittacomorpha sp.*	76	55	418	68
	Blephariceridae	*Blepharicera sp.*	52	81	419	76
	Deuterophlebiidae	*Deuterophlebia sp.*	52	69	358	70
	Dixidae	*Dixa sp.*	64	55	352	49
	Chaoboridae	*Chaoborus sp.*	76	58	443	60
	Culicidae	*Amnopheles sp.*	76	55	418	57
	Thaumaleidae	*Thaumalea sp.*	76	69	524	65

Table 4.12 (Continued)

	Ceratopogonidae	*Atrichopogon sp.*	52	65	338	44
	Chironomidae	*Chironomus sp.*	76	65	494	31
	Simuliidae	*Simulium sp.*	52	72	373	31
	Peleocorhynchidae	*Glutops sp.*	76	58	443	58
	Stratiomyidae	*Euparyphus sp.*	64	38	245	58
	Tabanidae	*Chrysops sp.*	76	40	304	50
	Athericidae	*Atherix sp.*	64	58	373	42
	Emphidaidae	*Hemerodromia sp.*	64	58	373	42
	Dolichopodidae	*Hercostomus sp.*	52	48	251	58
	Syprhidae	*Eristalis sp.*	52	55	286	64
	Ephydridae	*Discocerina sp.*	76	55	418	57
	Scatophagidae	*Orthacheta sp.*	76	58	443	64
	Muscidae	*Limnophora sp.*	52	58	303	64
Coleoptera	Dytiscidae	*Agabus sp.*	64	32	203	42
	Gyrinidae	*Gyrinus sp.*	76	70	532	50
	Haliplidae	*Brychius sp.*	52	42	220	50
	Hydrophilidae	*Ametor sp.*	76	40	304	34
	Elmidae	*Optioservus sp.*	64	68	437	52
	Psephenidae	*Dicranopselapus sp.*	52	64	332	60
	Ptilodactilidae	*Anchycteis sp.*	64	74	476	68
	Scirtidae	*Scirtes sp.*	76	64	486	65
	Staphylinidae	*Carpelimus sp.*	76	42	317	65
	Amphizoidae	*Amphizoa sp.*	52	32	165	65
	Carabidae	*Thalassotrechus sp.*	76	23	177	73

Table 4.12 (Continued)

	Noteridae	*Suphiselus sp.*	76	44	338	65
	Helophoridae	*Helophorus sp.*	52	46	240	65
	Hydraenidae	*Hydraena sp.*	52	48	251	65
	Dryopidae	*Helichus sp.*	52	54	283	60
	Chrysomelidae	*Donacia sp.*	76	44	338	59
	Heteroceridae	*Lanternarius sp.*	76	38	291	73
	Curculionidae	*Bagous sp.*	76	53	401	73
Odonata	Aeshnidae	*Aeshna sp.*	76	43	329	58
	Petaluridae	*Tanypteryx (hageni)*	76	43	329	68
	Gomphidae	*Gomphus sp.*	76	43	329	60
	Cordulegastridae	*Cordulegaster sp.*	76	43	329	50
	Corduliidae	*Neurocordulia sp.*	76	43	329	58
	Libellulidae	*Libellula sp.*	76	52	393	58
	Calopterygidae	*Calopteryx sp.*	76	62	469	50
	Lestidae	*Lestes sp.*	76	62	469	50
	Coenagrionidae	*Agria sp.*	76	70	532	50
Hemiptera	Belostomatidae	*Belostoma sp.*	76	23	177	62
	Corixidae	*Trichocorixa sp.*	76	32	241	31
	Gelastocoridae	*Gelastocoris sp.*	76	32	241	73
	Naucoridae	*Ambrysus sp.*	52	32	165	53
	Nepidae	*Ranatra sp.*	76	23	177	72
	Notonectidae	*Notonecta sp.*	76	32	241	40
	Pleidae	*Neoplea sp.*	76	40	304	83
	Ochteridae	*Ochterus sp.*	76	40	304	73
	Saldidae	*Isocytus sp.*	64	40	256	69

Table 4.12 (Continued)						
Megaloptera	Corydalidae	*Corydalus sp.*	64	80	512	87
	Sialidae	*Sialis sp.*	64	80	512	62

Exposure

The frequency distribution of relative exposure risk for all genera from families tested within the 8 orders of aquatic insects indicate that most genera fall into four categories of exposure corresponding to the three stream habitats used in the model including hyporheic (21-30%), erosional (51-60%), depositional (71-80%), or a combination of the two (61-70%) (figure 4.3). Hyporheic organisms were not examined extensively in this analysis, which resulted in the majority of exposure values falling between 51 and 80%.
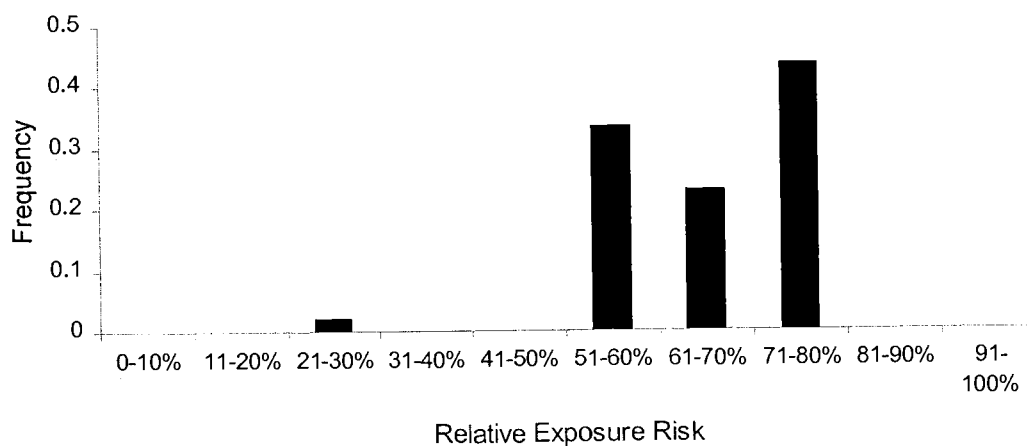
Figure 4.3. The frequency distribution of the relative exposure index for representative genera within each family (96 total) representing 8 orders of aquatic insects (Plecoptera (9), Trichoptera (17), Ephemeroptera (12), Diptera (22), Coleoptera (18), Odonata (9), Hemiptera (9)and Megaloptera (2)).

Frequency distributions representing the individual genera reveal how this is broken down by Order. Genera within the orders Trichoptera, Ephmeroptera, Diptera and Coleoptera all had distributions that spanned the 51 to 80% range

(figure 4.4 B-D). Frequency distributions for other orders fell within single exposure categories, including the Odonata (71-80%; figure 4.4 F), Hemiptera (51-60%; figure 4.4 G) and Megaloptera (51-60%; figure 4.2 H), reflecting preference for one specific type of stream habitat. Organisms falling within the lowest relative risk category (21-30%) occupy hyporheic stream habitats which incurred lower risk values. These included genera within the families Capniidae and Leuctridae in the order Plecoptera (figure 4.4 A).

Figure 4.4 A – H. Frequency distributions for the model component of exposure for representative genera within each family representing 8 orders of aquatic insects (Plecoptera (9), Trichoptera (17), Ephemeroptera (12), Diptera (22), Coleoptera (18), Odonata (9), Hemiptera (9) and Megaloptera (2)). Frequency of genera occurring within categories of percent relative risk as calculated by the model.

A: Plecoptera
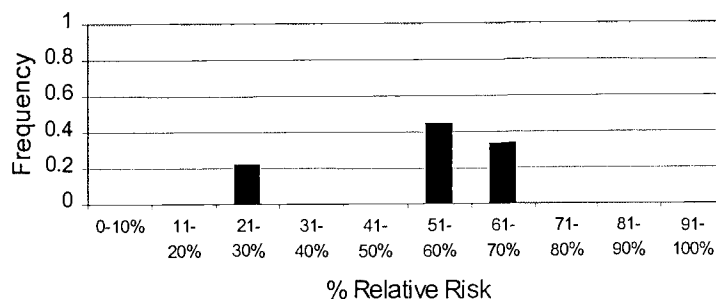
B: Trichoptera
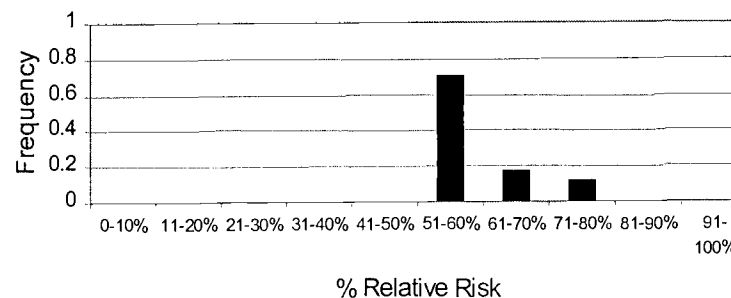
C: Ephemeroptera
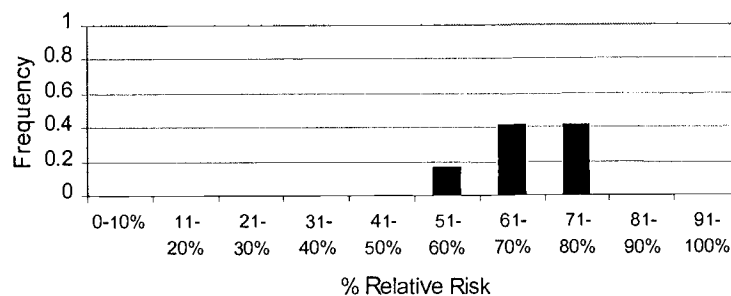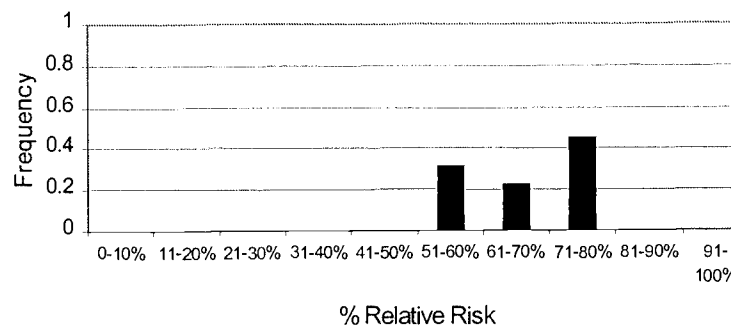
D: Diptera

Figure 4.4 A – H (Continued)

E: Coleoptera
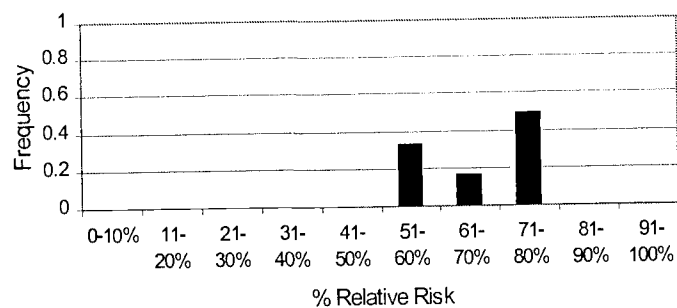
F: Odonata
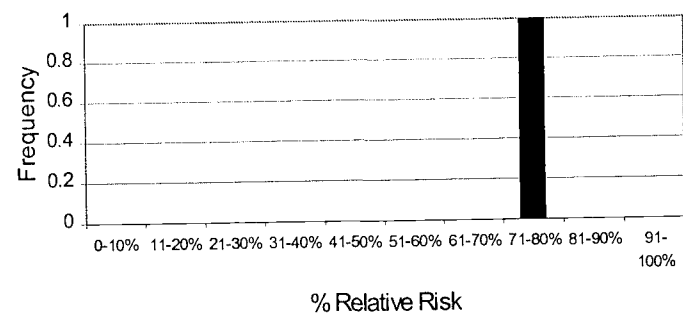
G: Hemiptera
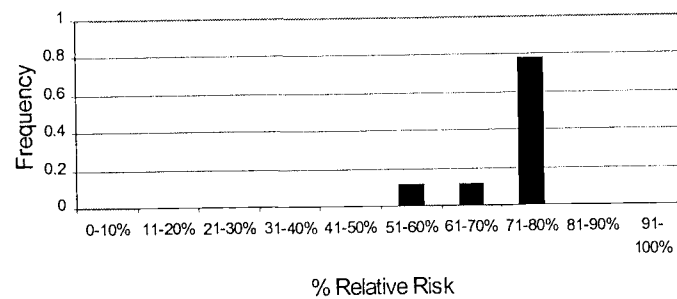
H: Megaloptera
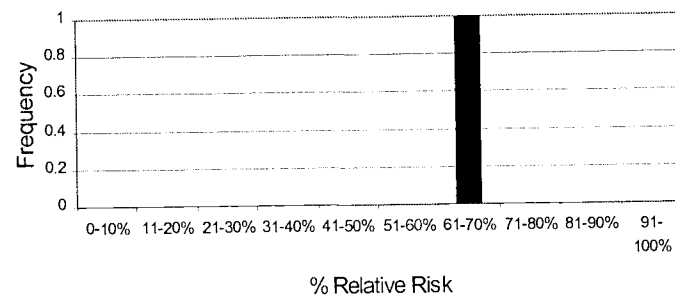
Uptake

The uptake frequency distributions for species within all eight orders is evenly distributed across the relative risk values, with a peak in the 61-70% range (figure 4.5). This reflects the wide distribution in different body forms and functional feeding categories across the orders.
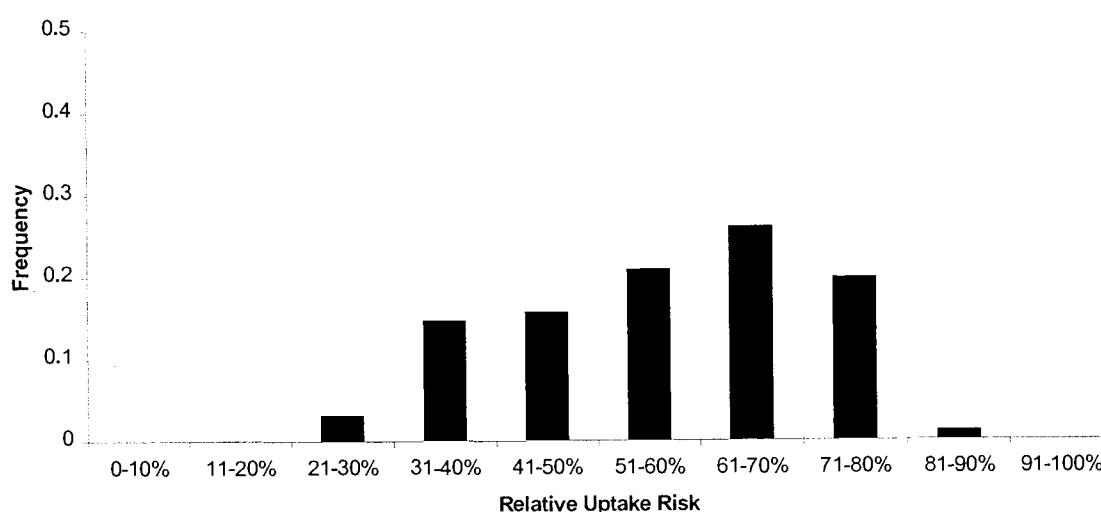
Figure 4.5. The frequency distribution of the relative uptake index for representative genera within each family (96 total) representing 8 orders of aquatic insects (Plecoptera (9), Trichoptera (17), Ephemeroptera (12), Diptera (22), Coleoptera (18), Odonata (9), Hemiptera (9)and Megaloptera (2)).

Representative genera from within families of the Plecoptera (9 families; figure 4.6 A), Trichoptera (17 families; figure 4.6 B) and Ephemeroptera (12 families; figure 4.6 C), which are reported to be more sensitive to pollutant stress, exhibited a trend for higher uptake indices relative to the other orders tested, with the Ephemeroptera having some genera within the highest uptake index of the three (the 71-80% range). The Diptera (22 families; figure 4.6 D) and Coleoptera (18

families; figure 4.6 D) both exhibited a wide frequency distribution, spanning the range from 21 to 80%. This is most likely a result of the wide variety of body forms and functional feeding strategies found in these orders. The odonate frequency distribution (9 families, figure 4.6 F) is somewhat biomodal, revealing differences in relative risk between the two suborders of dragonflies (Anisoptera) (6 families; 52-76%) and damselflies (Zygoptera) (4 families; 43%), which have different body form characteristics. The lowest range of uptake frequency distribution classes were found in the Hemiptera (figure 4.6 G), where risk for the majority of the 9 families fell within the 21 to 40 % range. The uptake distribution for genera within the two families of Megaloptera (2 families; figure 4.6 H), was high, entirely in the 71-81% range.

Figure 4.6 A-H. Frequency distributions for the model component of uptake for representative genera within each family representing 8 orders of aquatic insects (Plecoptera (9), Trichoptera (17), Ephemeroptera (12), Diptera (22), Coleoptera (18), Odonata (9), Hemiptera (9) and Megaloptera (2)). Frequency of genera occurring within categories of percent relative risk as calculated by the model.

A: Plecoptera
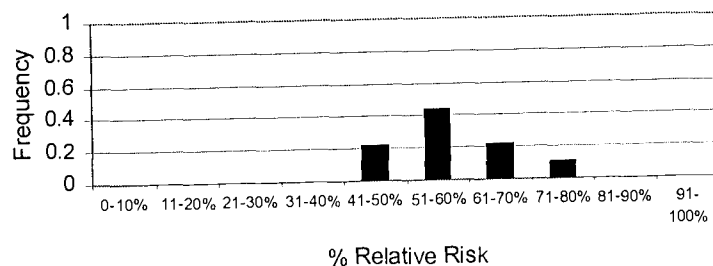
B: Trichoptera
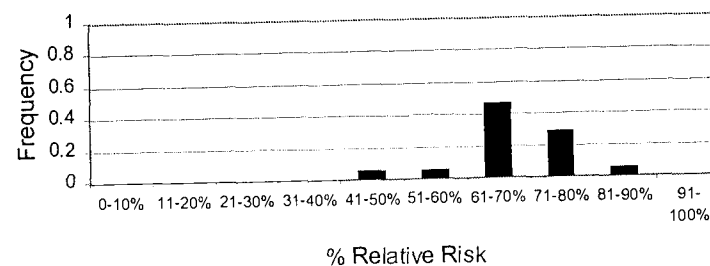
C: Ephemeroptera
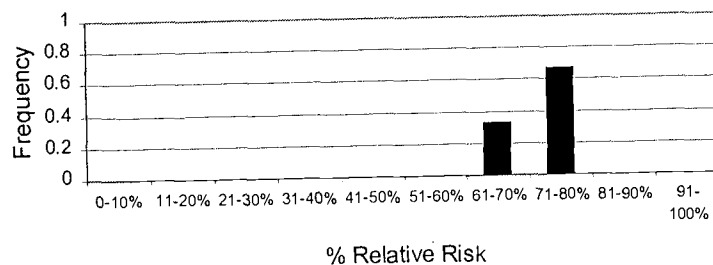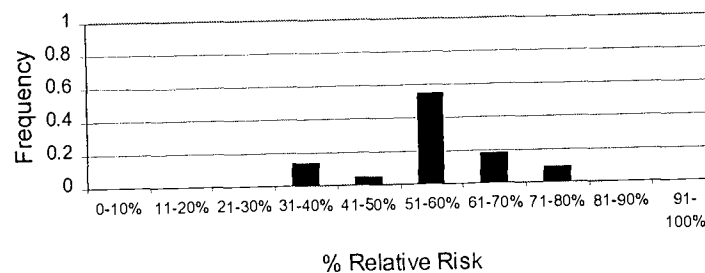
D: Diptera

Figure 4.6 Continued

E: Coleoptera

% Relative Risk

F: Odonata
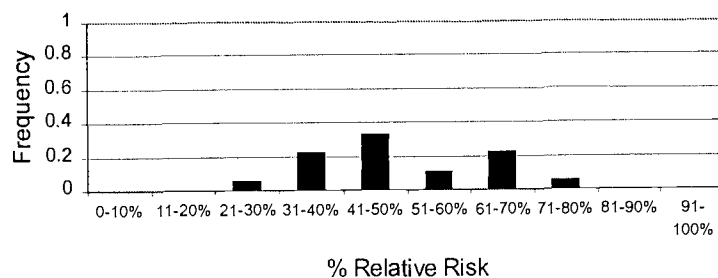
% Relative Risk
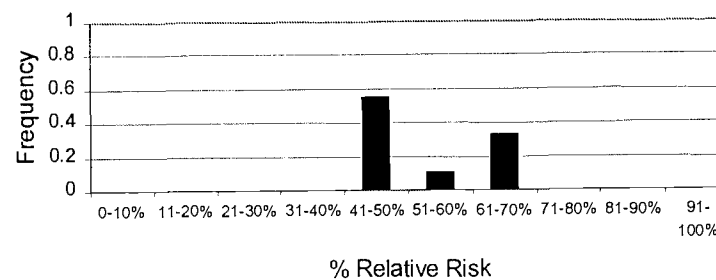
G: Hemiptera
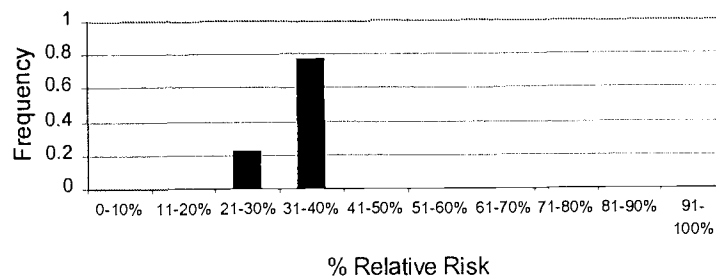
% Relative Risk

H: Megaloptera
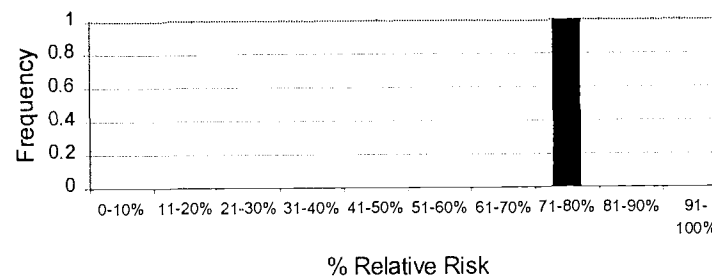
% Relative Risk

Relative Levels of Exposure and Uptake

Organisms with high or low indices for both components of exposure and uptake, would be expected to be at particularly high or low risk respectively, of accumulating large doses. Exposure and uptake risk indices were analyzed using exposure versus uptake plots in order to identify organisms with extreme values (low or high) of exposure and uptake. Based on these plots, a high exposure risk index was defined as greater than or equal to 76%, and low exposure as less than 52%. High uptake was defined as a risk index greater than or equal to 68%, and low uptake as less than 50%.

Based on the exposure and uptake risk indices calculated in Table 4.12, genera were identified that fell into four categories encompassing the range of values. The categories are defined as: category 1, high exposure ($\geq$76%) / high uptake ($\geq$66%); category 2, high exposure / low uptake; category 3, low exposure / high uptake; and category 4, low exposure / low uptake. This process divided the organisms into four sub-groups, leaving out the median groups.

Genera from families within the Orders Coleoptera and Diptera showed a variety of different exposure and uptake combinations, and had representatives in all four categories of exposure and uptake. This is most likely a result of the wide range of morphology and habitat association of the different genera within these orders. Genera falling into category 1 (high exposure / high uptake) categories include representatives of several Ephemeroptera families and from other families in other orders including the Calamocertidae (Trichoptera; *Heteroplecton* sp.), Thaumaleidae (Diptera, *Thaualea* sp., Gyrinidae (Coleoptera, *Gyrinus* sp.) and Coenagrionidae (Odonata, *Agria* sp) (Table 4.12).

Category 2 organisms (high exposure / low uptake) included the most of the Hemiptera, and all of the Odonata with the exception of Coenagrionidae. These are largely organisms with a high exposure index (pool dwelling), and a low uptake index (hard integument, no gills).

Organisms with attributes of low exposure and high uptake included many trichoptera families, and some Ephemeroptera and Diptera (Table 4.13). These organisms all have body forms that lead to high uptake, including small size and soft body form, but also inhabit riffles and areas of low chemical accumulation

Table 4.13. Families falling within four classes of exposure and uptake indices. High exposure / high uptake (category 1) implies the organism is at the highest risk for short-term effects. High exposure / low uptake (category 2) implies that while the organism is at a high risk for exposure based on the organisms' habitat association, but uptake rates may be low, thereby reducing effects. Low exposure / low uptake (category 3) implies the organism has attributes of low risk for short-term effects. Low exposure / high uptake (category 4) implies that exposure will be low, but that uptake will be high. Groups with median levels of exposure or uptake are not included.

	High Exposure (≥76%)	Low Exposure (≤52%)
High Uptake (≥66%)	**Trichoptera:** Calamocertidae, *Heteroplecton* sp. **Ephemeroptera:** Siphlonuridae, *Siphlonurus* sp. Ephemeridae, *Hexagenia* sp. Trichorythidae, *Tricorythodes* sp. Caenidae, *Caenis* sp. Baetiscidae, *Baetisca* sp. **Diptera:** Thaumaleidae, *Thaumalea* sp. **Coleoptera:** Gyrinidae, *Gyrinus* sp. **Odonata:** Coenagrionidae, *Agria* sp.	**Trichoptera:** Rhyacophilidae, *Rhyacophila* sp. Hydropsychidae, *Arctopsyche* sp. Brachycentridae, *Brachycentrus* sp. Helicopsychidae, *Helicopsyche* sp. Uenoidae, *Neothremma* sp. Sericostomatidae, *Gumma* sp. Philopotamidae, *Dolophilodes* sp. Hydroptilidae, *Hydroptila* sp. **Ephemeroptera:** Isonychiidae, *Isonychia* sp. Heptageniidae, *Cinygma* sp. **Diptera:** Blephariceridae, *Blepharicera* sp. Similidae, *Simulium* sp.

Table 4.13 (Continued)

	High Exposure (≥76%)	Low Exposure (≤52%)
Low Uptake (≤50%)	**Coleoptera:** Carabidae, *Thalassotrechus* sp. Noteridae, *Suphiselus* sp. Hydrophilidae, *Ametor* sp. Staphylinidae, *Carpelimus* sp. Chrysomelidae, *Donacia* sp. Heteroceridae, *Lantenarius* sp. **Diptera:** Tabanidae, *Chrysops* sp. **Hemiptera:** Belastomatidae, *Belostoma* sp. Corixidae, *Trichocorixa* sp. Gelastocoridae, *Gelastocoris* sp. Nepidae, *Ranatra* sp. Notonectidae, *Notonecta* sp. Pleidae, *Neoplea* sp. Ochteridae, *Ochterus* sp. **Odonata:** Aeshnidae, Aeshna sp. Petaluridae, *Tanypteryx hageni* Gomphidae, *Gomphus* sp. Cordulegastridae, *Cordulegaster* sp. Corduliidae, *Neurocordulia* sp.	**Plecoptera:** Capnidae, *Eucapnopsis* sp. Leuctridae, *Paraleuctra* sp. Perlodidae, *Isoperla* sp. Choroperlidae, *Swelsta* sp. **Diptera:** Dolichopodidae, *Hercostomus* sp. **Coleoptera:** Haliplidae, *Brychius* sp. Amphizoidae, *Amphizoa* sp. Helophoridae, *Helophorus* sp. Hydraenidae. *Hydraena* sp. **Hemiptera:** Naucoridae, *Ambrysus* sp.

Effect Values

Short-term effect values (Table 4.12) were calculated utilizing component indices of exposure (C_E) and uptake (C_U) (equation 3). The majority of the genera fell in the 300-400 and the 401-500 range, which are intermediate to low short-term effect indices. Genera with the lowest calculated short-term effect values (0-100) include representatives of families within the hyporheic stoneflies Capniidae (*Eucapnopsis* sp.) and Leuctridae (*Paraleuctra* sp.), the Coleopera family Carabidae (*Thalassotrechus* sp.), the Hemiptera families Belostomatidae (*Belostoma* sp.) and Nepidae (*Ranatra* sp.). Those genera with high effect values came mostly from families within the Ephemeroptera (Siphlonuridae, *Siphlonurus* sp.; Ephemeridae, *Hexagenia* sp.; Tricorythidae, *Tricorythodes* sp. Caenidae, *Caenis* sp.; and Baetiscidae, *Baetisca* sp.) but also included one Diptera (Thaumaleidae, *Thaumalea* sp.), one Coleoptera (Gyrinidae, *Gyrinus* sp.), and the Megaloptera (Corydalidae, *Corydalus* sp. and Sialidae, *Sialis* sp.).

LONG-TERM EFFECTS

Recovery component (C_R) values for each family are presented in Table 4.12. Overall trends in recovery summed across the 8 orders show wide distribution with a median in the 51-70% class, and extremes of 11-20% (low recovery potential) and 91-100% (high recovery potential) (figure 4.7). Only one genus fell in the 91-100% range (Trichoptera: *Glossotoma* sp.), and no genera fell in the 0-10% range.
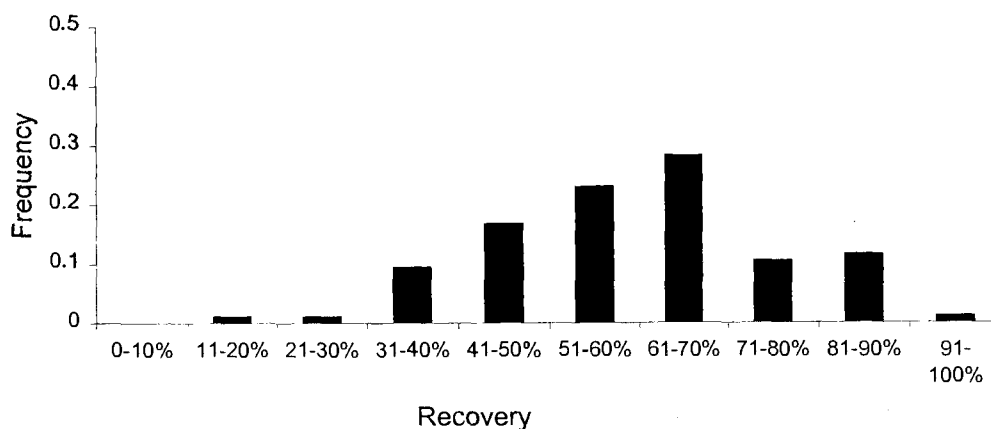
Figure 4.7. The frequency distribution for recovery combined over 8 orders of aquatic insects (Plecoptera (9), Trichoptera (17), Ephemeroptera (12), Diptera (22), Coleoptera (18), Odonata (9), Hemiptera (9) and Megaloptera(2)).

Frequency distributions of recovery indices (figure 4.8 A-H), show differences in the patterning of recovery characteristics. The orders Ephemeroptera (figure 4.8 C) and Diptera (figure 4.8 D) show a relatively wide distribution of recovery indices, indicating a wide variety of possible recovery characteristics between the selected taxa (i.e. number of generations per year, drift behavior, adult flight and adult life-span).

Genera with combinations of attributes that are suggestive of low potential rates of recovery (<50%) were found in the Orders Megaloptera (Corydalidae, *Corydalus* sp.) (15%), Ephemeroptera (Baetiscidae, *Baetisca* sp.) (36%), Diptera (Blephariceridae, *Blepharicera* sp.) (36%), Odonata (Petaluridae, *Tanypteryx hageni*) (39%), and Hemiptera (Gelastocoridae, *Gelastocoris* sp. and Ochteridae, *Ochterus* sp.) (39%). Other families including the Trichopteran family Glossostomadidae (*Glossosoma* sp.), had combinations of attributes that conferred high relative contributions to recovery (>70%) in the model. Other families in this category included several Ephemeropteran families (Isonychiidae, *Isonychia* sp.

(90%), Ameletidae, *Ameletus* sp. (86%), Siphlonuridae, *Siphlonurus* sp. (86%) and the dipteran families Chironomidae (81%) and Similiidae, *Simulium* sp. (84%).

Figure 4.8 A-H. Frequency distributions for the model component of recovery for representative genera within each family representing 8 orders of aquatic insects (Plecoptera (9), Trichoptera (17), Ephemeroptera (12), Diptera (22), Coleoptera (18), Odonata (9), Hemiptera (9) and Megaloptera (2)). Frequency of genera occuring within categories of percent relative risk as calculated by the model

A: Plecoptera
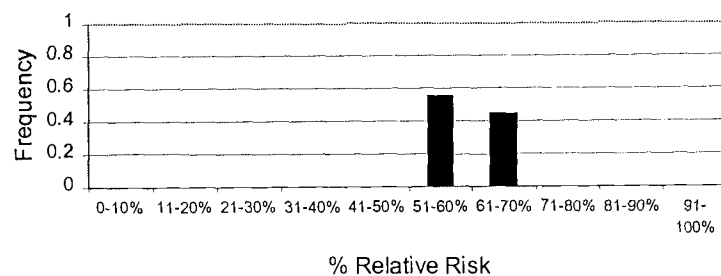
B: Trichoptera
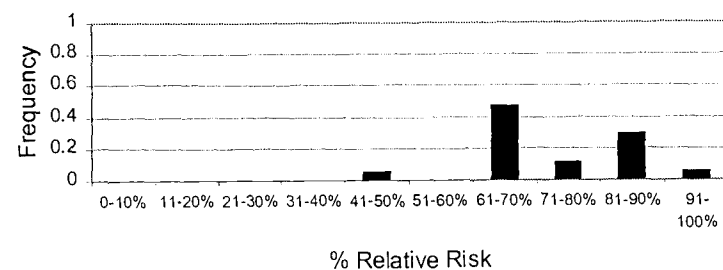
C: Ephemeroptera
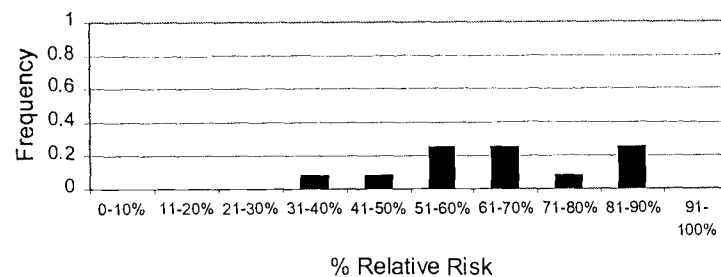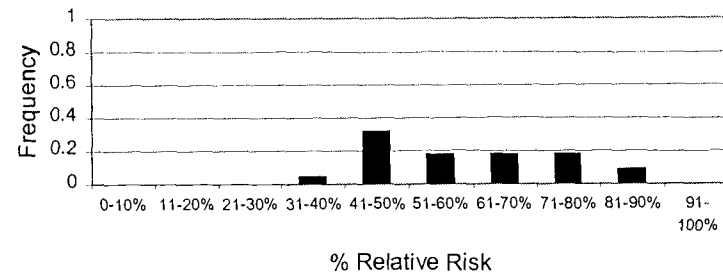
D: Diptera

Figures 4.8 (Continued)

E: Coleoptera
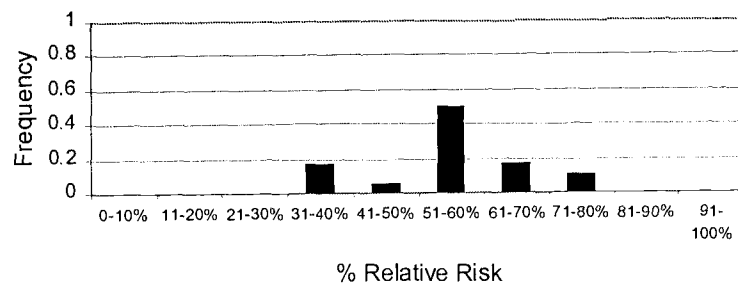
F: Odonata
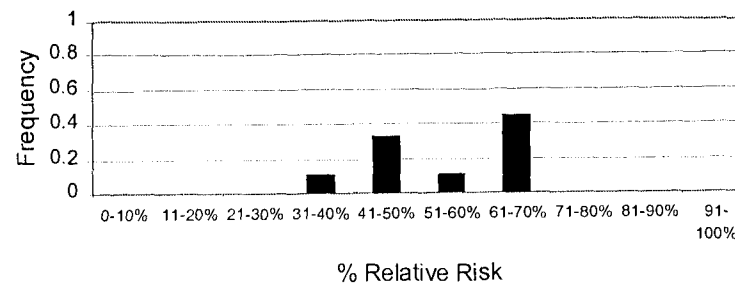
G: Hemiptera
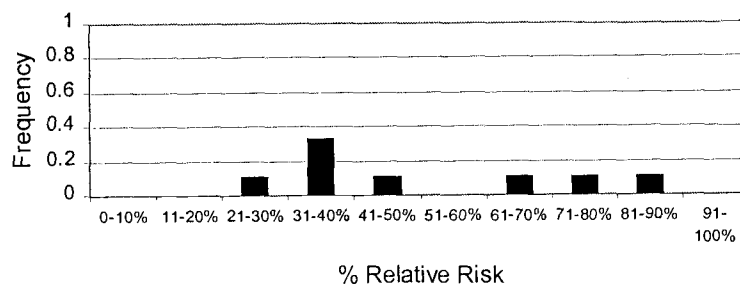
H: Megaloptera
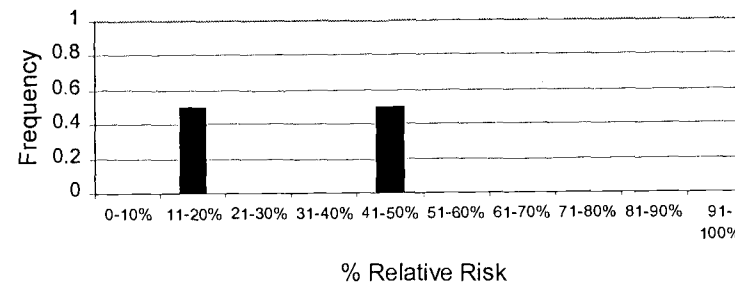
COMPARISONS OF SHORT AND LONG-TERM RISK INDICES

In order to compare combinations of short and long term risk indices between organisms, rank values were assigned to index values according to Table 4.14. A high rank value (10) indicates a high potential for short-term effects, while a high rank value for long-term indicates a high contribution for recovery. The number and pattern of different combinations were analyzed for the organisms used in this model (Table 4.15).

The combination of short- and long- term effect indices with the highest number of genera was a moderate short-term effect index (5), with a slightly higher recovery index (7) (Table 4.15). This corresponds to a short-term effect index between 401 and 500 and a recovery index between 61 and 70. Organisms were identified that fell into three categories of short-and long-term index combinations including: 1) High overall impact from a combination of high short-term (index value >460) and high long-term (low recovery) indices (<55); 2). Low overall impact from low short-term (<300) and low long-term (high recovery) indices (>70); and 3) Intermediate impact, which includes combinations of high short-term and low long-term, and low short-term and high long-term indices.

Table 4.14. Rank values assigned to short- and long-term index values for organisms used in the model. Ranking allowed for values of short- and long-term effects, which operate on different time scales, to be compared between taxa. A high rank value (10) indicates a high potential for short-term effects, while a high rank value for long-term indicates a high contribution for recovery.

Short-term Effects Index	Short-term Effects Index Rank Value	Long-term Effects Index	Long-term Effects Index Rank Value
1-100	1	1-10	1
101-200	2	11-20	2

Table 4.14 (Continued)			
201-300	3	21-30	3
301-400	4	31-40	4
401-500	5	41-50	5
501-600	6	51-60	6
601-700	7	61-70	7
701-800	8	71-80	8
801-900	9	81-90	9
901-1000	10	91-100	10

Table 4.15. A matrix showing the number of genera with ranks of different combinations of short- and long-term effect indices. A high rank value (10) indicates a high potential for short-term effects, while a high rank value for long-term indicates a high contribution for recovery.

	Long-term Recovery Rank Value									
Short-term Effects Rank Value	1	2	3	4	5	6	7	8	9	10
1										
2				2	1	2	2			
3				2	3	6	5	3	1	
4			1	2	6	5	7	4	7	1
5				2	3	8	11	3	1	
6		1		1	3	1	3		1	
7										
8										
9										
10										

Organisms in the high category included the Baetiscidae (Ephemeroptera) and Corydalidae (Megaloptera). Organisms at low risk included several families of Hemiptera (Corixidae, Naucoridae, and Notonectidae), and Diptera (Simulidae, Tabanidae, Athericidae, Emphididae, and Dolichopodidae) (Table 4.16).

The majority of organisms fell in the intermediate category of high short-term effects and low long-term effects (high recovery) portion of the matrix. Genera falling into this intermediate category included the mayflies Siphlonuridae and Tricorythidae, and the Coenagrionidae (Odonata) (Table 4.16). Families with combinations of low short-term effects and high long-term effects (low recovery) included the Carabidae and Heteroceridae (Coleoptera), and the Nepidae and Gelastocoridae (Hemiptera).

Table 4.16: Organisms identified by the model (31 genera) as falling into different combinations of short- and long-term effects. These categories included high overall impact from a combination of high short-term (index value >460) and high long-term (low recovery) indices (<55%), low overall impact from low short-term (<300) and low long-term (high recovery) indices (>70%), and intermediate impact, which includes combinations of high short-term and low long-term, and low short-term and high long-term indices. Genera with median short or long term index values are not included (65 genera).

	Low Long-term Effects (High Recovery) (>70%)	High Long-term Effects (Low Recovery) (<55%)
High Short-term Effects (>460)	**Trichoptera:** Lepidostomatidae, *Lepidostoma* sp. **Ephemeroptera:** Baetidae, *Baetis* sp. Ameletidae, *Ameletus* sp. Siphlonuridae, *Siphlonurus* sp. **Diptera:** Chironomidae	**Ephemeroptera:** Baetiscidae, *Baetisca* sp. Ephemeridae, *Hexagenia* sp. **Trichoptera:** Phyrganeidae, *Agrypnia* sp. **Diptera:** Thaumaleidae, *Thaumalea* sp. **Coleoptera:** Ptilodactilidae, *Anchycteis* sp. Scirtidae, *Scirtes* sp. **Megaloptera:** Corydalidae, *Corydalus* sp. Sialidae, *Sialis* sp.

Table 4.16 (Continued)

	Low Long-term Effects (High Recovery) (>70%)	High Long-term Effects (Low Recovery) (<55%)
Low Short-term Effects (<300)	**Plecoptera:** Capniidae, *Eucapnopsis* sp. **Trichoptera:** Polycentropodidae, *Polycentropus* sp. **Hemiptera**: Corixidae, *Trichocorixa* sp. Notonectidae, *Notonecta* sp. Naucoridae, *Ambrysus* sp. **Coleoptera**: Dytiscidae, *Agabus* sp.	**Plecoptera:** Perlidae, *Calineuria* sp. **Diptera:** Syprhidae, *Eristalis* sp. Stratiomyidae, *Euparyphus* sp. **Coleoptera:** Carabidae, *Thalassotrechus* sp. Heteroceridae, *Lanternarius* sp. Amphizoidae, *Amphizoa* sp. Helophoridae, *Helophorus* sp. Hydraenidae, *Hydraena* sp. **Hemiptera**: Nepidae, *Rantra* sp. Gelastocoridae, *Gelastocoris* sp. Saldidae, *Isocytus* sp. Belostomatidae, *Belostoma* sp.

CONCLUSIONS / DISCUSSION

This research provides a general approach for incorporating biological and ecological information into the risk assessment process. Methods include criteria for the identification, collection and analysis of this information in order to help explain variation in field effects between species. Parameters within components of exposure, uptake and recovery that may be important in determining the potential for effects were included, and specific information relating to these parameters was collected for the organisms relevant to the environment under study. Information of this kind provides an easily accessible biological database for aquatic insects that will help improve the understanding of the short and long-term impacts of chemical pollution.

Organism information used in the model was at the genus level, and it was assumed that this information could be taken to a higher taxonomic level to explain variation between families. However, indices of short and long-term effects need to be analyzed for different genera within a family in order to better understand variation in these characteristics at this level.

Ranks and weightings were used to distinguish between potential for short – and long-term effects between organisms based on parameters of exposure, uptake and recovery. Ranking and weighting of these characteristics were based on hypotheses generated from literature on the variation in invertebrate characteristics in morphology, behavior and life history, and first principles that determine rates of exposure and uptake in the environment. These hypothesis need to be tested in the laboratory and the field. For example, assumptions made in this research on the influence of the degree of respiratory exchange tissue and properties of the integument that determine permeability, can be determined in the laboratory.

SHORT-TERM EFFECTS

Comparisons of the short- term effects indices between different species found a wide variation in combinations of different attributes, which were reflected in the frequency distributions of indices. Variation in the short-term effects index suggests that some families may be at greater risk for high rates of exposure and uptake than others, based on characteristics of habitat association, activity level, food source and morphology. These attributes could in theory be as important as physiological susceptibility data in determining the outcome of short exposures. Genera with high short-term effects indices (>500) included many from the order Ephemeroptera and Trichoptera. These organisms had morphological attributes leading to high indices of uptake. However, variation in the indices occurred between genera within some orders, especially in the Plecoptera. This was a function of a reduced exposure index for some species classified as associated with the hyporheic zone, in addition to morphological differences between species. However, the Megaloptera and some species of Coleoptera and Diptera were also identified as having attributes that could lead to high short-term effects.

Studies examining the short-term effects of pesticide exposure in the field concentrate to a large degree on observations of communities before and after disturbance, including monitoring drift rates and quantitative sampling. Through these studies, some genera have been identified as being highly affected within the orders Plectoptera (Leuctridae *Leuctra* sp.; Nemouridae, *Nemoura* sp.; and Peltoperlidae, *Peltoperla* sp.), Ephemeroptera (Leptophlebiidae, *Paraleptophlebia* sp.; Baetidae, *Baetis* sp.; Heptageniidae, *Epeorus* sp. and *Heptagenia* sp.), the Trichoptera (Philopotamidae, *Dolophilodes* sp.; Hydropsychidae, *Parapsyche* sp.; and Limnephilidae, *Pycnopsyche* sp.), and the Diptera (Chironomidae and Simuliidae, *Simulium* sp.) (Wallace et al., 1989; Courtemanch & Gibbs, 1980; Cuffney et al., 1984; Gruessner & Watzin, 1996; Kreutzweiser & Sibley, 1991; Eidt & Weaver, 1983). Observations that the Ephemeroptera, Trichoptera and

Plecoptera are in general more sensitive to pollution relative to other aquatic orders in the field (Lenat, 1988) has lead to their use in the EPT index, which attempts to establish degree of community impairment using the proportion of these organisms as an indicator (Plafkin, 1989). Less information is available on effects within the orders Coleoptera, Odonata and Hemiptera, but genera cited as sensitive include *Optioservus* sp. (Coleoptera) and *Lanthus* sp. (Odonata) (Cuffney et al., 1984).

Many of the same organisms identified in field studies above were shown in this analysis as having characteristics of morphology and behavior that might lead to a high potential for short-term effects (i.e. most Ephemeroptera had high short-term effect indices). This type of analysis may provide a basis for interpreting the underlying reasons for effects in the field, some of which were analyzed here (i.e. morphology, stream position, behavior.

LONG-TERM EFFECTS

Unlike uptake and exposure characteristics, long-term effects at the population level are not addressed at all in toxicological bioassays. The recovery of a population after pesticide exposure may not be predictable from toxicity data alone (Sherratt & Jepson, 1993; Maund et al., 1997). A knowledge of the life histories of aquatic invertebrates has been shown to improve the ability to predict differences in recovery (Sherratt et al, 1999) and therefore long-term effects.

Wide variation in the potential for long-term effects was found between different species in the model, highlighting the need to identify organisms that are ecologically as well as physiologically susceptible to pesticide impacts. Genera with low recovery indices were found mostly in the Hemiptera, Coleoptera and Megaloptera. Genera with high recovery indices (>80%) were found mostly within the Ephemeroptera and Trichoptera orders, and a few in the Diptera (Chironomidae and *Simulium* sp.).

Based on a literature search of documented recovery times by Niemi et al, 1990, organisms were ranked at the ordinal level for time to recovery from quickest to slowest as Diptera, Ephemeroptera, Trichoptera, and Plectoptera. Coleoptera were not found to be well represented in the literature, but appear to recover similarly or more slowly than the Trichoptera. The propensity for Ephemeroptera and Diptera to recovery rather quickly may be a function of their drift rates relative to standing stock (Townsend & Hildrew, 1976), and the variety of life history strategies (i.e. univoltine, multivoltine, syncronous and asynchronous emergence) which increases the likelihood that reproductive adults will be present (Niemi et al., 1990). Recovery has been hypothesized to be slower for Trichopterans because they are often sessile or attached, thereby reducing their presence in drift, and some may have a generation time of more than a year.

The analysis of contribution to recovery did not address all components related to the potential for long-term effects. The timing of the disturbance can also influence rate of recovery if the organism is in a critical life stage or occurs in the autumn when lower drift rates and lack of winter reproduction may delay recovery until the following spring (Niemi et al., 1990). Various orders of aquatic invertebrates have been found to recover at different rates because organisms were in vulnerable stages of their life cycles at the time of pesticide application (Ide, 1968). In addition, for streams that depend on aerial recolonization, recovery may vary with latitude, depending on the timing of the disturbance in relation to the flight periods of adults (Wallace, 1990).

COMBINATIONS OF SHORT- AND LONG-TERM EFFECTS

Organisms with different combinations of short and long-term effect indices were identified in order to explore variation in the overall outcome of pesticide exposure. High rates of recovery could counteract high short-term acute impacts in

the field (i.e. many Ephemeroptera and Diptera). Organisms with attributes resulting in high short-term effects indices, but low rates of recovery may be highly affected in the treated area for an extended period of time relative to other aquatic insects (i.e. some mayfly genera (Baetiscidae, *Baetisca* sp.) and the Megaloptera). This brings into question the validity of risk assessments based upon sensitivity data alone.

Some combinations of attributes can confer less predictable responses in field populations. For example, rapid recovery can mask high short-term effects, which may be found with many Ephemeroptera (e.g. genera within the Siphlonuridae, Tricorythidae, Caenidae and Baetiscidae) and Diptera (Chironomidae). These organisms are highly affected immediately following disturbance, but have been found to return to pre-treatment numbers relatively quickly (Raven & George, 1989; Cuffney et al., 1984). In addition, attributes of low recovery can exacerbate the impact of low effects. This may be the case with some Coleoptera and Hemiptera, and the long-lived stoneflies (Perlidae).

This analysis evaluated characteristics of the organisms associated with ecotoxicological processes on two time scales, short- and long-term effects, that help explain variation in effects between organisms in the field. This analysis has the potential to identify underlying reasons for contraindicated between the laboratory and the field, and between two temporal scales, the short- and long-term. Although it is acknowledged that not all parameters were included in the model, the approach is general and flexible, such that new components and parameters can be added. This general approach, as summarized in appendix 4.1, can be applied to other biological systems, which may have different parameters , options and weights than what is described here.

Chapter 4 Appendix

Appendix 4.1. General approach for the identification of ecotoxicological component processes, parameters, parameter options, and rank values for a database model to determine the potential for short- and long-term effects for organisms exposed to pesticides in the field.

STEP 1: DETERMINATION OF MODEL COMPONENTS

For an organism exposed to a chemical in the environment, there are several major processes that determine the potential for toxicological impacts to individuals and subsequent impacts at the population level. These processes, termed components in the following model, span a range of morphological, physiological and ecological attributes that determine the potential for short and long-term side effects (sensu Jepson, 1993). As a broad generalization of ecotoxicological processes, the components that best capture the important phenomena in the present exercise were exposure and uptake, which make major contributions to the potential for short-term effects, and recovery, which includes all those aspects of population process and demography that determine longer-term impacts.

STEP 2: DETERMINING KEY PARAMETERS OF COMPONENT PROCESSES

The major components comprise of a number of parameters that describe the behavioral, physiological and morphological characteristics of organisms that underlie the component processes. In the following model, the minimum number of parameters required to accurately describe each component process are selected. Parameter number should be minimized by focusing on more general phenomena, and refining the possible parameters to include only those that are most important in determining the component process. Within each of the parameters, a set of options was defined that represents the range of features that exist for the habitat,

organisms or chemicals under consideration. These options are determined through knowledge of the system under study and literature search.

STEP 3: ASSIGNMENT OF RISK RANKINGS

Calculations of relative contribution to component processes are taxon specific. Each parameter option within a component is assigned a value, that when added together with the other parameter options within the same component results in an index of relative risk for each major component for a given species. The values assigned to the parameter options are determined using first principles, literature search or expert consensus. Within a parameter, the values assigned to particular options follow a simple sequences (1, 2, 3) or a more complex series (i.e. 0, 3, 4, 5, 9) to represent possible trends in the relative risk of an impact that each option conferred. In this analysis, high numbers contributed significantly to short-term risk. A rank of 0 represents a case of no additional risk. In the case of long-term impacts, high values indicate a large positive contribution to population level recovery.

STEP 4: PARAMETER AND COMPONENT CALCULATION

Component relative risk indices are calculated by adding the values assigned to parameter options. A weighting value is also assigned to each parameter within each component. This enables scaling and adjustment of the contributions that individual parameters make to the final risk index for each taxon.

Parameter values (equation 1) and component relative risk indices (equation 2) are calculated as follows. Parameter values (P) (converted to a % scale to aid in interpretation) are calculated as:

Equation 1 $\qquad$ $\mathbf{P = [R\,/\,R_{Max} \; x \; W] \; x \; 100}$

Where:

> $R = $ *Value assigned to a specific parameter option*
>
> $R_{Max} = $ *Maximum value assigned to any option within a specific parameter*
>
> $W = $ *Parameter weighting value*

A component risk index (C) for each taxon, is then calculated as the addition of the weighted parameter values (1-100) (equation 2).

Equation 2 $\qquad$ $\mathbf{C = \Sigma P_{1\text{-}n}}$

STEP 5: SELECTING REPRESENTATIVE TAXA

Taxa used in the model should be local and based on sampling of affected habitats. They should be taxonomically broad, but within a class and below phylum level.

STEP 6: DEVELOPMENT OF FREQUENCY DISTRIBUTIONS OF RISK INDICES

Frequency distributions are used to analyze the variation in component risk indices at range of taxonomic levels to examine patterns within and between orders and between selected species. This process establishes the range and relative occurrence of risk index values, and examines the overall pattern of characteristics of a particular community of organisms. In addition, families with extremes of risk for the different component processes are identified.

STEP 7: INTEGRATION OF SHORT AND LONG-TERM COMPONENT INDICES

Components that make up short-term risk are largely toxicological and physiological in nature, while components that describe long-term impacts are mostly ecological and landscape level processes. Component indices of short and long-term effects are not therefore combined quantitatively. Instead, the relative patterns of short and long-term risk between families of different orders may be investigated, and families with sets of attributes that might lead to high levels of risk could be identified. Categories of different combinations of risk are defined as: category 1 high short-term, high long-term; category 2: high short-term low long-term; category 3: low short-term, high long-term, and category 4: low short-term and low long-term risk. Category 1 organisms (high short-term and high long-term effects indices), are at the greatest risk for prolonged effects of pesticide exposure. Category 3 organisms may also show prolonged effects long-term effects, because although effects are low, recovery may be slow. Category 2 organisms (high short-term but low long-term effects indices) may be highly affected initially following pesticide exposure, but re-colonization is predicted to occur rather quickly. Category 4 organisms would be expected to be affected the least from the short- and long-term effects of pesticide exposure.

Chapter 5

A Database Analysis of Stream Invertebrate Community Level Variation
in the Potential for Short- and Long-term Effects of Pesticides

Jennifer L. Peterson and Paul C. Jepson

ABSTRACT

A database model was used to compare the potential for short- and long-term effects of pesticide exposure for macroinvertebrate stream communities from different geographical regions. The potential for exposure, uptake and recovery for stream macroinvertebrate assemblages from Oregon valley and cascade streams was analyzed using a model that described and ranked macroinvertebrate characteristics of morphology, behavior and life history to represent possible trends in relative risk. This analysis distinguished between the potential for short- and long-term risk between four geographically different stream types. Cascade streams exhibited a systematic trend toward a higher potential for short-term effects compared to valley streams. The potential for long-term effects varied between stream types and exhibited no trend.

INTRODUCTION

A variety of different approaches have been used to establish the effects of pollution on stream macroinvertebrate communities using community level indices of structure and composition. Monitoring data are often used to determine the degree of pollutant impact by examining properties of the assemblage present. Diversity indices attempt to provide a single value for the composition of a community based on the total number of different taxa present (i.e. Simpson, 1949; Shannon & Weaver, 1949). Biotic indices examine observed and expected community make-up are also used to determine degree of impact (e.g. Woodiwiss, 1964; Armitage et al., 1983; De Pauw & Vanhooren 1983; Ohio EPA, 1987; Karr et al., 1986). These methods are however, based solely upon enumeration of the taxa present, and do not take into consideration the morphological, behavioral or ecological attributes unique to the species present. These attributes may influence the potential for short- and long- term effects in the field (Chapter 4).

The relative toxicological risks that pesticides pose to macroinvertebrate communities from different streams could be determined using models that evaluate the attributes that determine relative risk between species in the short- and long-term including morphology, habitat, and life history characteristics. The analytical method proposed in chapter 4 is based on attributes that affect the potential for short-term effects (i.e. morphology, habitat association, functional feeding, body size) and those that affect population recovery (i.e. number of generations per year, adult life-span and vagility, and propensity to drift). This model explored the variation in these organismal characteristics that exist between different genera from the range of families from Pacific Northwest streams, and it tentatively identified taxa that may have an increased potential for suffering adverse effects in the field.

This model is used below to examine the effect that differences in community structure may have on the short and long-term sensitivity of aquatic invertebrate communities to pesticide effects. Using this approach, data that is collected for different stream types as a part of monitoring programs could be used to identify streams and watersheds that may be at an increased risk for the effects of pesticides.

Biological and physical data from Oregon Cascade streams have been collected through the U.S. Environmental Protection Agency's Environmental Monitoring Assessment Program (EMAP) regional pilot study for Western streams (Peck et al., 2000). The EMAP program was developed to assess the condition of the nation's ecological resources, and was designed to monitor indicators of pollution and habitat condition and seek links between human-caused stressors and ecological condition. Some objectives of the program are: 1) to collect high quality environmental data from streams and rivers across the region in order to describe their current ecological condition; 2) build a database for long-term monitoring, develop methodologies to advance the science and understanding of the ecological function of western ecosystems and the relation of human influence;

and 3) to build a program of ecological monitoring which will lead to better management and protection of these systems (Peck et al., 2000).

Measurements of taxon richness for Pacific Northwest streams has been found to be highly dependent on sampling effort (Li et al., in press). Monitoring data were made available from this program for streams in mountain Oregon Cascade and Willamette Valley streams that had been sampled extensively (approx. 50 samples), providing assemblage data for a variety of stream types (Li et al., in press). These data present a unique opportunity to explore the differences in potential short- and long-term risk within and between communities at the landscape scale using the model presented in chapter. This explores and develops a model approach and established the regime of data management and collection that is required in order to complete such an analysis. The values obtained should not however, be taken to be definitive, and are subject to validation through experiment and further observation.

MATERIALS AND METHODS

STREAM SELECTION AND SAMPLING

Streams used in this study were selected and sampled as a part of the EPA's initiative to develop methods for conducting western regional synoptic surveys for the Environmental Monitoring and Assessment Program in 1992 (Herlihy et al., 1997; Li et al., in press). Streams selected were wadeable valley and mountain streams (Cascades) located between 44°N and 45°N. Twinspan analysis (SAS, 1988) has been used to statistically separate these streams on the basis of macroinvertebrate assemblage data (Herlihy, unpublished data). These streams also fell into major geographical classifications of Cascades and Willamette Valley streams, which had physical characteristics according to Table 5.1. Cascade streams were higher gradient compared to valley streams (gradient 3-17%and <2,

respectively), cooler (7-12 °C and 10-20 °C), and had coarser substrates compared to valley streams (Li et al., in press). In addition, the riparian zones of cascade streams were more complex and the surrounding landscape was less disturbed than valley streams (Herlihy et al., 1997).

Within each cascade and valley stream classification, there were two sub-classifications (1 and 2). Within the cascade streams, cascade 1 streams tend to be cooler (8-11 °C) compared to cascade 2 streams (10-14 °C). Of the valley streams, valley 1 streams tended to be deeper and have a larger surrounding watershed than valley 2 streams (Herlihy et al., 1997).

Table 5.1: General physical characteristics of stream types separated by twinspan analysis. Data collected as a part of the Environmental Protection Agency's Environmental Monitoring Assessment Program (EMAP) (Herlihy et al., 1997).

Stream Name	Stream Classification	Elevation (feet)	Stream Temp. (°C)	Slope (%)	Mean Wetted Width (m)	Substrate & Fines (%)	Total Nitrogen (µg/L)	Total Phosphorus (µg/L)	% Riparian Zone
Beaver Creek	Valley 1	270	16.0	0.0	6.35	70.9	518.0	57.0	16.5
Camous Creek	Valley 2	280	21.0	0.150	4.07	7.27	249	76.0	0
Calapooia Creek	Cascade 1	2640	9.0	16.3	2.9	3.6	20.0	6.0	15.9
Gate Creek	Cascade 2	1110	11.0	2.9	7.7	1.8	28.0	6.0	98.5

COMMUNITY SENSITIVITY ANALYSIS

Out of six streams that were sampled extensively according to Li et al. (in press), one stream from each twinspan separation, valley 1 and 2 and cascade 1 and 2, was selected for this analysis (Table 5.2).

Table 5.2. Characteristics of streams selected for analysis. Data was collected by the Environmental Protection Agency's Environmental Monitoring Assessment Program (EMAP). Stream Classification signifies biological (assemblage) data between streams.

Stream Name	Stream Classification	Total No. Surber Samples Taken	Total No. Insect Genera Collected	Total No. Organisms
Beaver Creek	Valley 1	45	19	529
Camous Creek	Valley 2	45	17	2,809
Calapooia Creek	Cascade 1	45	51	2,237
Gate Creek	Cascade 2	59	59	4,154

Lists of genera from these four streams were analyzed using a model developed to examine relative differences in the potential for short-and long-term effects of stream insects to pesticides (Chapter 4). This model describes relative differences in the potential short- and long-term effects of pesticides by analyzing a set of attributes unique to each organism with respect to exposure, uptake, and recovery processes (Table 5.3). Parameters within the basic components of this model include characteristics that describe behavioral, physiological and morphological attributes of the organisms. The parameters used by the model are listed in Table 5.3. Parameter values describe the potential differences in relative ecotoxicological risk associated with these attributes, and are weighted according to first principles according to their perceived contribution to the component process.

Model output includes risk indices for each component process, and overall values for predictions of relative short and long-term effects, which are calculated according to the formulae presented in chapter 4. The model approach and associated calculation of risk indices was used to develop a method for comparing organismal attributes concerning the potential for field effects, rather then to establish definitive results.

Table 5.3. Summary of components of short-term and long-term effects evaluated in the macroinvertebrate susceptibility model (Chapter 4). Components are major processes that determine the potential for ecotoxicological impacts. Parameters describe behavioral, physiological and morphological characteristics of organisms that underlie component processes. Each parameter has several options which represent the range of potential attributes that exist within a macroinvertebrate community. These options are ranked based on first principles, literature search, or expert consensus, to represent possible trends in relative risk of impact that each option may confer. For components of short-term effects, exposure and uptake, a high rank value contributes significantly to short-term risk. For the long-term effect component of recovery, a high value indicates a large positive contribution to recovery. Weight values were assigned to each parameter to allow for scaling and adjustment of individual parameters.

COMPONENT	Parameter	Weight	Parameter Options	Option Rank Value
EXPOSURE				
	Habitat	0.8		
			Hyporheic Zone	1
			Erosional	4
			Depositional	7
			Laboratory	10
	Life Stage	0.2		
			Resting	1
			Active	2

Table 5.3 (Continued)

UPTAKE

Respiratory Exchange Mechanism	0.3		
		Atmospheric	0
		Cuticle	1
		Cased Gills	2
		Uncased Gills	3
Integument Permeability	0.25		
		Hard	1
		Mixture	2
		Soft	3
Food Source	0.2		
		Nothing	0
		Other Animals	3
		Algae	4
		Detritus / CPOM	5
		FPOM (no fan)	6
		FPOM (fan appendage)	9
Body Size	0.25		
		Large (20.1 mm and up)	1
		Medium (5.1 to 20.0 mm)	2
		Small (0 to 5.0 mm)	3

Table 5.3 (Continued)

RECOVERY

Generation Time	0.25			
		Multivoltine	7	
		Bivoltine	5	
		Univoltine	4	
		Semivoltine	3	
		Merovoltine	1	
Drift Behavior	0.4			
		High	5	
		Low	3	
		None	0	
Adult Flight	0.25			
		Strong	5	
		Weak	3	
		None	0	
Adult Life-span	0.1			
		Weeks	3	
		Days	2	
		Hours	1	

For each stream, data related to specific parameters of component processes for short-term (exposure and uptake) and long-term (recovery) were collected and parameter and component indices were calculated for each genus, according to the methods described in chapter 4 (Box 5.1). Species data collected from the literature for each species within each stream analyzed can be found in appendices 5.1 to 5.4 A for Beaver Creek, Camous Creek, Calapooia Trib and Gate Creek, respectively. Option values, calculated component risk indices within categories of short- (exposure and uptake) and long-term (recovery) effects, and index value summary distributions for each stream can be found in appendices 5.1 to 5.4 B - C.

Box 5.1 Steps Involved in Data Collection For Stream Genera

1) Organism characteristics were classified according to the model criteria presented in Table 5.3. The primary reference for the classification of organism characteristics was An Introduction to the Aquatic Insects of North America (Merritt & Cummins, 1996). If detailed information at the genus level was not available in Merritt & Cummins, it was obtained from literature more specific to each order including:

Plecoptera, Stewart & Stark, 1998
Trichoptera, Wiggins, 1977
Ephemeroptera, Edmunds et al., 1976
Diptera, Coleoptera, Odonata, Hemiptera and Megaloptera, Stehr, 1993.

2) Organism data within components of exposure and uptake (short-term) and recovery (long-term) were collected from the literature and assimilated in Excel (Microsoft, 1997) spreadsheets by Order. If data was unavailable for an organism in the calculation of a parameter, it was omitted from analysis

3) Option ranks were assigned according to Table 5.3, and exposure, uptake, effects and recovery indices were calculated for each species according to the formulae presented in chapter 4.

ANALYSIS

Frequency distributions were used to analyze variation in calculated indices of short-term effects (exposure and uptake) and long-term effects (recovery) (Tables 5.4-5.7). Frequency distributions for different streams were compared by creating distributions of differences between the frequency distributions, by subtracting one from another, to determine if risk indices for short- and long-term effects varied significantly between different stream types. Shifts in community frequency distributions between streams may indicate differences in potential for short- or long-term effects. For example, when the frequencies of one stream are compared with another, a shift to the right of the frequency distribution would indicate a higher potential for effects for one stream, whereas a shift to the left would indicate a lower potential.

RESULTS

Complete species lists along with associated indices of short-term effects (exposure and uptake) and long-term effects (recovery) from each stream are presented for Beaver Creek (valley 1), Camous Creek (valley 2), Calapooia Creek (cascade 1) and Gate Creek (cascade 2) in Tables 5.4 – 5.7, respectively.

Table 5.4. Genera risk indices for organisms collected at Beaver Creek (valley 1 classification).

Order	Family	Genus	Exposure	Uptake	Short-Term Effects Index	Recovery
Coleoptera	Elmidae	*Ampumixis*	48	37	179	68
Coleoptera	Elmidae	*Cleptelmis*	48	37	179	68
Coleoptera	Elmidae	*Lara*	48	36	173	61
Coleoptera	Elmidae	*Narpus*	48	37	179	68
Coleoptera	Elmidae	*Optioservus*	60	34	203	68
Diptera	Ceratopogonidae	*Culicoiidinae*	60	58	350	76
Diptera	Chironomidae	*Chironomini*[a]	48	57	272	92
Diptera	Chironomidae	*Orthocladinae*[a]	60	57	340	92
Diptera	Chironomidae	*Tanypodinae*[a]	60	50	300	92
Diptera	Chironomidae	*Tanytarsini*[a]	60	60	360	92
Diptera	Tipulidae	*Chrysops*	72	40	288	65
Diptera	Tipulidae	*Tipula*	60	53	317	62
Ephemeroptera	Caenidae	*Caenis*	72	65	468	62
Ephemeroptera	Ephemeridae	*Hexagenia*	72	68	492	46
Ephemeroptera	Leptophlebidae	*Paraleptophlebia*	48	73	352	60
Plecoptera	Nemouridae	*Malenka*	60	66	397	63
Plecoptera	Perlodidae	*Isoperla*	48	42	200	63
Trichoptera	Leptidostomatidae	*Lepidostoma*	60	73	437	73
Trichoptera	Psychomyiidae	*Psychomyia*	48	53	256	83

[a] chironomidae identified to the tribe level.

Table 5.5. Genera risk indices for organisms collected at Camous Creek (valley 2 classification).

Order	Family	Genus	Exposure	Uptake	Effects Value	Recovery
Coleoptera	Elmidae	*Dubiraphia*	48	34	163	68
Coleoptera	Haliplidae	*Haliplus*	48	46	221	64
Coleoptera	Staphylinidae	*sp.*	72	42	300	55
Diptera	Ceratopogonidae	*Culicoidinae*	60	58	350	76
Diptera	Chironomidae	*Chironomini*[a]	60	58	350	76
Diptera	Chironomidae	*Orthocladinae*[a]	60	57	340	92
Diptera	Chironomidae	*Tanypodinae*[a]	60	50	300	92
Diptera	Chironomidae	*Tanytarsini*[a]	60	60	360	92
Diptera	Empididae	*Hemerodromia*	60	58	350	77
Diptera	Tipulidae	*sp.*	60	40	240	63
Ephemeroptera	Caenidae	*Caenis*	72	65	468	62
Hemiptera	Corixidae	*Trichocorixa*	72	32	228	84
Odonata	Coenagrionidae	*Argia*	72	70	504	65
Odonata	Gomphidae	*Gomphus*	72	43	312	55
Trichoptera	Hydropsychidae	*Hydropsyche*	48	70	336	80
Trichoptera	Hydroptilidae	*Hydroptila*	48	89	427	67
Trichoptera	Leptoceridae	*Oecetis*	60	68	410	65

[a] Chironomidae identified to the tribe level.

Table 5.6. Genera risk indices for organisms collected at Calapooia Creek (cascade 1 classification).

Order	Family	Genus	Exposure	Uptake	Short-Term Effects Index	Recovery
Coleoptera	Elmidae	*Heterlimnius*	52	35	182	68
Coleoptera	Elmidae	*Lara*	52	36	188	61
Diptera	Ceratopogonidae	*Culicoidinae*	64	58	373	76
Diptera	Chironomidae	*Chironomini*[a]	52	57	295	92
Diptera	Chironomidae	*Orthocladinae*[a]	64	57	363	92
Diptera	Chironomidae	*Tanypodinae*[a]	64	50	320	92
Diptera	Chironomidae	*Tanytarsini*[a]	64	60	384	92
Diptera	Dixidae	*Dixa*	64	55	352	71
Diptera	Dixidae	*Meringodixa*	64	55	352	71
Diptera	Empididae	*Chelifera*	76	70	532	73
Diptera	Pelecorhynchidae	*Glutops*	76	58	443	*
Diptera	Psychodidae	*Maruina*	52	43	225	*
Diptera	Psychodidae	*Pericoma / Telmatoscopus*	76	55	418	63
Diptera	Simulidae	*Prosimulium*	76	80	608	73
Diptera	Simulidae	*Simulium*	52	72	373	84
Diptera	Tipulidae	*Antocha*	76	53	401	67
Diptera	Tipulidae	*Dicranota*	64	40	256	63
Ephemeroptera	Ameletidae	*Ameletus*	64	73	469	86
Ephemeroptera	Baetidae	*Baetis*	64	73	469	78
Ephemeroptera	Ephemerellidae	*Caudatella*	64	65	416	68
Ephemeroptera	Ephemerellidae	*Drunella*	64	63	402	68
Ephemeroptera	Ephemerellidae	*Serratella*	64	66	423	68

Table 5.6 (Continued)

Ephemeroptera	Heptageniidae	*Cinygma*	52	74	387	60
Ephemeroptera	Heptageniidae	*Epeorus*	52	73	381	60
Ephemeroptera	Heptageniidae	*Ironodes*	52	73	381	60
Ephemeroptera	Heptageniidae	*Rhithrogena*	52	73	381	60
Ephemeroptera	Leptophlebiidae	*Paraleptophlebia*	52	73	381	60
Plecoptera	Chloroperlidae	*Swelsta*	52	42	217	60
Plecoptera	Leuctridae	*Despaxia*	52	46	240	62
Plecoptera	Leucridae	*Megaleuctra*	52	46	240	62
Plecoptera	Nemouridae	*Malenka*	64	66	423	63
Plecoptera	Nemouridae	*Zapada*	64	74	476	63
Plecoptera	Peltoperlidae	*Yoraperla*	64	66	423	60
Plecoptera	Perlidae	*Calineuria*	52	53	277	53
Plecoptera	Perlodidae	*Isoperla*	52	42	217	63
Plecoptera	Perlodidae	*Perlinodes*	52	53	277	63
Plecoptera	Perlodidae	*Skwala*	52	33	173	63
Trichoptera	Brachycentridae	*Micrasema*	52	71	370	81
Trichoptera	Calamoceratidae	*Heteroplectron*	76	64	490	62
Trichoptera	Glossosomatidae	*Glossosoma / Anagapetus*	52	61	315	93
Trichoptera	Hydropsychidae	*Diplectrona*	52	83	430	81
Trichoptera	Hydropsychidae	*Parapsyche*	52	83	430	81
Trichoptera	Hydroptilidae	*Palaeagapetus*	52	81	422	70
Trichoptera	Lepidostomatidae	*Leptidostoma*	64	73	466	73
Trichoptera	Limnephilidae	*Apatania*	52	79	410	73
Trichoptera	Limnephilidae	*Ecclisomyia*	76	75	570	73
Trichoptera	Limnephilidae	*Oligophlebodes*	52	62	324	73
Trichoptera	Philopotamidae	*Dolophilodes*	52	75	390	85

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Table 5.6 (Continued)

Trichoptera	Rhyacophilidae	*Rhyacophila*	52	70	364	81
Trichoptera	Uenoidae	*Neothremma*	52	73	378	70

* No information in literature on generations per year; recovery index not calculated.
[a] Chironomidae identified to the tribe level.

Table 5.7. Genera abundance values and associated risk indices for organisms collected at Gate Creek (cascade2 classification).

Order	Family	Genus	Exposure	Uptake	Short-Term Effects Index	Recovery
Coleoptera	Dytiscidae	*Hydrovatus*	76	32	241	73
Coleoptera	Elmidae	*Ampumixis*	52	37	194	68
Coleoptera	Elmidae	*Cleptelmis*	52	37	194	68
Coleoptera	Elmidae	*Heterlimnius*	52	35	182	68
Coleoptera	Elmidae	*Lara*	52	36	188	61
Coleoptera	Elmidae	*Narpus*	52	37	194	68
Coleoptera	Elmidae	*Optioservus*	64	34	217	68
Coleoptera	Elmidae	*Ordobrevia*	52	37	194	68
Coleoptera	Elmidae	*Zaitzevia*	52	37	194	68
Diptera	Ceratopogonidae	*Culicoidinae*	64	58	373	76
Diptera	Chironomidae	*Chironomini*[a]	52	57	295	92
Diptera	Chironomidae	*Orthocladinae*[a]	64	57	363	92
Diptera	Chironomidae	*Tanypodinae*[a]	64	50	320	92

Table 5.7 (Continued)

Diptera	Chironomidae	*Tanytarsini*[a]	64	60	384	92
Diptera	Dixidae	*Dixa*	64	55	352	71
Diptera	Dixidae	*Meringodixa*	64	55	352	71
Diptera	Empidae	*Chelifera*	76	70	532	73
Diptera	Empidae	*Hemerodromia*	64	58	373	77
Diptera	Pelecorhynchidae	*Glutops*	76	58	443	*
Diptera	Simulidae	*Prosimulium*	76	80	608	73
Diptera	Simulidae	*Simulium*	52	72	373	84
Diptera	Tipulidae	*Antocha*	76	53	401	67
Diptera	Tipulidae	*Cryptolabis*	76	55	418	63
Diptera	Tipulidae	*Dicranota*	64	40	256	63
Diptera	Tipulidae	*Hexatoma*	64	48	309	63
Diptera	Tipulidae	*Rhabdomastix*	64	48	309	63
Diptera	Tipulidae	*Tipula*	64	53	338	62
Ephemeroptera	Ameletidae	*Ameletus*	64	73	469	86
Ephemeroptera	Baetidae	*Baetis*	64	73	469	78
Ephemeroptera	Caenidae	*Caenis*	76	65	494	62
Ephemeroptera	Ephemerellidae	*Caudatella*	64	65	416	68
Ephemeroptera	Ephemerellidae	*Drunella*	64	63	402	68
Ephemeroptera	Ephemerellidae	*Serratella*	64	66	423	68
Ephemeroptera	Heptageniidae	*Epeorus*	52	73	381	60
Ephemeroptera	Heptageniidae	*Ironodes*	52	73	381	60
Ephemeroptera	Heptageniidae	*Rhithrogena*	52	73	381	60
Ephemeroptera	Leptophlebiidae	*Paraleptophlebia*	52	73	381	60
Megaloptera	Sialidae	*Sialis*	64	70	448	48
Odonata	Gomphidae	*Octogomphus*	76	70	532	62
Plecoptera	Leuctridae	*Despaxia*	52	46	240	62

Table 5.7 (Continued)

Plecoptera	Nemouridae	*Malenka*	64	66	423	63
Plecoptera	Nemouridae	*Zapada*	64	74	476	63
Plecoptera	Peltoperlidae	*Yoraperla*	64	66	423	60
Plecoptera	Perlidae	*Calineuria*	52	53	277	53
Plecoptera	Perlidae	*Hesperoperla*	52	70	364	60
Plecoptera	Perlodidae	*Isoperla*	52	42	217	63
Plecoptera	Perlodidae	*Skwala*	52	33	173	63
Trichoptera	Brachycentridae	*Micrasema*	52	71	370	81
Trichoptera	Calamoceratidae	*Heteroplectron*	76	64	490	62
Trichoptera	Glossosomatidae	*Glossosoma / Anagapetus*	52	61	315	93
Trichoptera	Hydropsychidae	*Arctospsyche*	52	83	430	81
Trichoptera	Hydropsychidae	*Hydropsyche*	52	83	430	81
Trichoptera	Hydroptilidae	*Hydroptila*	64	62	398	68
Trichoptera	Lepidostomatidae	*Lepidostoma*	64	73	466	73
Trichoptera	Limnephilidae	*Hydatophylax*	76	73	553	73
Trichoptera	Philopotamidae	*Wormaldia*	52	65	338	81
Trichoptera	Polycentropodida	*Polycentropus*	52	57	295	77
Trichoptera	Psychomyiidae	*Psychomyia*	52	53	277	68
Trichoptera	Rhyacophilidae	*Rhyacophila*	52	78	407	68

* No information in literature on generations per year; recovery index not calculated.
[a] Chironomidae identified to the tribe level.

Frequency distributions for risk indices of short-term effects (exposure and uptake) and long-term effects (recovery) are presented in figures 5.1-5.4. In order to evaluate differences in frequency distributions between streams, and the magnitude of the differences, difference frequency distributions were developed. These distributions were created by subtracting the frequency distribution of one stream from the frequency of another. These difference frequency distributions show where differences in the potential for risk occurs between two stream assemblages. For example, if the distribution of stream A is subtracted from stream B, positive numbers indicate where stream A exhibits a higher frequency of organisms exhibiting a high potential for effects. Likewise, negative numbers indicate where stream B exhibits a higher frequency. Difference distributions of all pairwise comparisons between the four stream assemblages used in this research are presented in figures 5.5 – 5.9. Table 5.8 summarizes the differences between the four streams, which will be discussed further below.

Figures 5.1 A-D. Frequency distributions for the Beaver Creek (Valley 1 classification) species assemblage related to uptake (A), exposure (B), short-term effects (C), and recovery (long-term effects) (C).

A. Beaver Creek Exposure
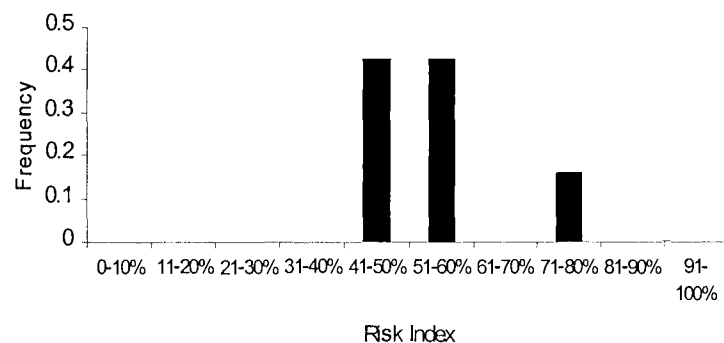
B. Beaver Creek Uptake
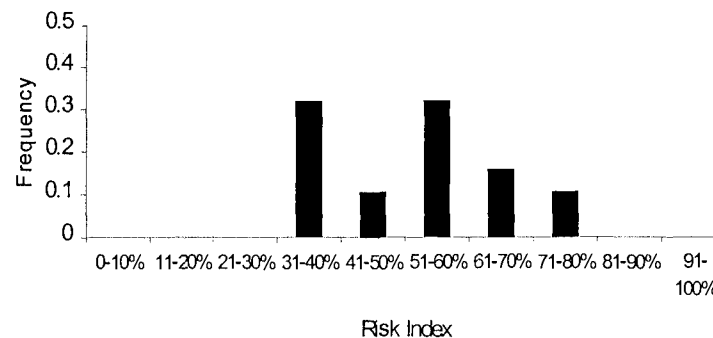
C. Beaver Creek Short-term Effect Index
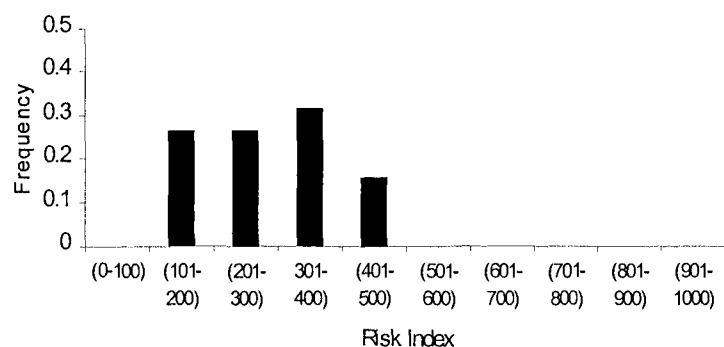
C. Beaver Creek Recovery Contribution
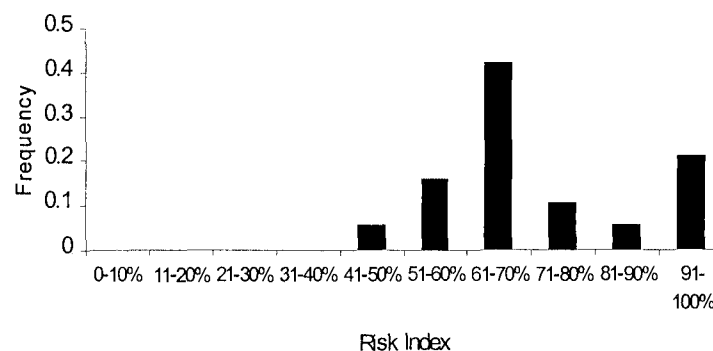
Figures 5.2 A-D. Frequency distributions for the Camous Creek (Valley 2 classification) species assemblage related to uptake (A), exposure (B), short-term effects (C), and recovery (long-term effects) (C).

A. Camous Creek Exposure
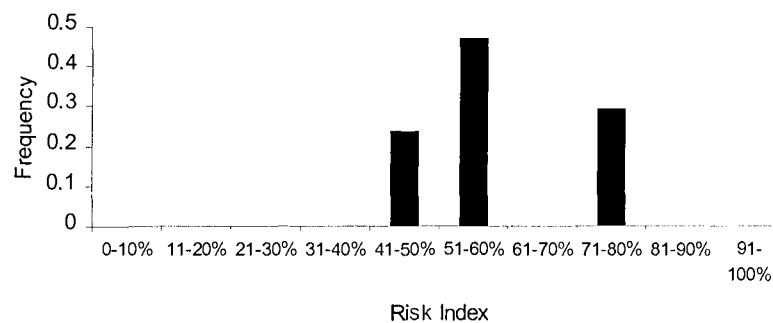
B. Camous Creek Uptake
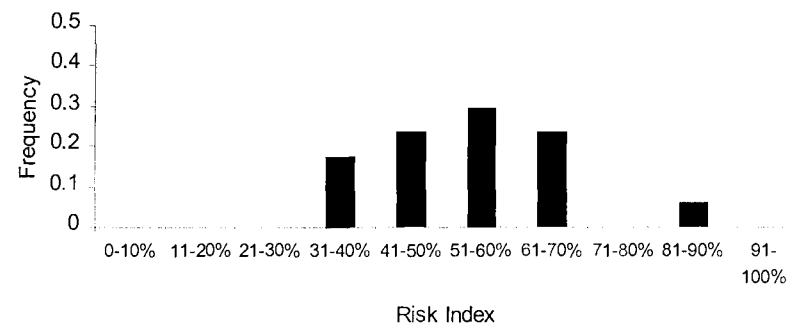
C. Camous Creek Short-term Effect Index
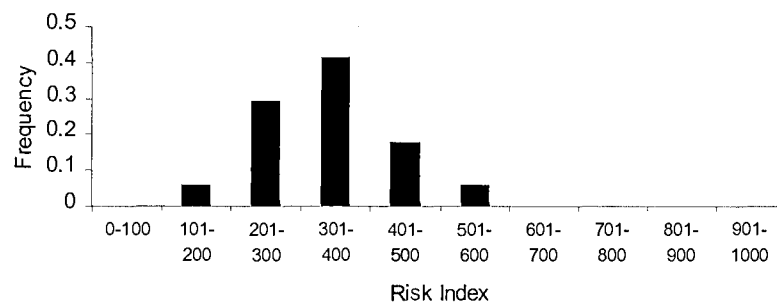
D. Camous Creek Recovery Contribution
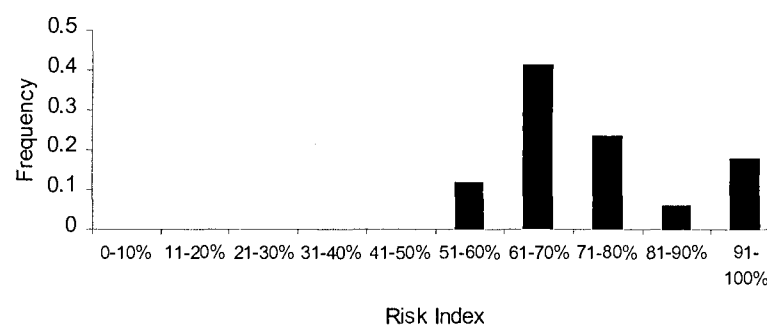
Figures 5.3 A-D. Frequency distributions for the Calapooia Trib (Cascade 1 classification) species assemblage related to uptake (A), exposure (B), short-term effects (C), and recovery (long-term effects) (C).

A. Calapooia Trib Exposure
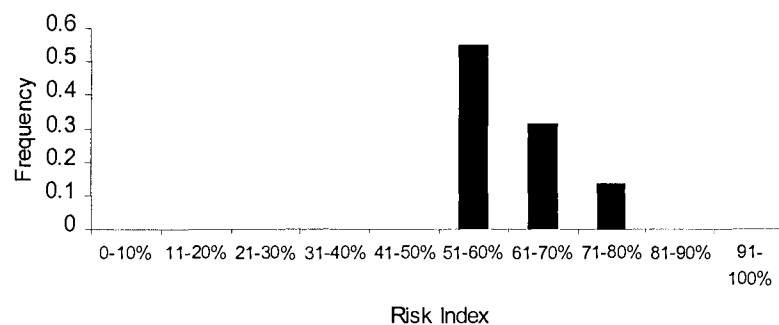
B. Calapooia Trib Uptake
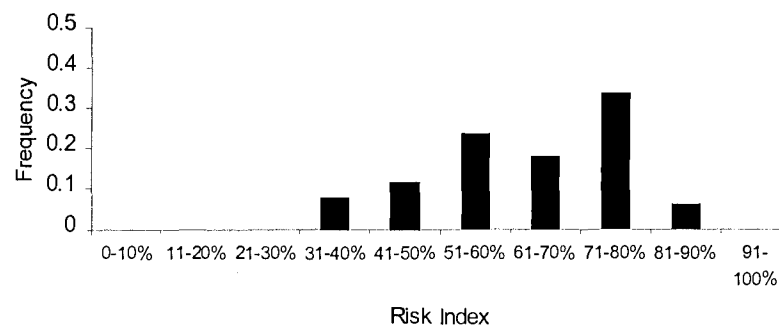
C. Calapooia Trib Short-term Effect Index
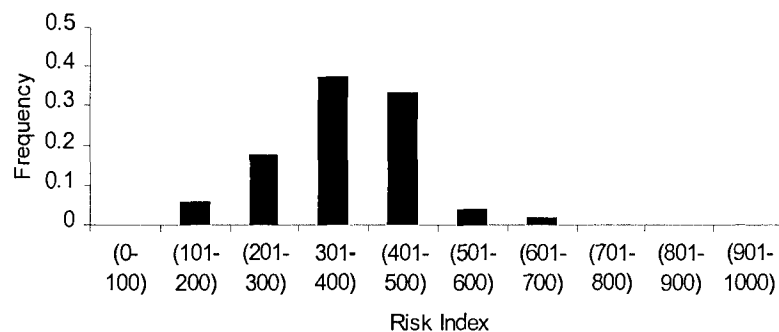
D. Calapooia Trib Recovery Contribution
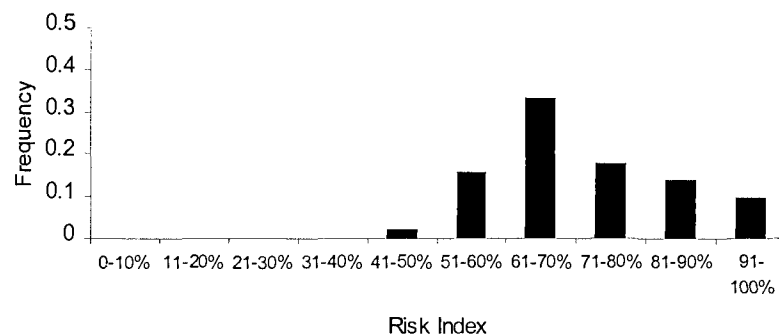
Figures 5.4 A-D. Frequency distributions for the Gate Creek (Cascade 2 classification) species assemblage related to uptake (A), exposure (B), short-term effects (C), and recovery (long-term effects) (C).

A. Gate Creek Exposure
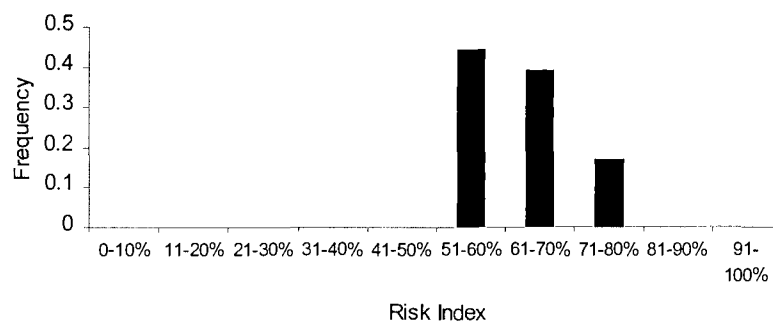
B. Gate Creek Uptake
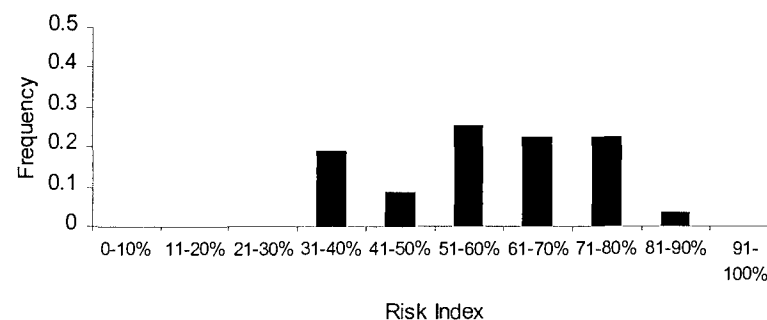
C. Gate Creek Short-term Effect Index
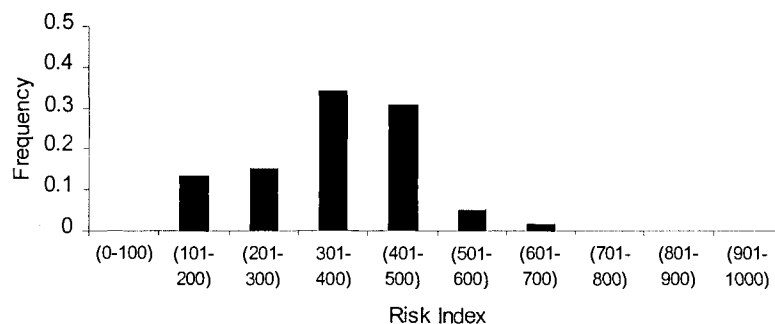
D. Gate Creek Recovery Contribution
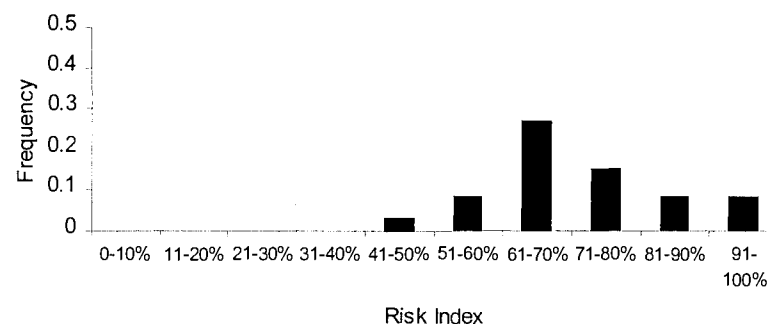
Table 5.8. Differences in indices of the short-term risk of pesticide exposure in categories of exposure and uptake, and long-term effects in the category of recovery as defined by the model developed in chapter 4. In addition to degree of difference between the two, shifts to the right or left indicate a systematic shift in the frequency distribution, indicating a higher potential (shift to the right) or a lower potential (shift to the left) for a given effect.

	Cascade / Cascade	Valley / Valley	Cascade / Valley C 1 / V 1 C1 / V2 C2 / V1 C2 / V2			
	Gate / Calapooia	*Beaver / Camous*	*Gate / Beaver*	*Gate / Camous*	*Calapooia / Beaver*	*Calapooia / Camous*
Exposure	10.8% difference. *Systematic trend for higher potential for risk in Gate Creek*	18.6% difference. *Systematic trend for higher potential for risk in Camous Creek.*	42% difference. *Systematic trend for higher potential for risk in Gate Creek.*	39% difference. *No systematic trend.*	44.2% difference. *Systematic trend for a higher potential in Calapooia Creek..*	39% difference. *No systematic trend.* Camous had a higher frequency of organisms at extremes (41-50 and 71-80).
Uptake	17% difference. *No systematic trend.*	26.6% difference. *No systematic trend.*	21.1% difference. *Systematic trend toward a higher potential for Gate Creek.*	23% difference. *Systematic trend toward a higher potential for Gate Creek.*	31.7% difference. *Systematic trend toward a higher potential for Calapooia Creek.*	33% difference. *Systematic trend toward a higher potential for Calapooia Creek.*

Table 5.8 Cont.

EFFECTS	17% difference. *No systematic trend.*	20.4% difference. *Systematic trend toward a higher potential for Camous Creek.*	23.8% difference. *Systematic trend toward a higher potential for Gate Creek.*	22% difference. *Systematic trend toward a higher potential for Gate Creek.*	29.1% difference. *Systematic trend toward a higher potential for Calapooia Creek.*	17.5% difference. *Systematic trend toward a higher potential for Calapooia Creek.*
Recovery	11.9% difference. *No systematic trend.*	13.6% difference. *No systematic trend.*	22.3% difference. *No systematic trend.*	6% difference. *No systematic trend.*	19.5% difference. *No systematic trend.*	17.6% difference. *No systematic trend.*

Figures 5.5 A-B. Frequency difference distributions for Gate Creek compared with Calapooia Creek in categories of exposure (A), uptake (B), the potential for short-term effects (C) and recovery (D).

A. Gate / Calapooia Exposure
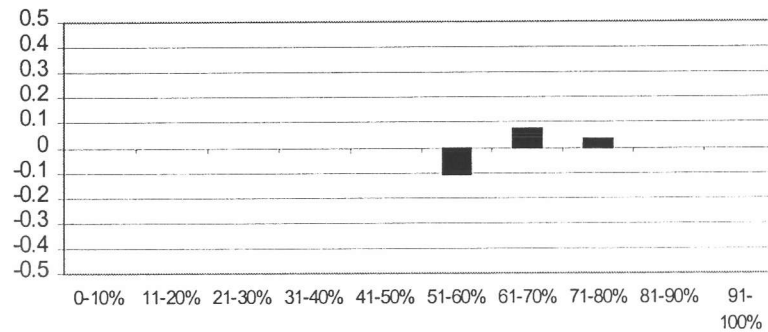
B. Gate / Calapooia Uptake
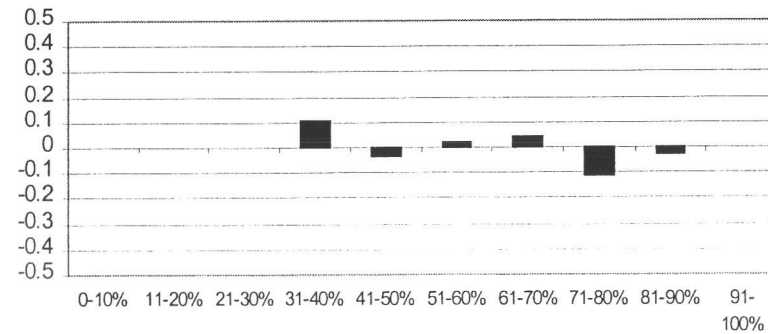
C. Gate / Calapooia Short-term Effects Indices
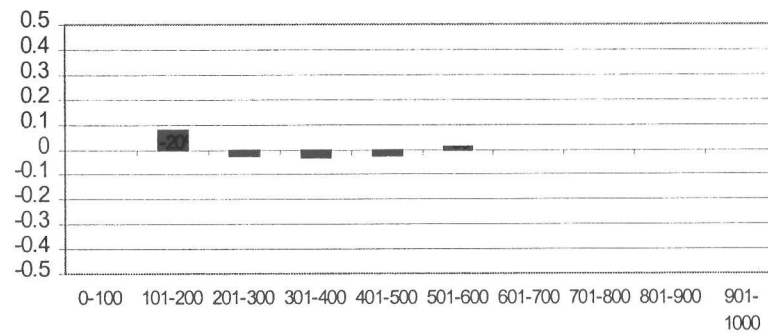
D. Gate / Calapooia Recovery
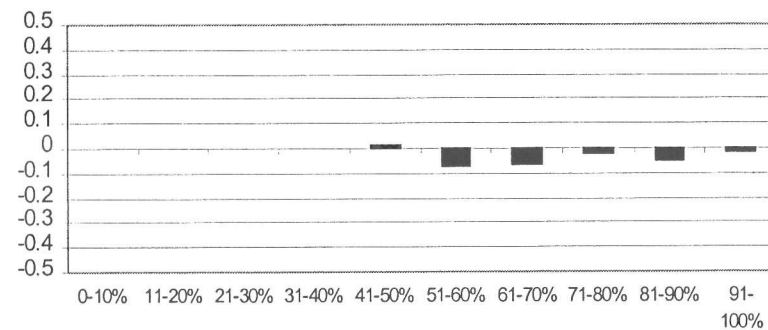
Figures 5.6 A-B. Frequency difference distributions for Beaver Creek compared with Camous Creek in categories of exposure (A), uptake (B), the potential for short-term effects (C) and recovery (D).

A. Beaver / Camous Exposure
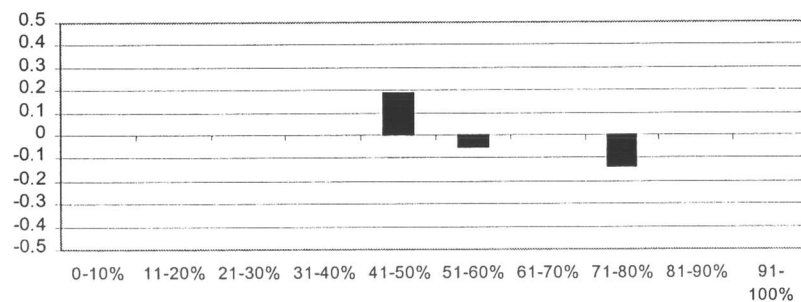
B. Beaver / Camous Uptake
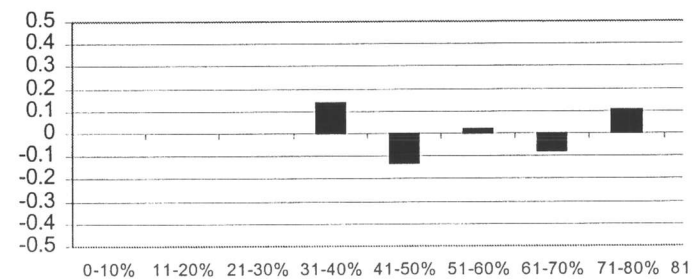
C. Beaver / Camous Short-term Effects Index
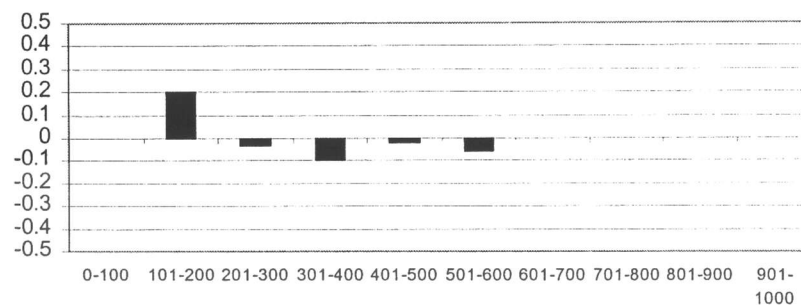
D. Beaver / Camous Recovery
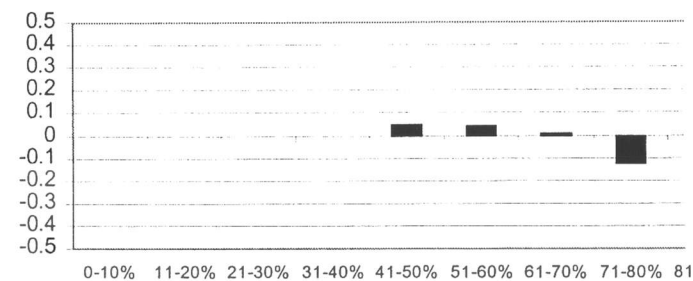
Figures 5.7 A-B. Frequency difference distributions for Gate Creek compared with Beaver Creek in categories of exposure (A), uptake (B), the potential for short-term effects (C) and recovery (D).

A. Gate / Beaver Exposure
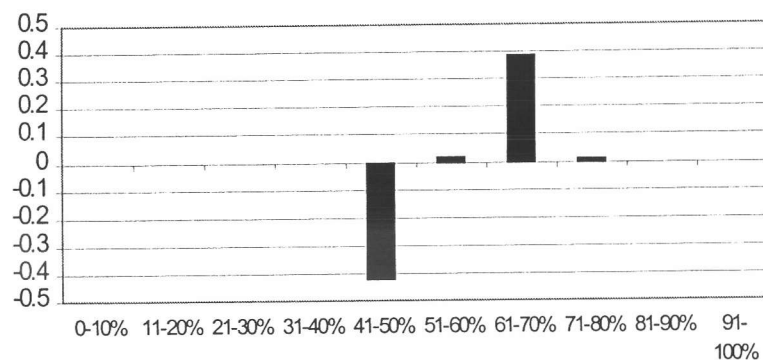
B. Gate / Beaver Uptake
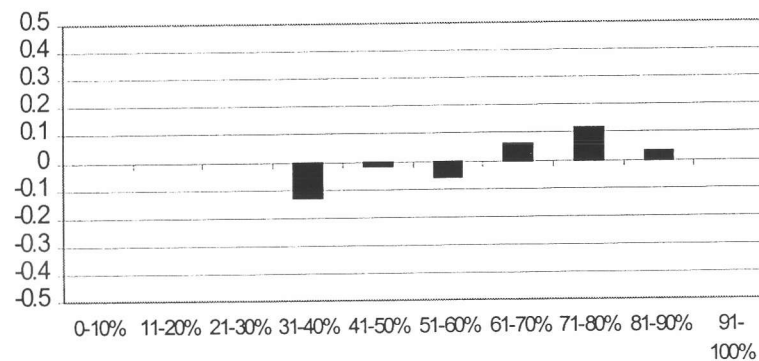
C. Gate / Beaver Short-term Effects Index
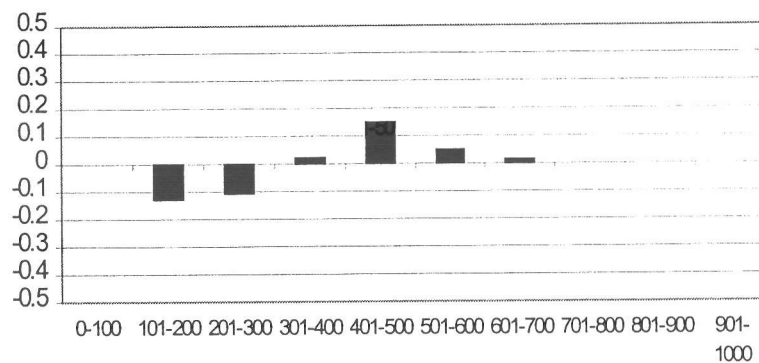
D. Gate / Beaver Recovery
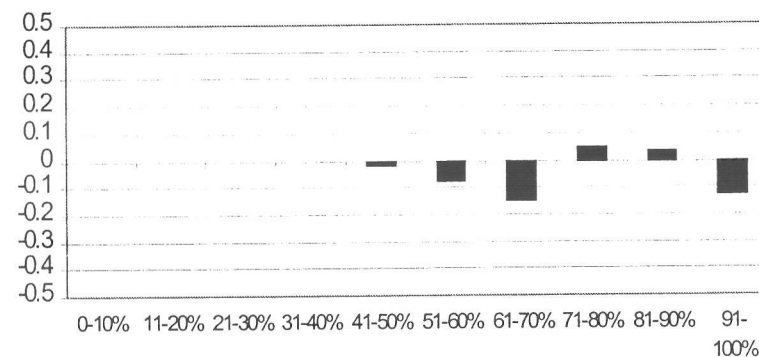
Figures 5.8 A-B. Frequency difference distributions for Gate Creek compared with Camous Creek in categories of exposure (A), uptake (B), the potential for short-term effects (C) and recovery (D)

A. Gate / Camous Exposure
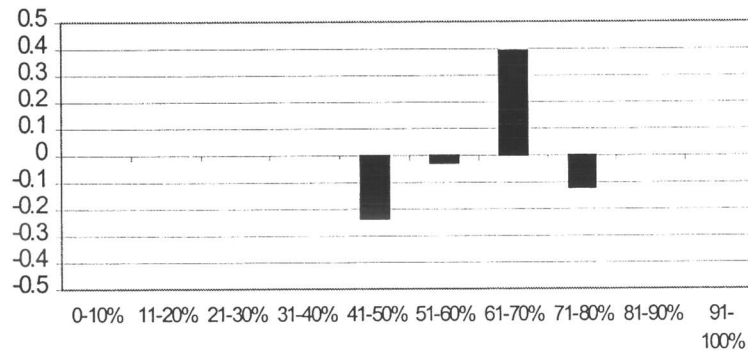
B. Gate / Camous Uptake
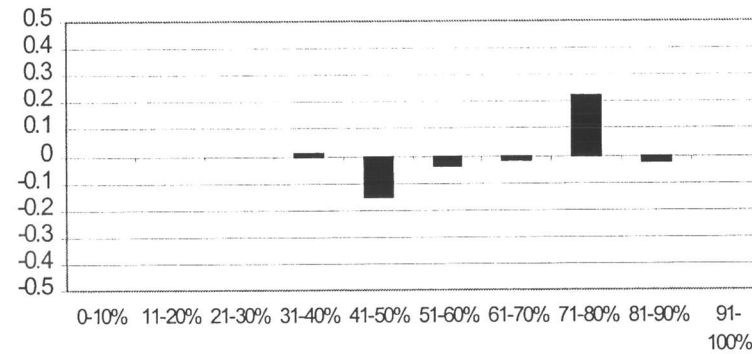
C. Gate / Camous Effects
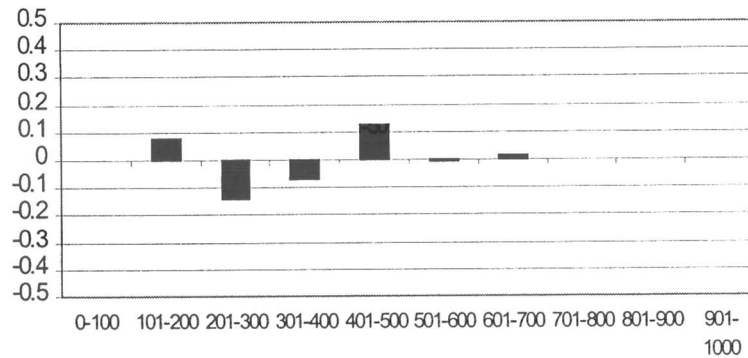
D. Gate / Camous Recovery
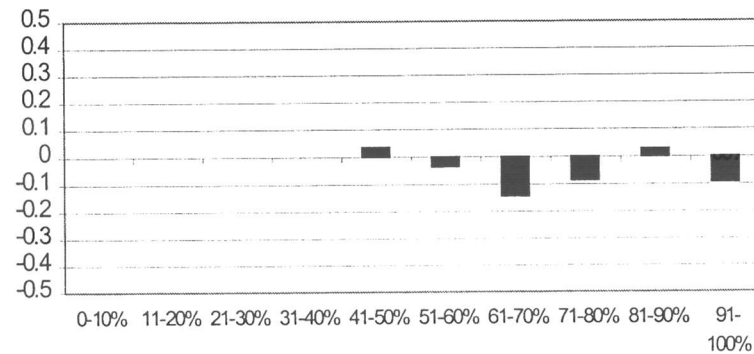
Figures 5.9 A-B. Frequency difference distributions for Calapooia Creek compared with Beaver Creek in categories of exposure (A), uptake (B), the potential for short-term effects (C) and recovery (D)

A. Calapooia / Beaver Creek Exposure
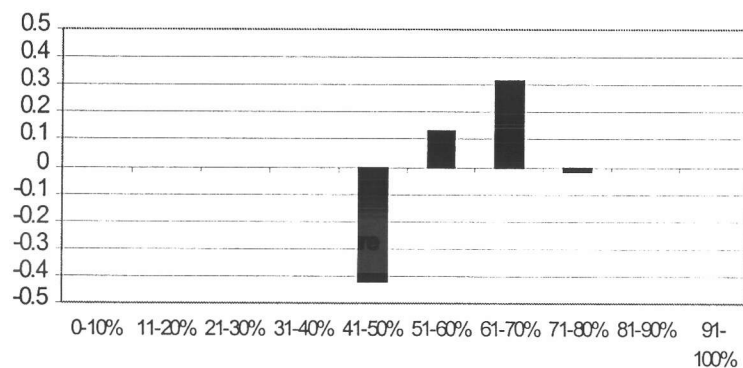
B. Calapooia / Beaver Creek Uptake
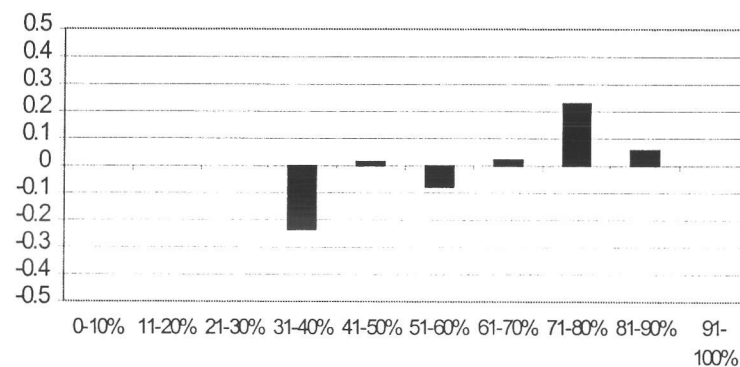
C. Calapooia / Beaver Creek Short-term Effects Index
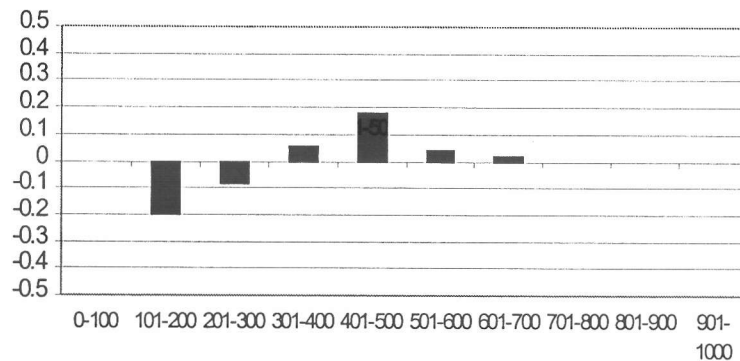
C. Calapooia / Beaver Creek Recovery
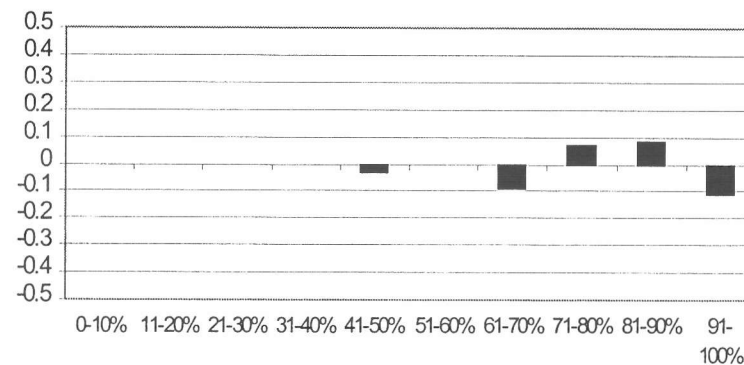
Figures 5.10 A-B. Frequency difference distributions for Calapooia Creek compared with Camous Creek in categories of exposure (A), uptake (B), the potential for short-term effects (C) and recovery (D)

A. Calapooia / Camous Creek Exposure
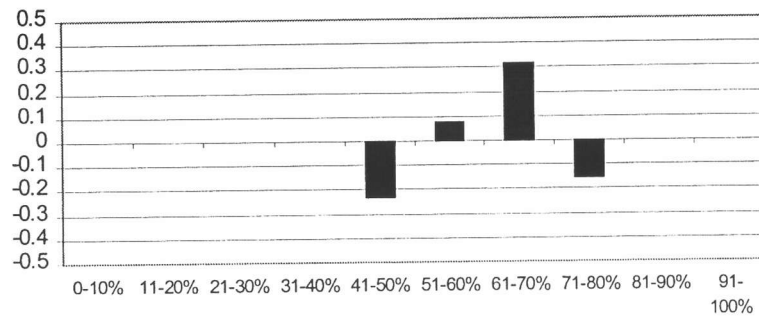
B. Calapooia / Camous Creek Uptake
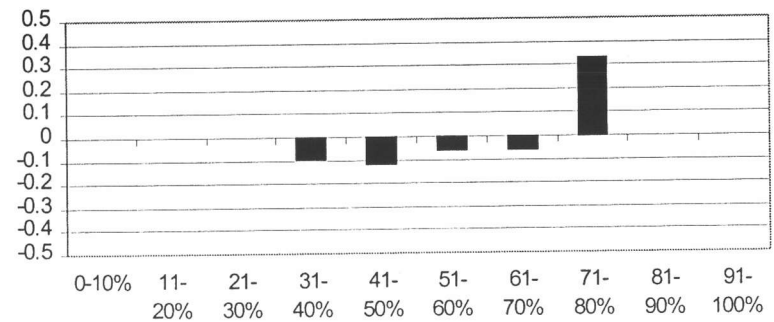
C. Calapooia / Camous Creek Effects
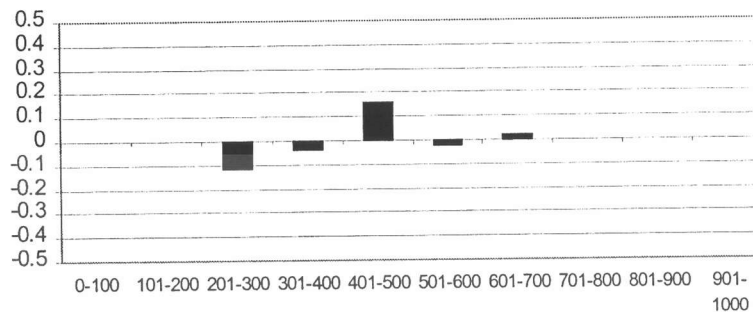
D. Calapooia / Camous Creek Recovery
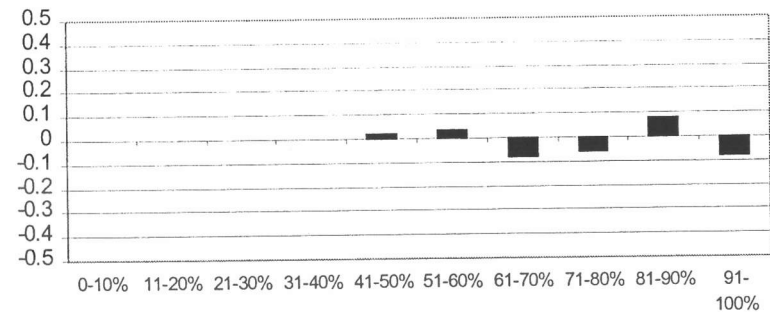
Overall, cascade and valley comparisons showed the most difference in the potential for short-term effects. Cascade streams showed a higher frequency of organisms exhibiting higher index values of short-term effects. These streams contained organisms determined to have a high potential for short-term effects according to the model (>400), including many genera in the Ephemeroptera *(Ameletus, Baetis, Caenis, Caudatella, Drunella*, and *Serratella)*, Plecoptera *(Malenka, Zapada,* and *Yoraperla)*, and Trichoptera *(Heteroplecton, Arctospsyche, Hydrophsyche, Lepidostoma, Hydatophylax,* and *Rhyacophila)*. Valley stream assemblages contained less organisms with a high potential for short-term effects. For example, each valley stream only contained one organism with an effects value >400 (Camous: Plecoptera, Cainidae, *Caenis* sp.; Beaver: Plecoptera, Nemouridae, *Malenka* sp.).

CASCADE 1 / CASCADE 2

Species lists from the two cascade streams were more diverse than the valley streams, with 51 and 59 different genera collected from Calapooia and Gate Creek, respectively (Li et al., *in press*) (Table 5.2).

Difference comparisons of frequency distributions between Calapooia (cascade 1) and Gate Creek (cascade 2) were small to intermediate for indices of exposure (10.8%), uptake (17%) and calculated short-term effects (17%) (5.5 A-C; Table 5.8). In addition, there was a systematic trend for a higher potential for exposure in Gate Creek compared to Calapooia Creek. This may suggest Gate has a higher composition of organisms inhabiting pools or areas of slower moving water, which according to the model will have a higher potential for exposure. Comparisons uptake and calculated short-term effect index frequency distributions between the two stream assemblages shows mixed differences, with no trends toward either one having a higher potential (figure 5.5 B-C). Based on indices of exposure and uptake, comparisons of the potential for short-term effects indices

between the two streams showed similar frequency distributions, with the exception of low effects (101-200), where Calapooia had a higher frequency of organisms (figure 5.5 C). Differences in the potential for long-term effects was small (11.9%), but Calapooia Creek consistently had a lower potential for recovery in most categories (figure 5.5D).

VALLEY 1 / VALLEY 2

Valley stream species less was considerably less diverse than those from the valley streams, with about 30 fewer genera collected compared to both of the cascade streams. In addition, the number of organisms collected from Beaver Creek was less (529) compared to the other three streams analyzed (Camous 2,809, Calapooia 2,237, and Gate Creek 4,154) (Table5.2).

Comparisons in the potential for short- term effects showed greater differences between the two valley streams than compared to the two cascade streams (Table 5.8). There were intermediate differences between the two in components of exposure (18.6%), uptake (26.6%) and overall potential for short-term effects (23.8%) (figure 5.6 A-C). There was no trend between the two in potential for uptake, but exposure and calculated short-term effects both showed a trend toward a higher potential in Camous Creek. Beaver Creek had a higher frequency of organisms with very low calculated short-term effects indices (101-200) (figure 5.6 C), which may be attributable to a more diverse assemblage of organisms incurring lower uptake indices, such as Coleoptera.

Differences between the potential for long-term effects between the two were small (<13.6%), but showed a trend toward a lower potential for recovery in Beaver Creek compared to Camous. The exception was high recovery index values (91-100%), where Beaver Creek had a higher frequency.

CASCADE / VALLEY

Most comparisons between cascade and valley streams showed a trend toward the cascade streams having a higher potential for short-term effects (Table 5.8). Differences in potential for exposure between the valley and cascade streams were large (39-44.2%). Comparisons between Beaver Creek and the two cascade streams (Gate Creek and Calapooia Creek) showed a systematic trend toward a higher potential risk in the cascade streams (figures 5.7A and 5.9A), while comparisons with Camous Creek showed no trend (figures 5.8 A and 5.10A).

Differences in frequency distributions for uptake potential between cascade and valley streams was intermediate in all cases (21.1-33%), with all pairwise comparisons between valley and cascade streams showing the two cascade streams with a trend toward a higher potential for uptake (figures 5.7 – 5.10 B). However, differences in uptake were greater between Beaver Creek and Camous Creek compared to the cascade stream Calapooia Creek (31.7 and 33%, respectively) compared to Gate Creek (21.1 and 23%, respectively). This may indicate that the Gate Creek assemblage is closer in potential for uptake to the two valley streams (higher overall) compared Calapooia Creek. Short-term effect index comparisons showed systematic trends toward the cascade streams having a higher potential compared to the valley streams. However, the difference was slightly greater for Gate and Calapooia comparisons with Beaver (23.8 and 29.1%, respectively) than for Camous (22% and 17.5%, respectively). This may indicate that Camous may be closer to the cascade stream assemblages for the potential for short-term effects compared to Beaver.

Differences in frequency distribution for the potential for recovery ranged from 6% to 22.3% for pairwise comparisons between the two valley (Beaver and Camous) and cascade (Gate and Calapooia) streams (figures 5.5 – 5.10 D). Difference frequency distributions showed a systematic trend toward a higher potential for recovery for Calapooia Creek compared to Beaver. Recovery showed trend between the cascade and valley streams, but Camous Creek exhibited lower

recovery potential overall compared to the two cascade streams Gate and Calapooia.

Analysis of the potential for long-term effects between different streams showed very few systematic trends, although the analysis identified the valley streams as consistently having more organisms in the highest category (91-100%). This may indicate the valley streams have a higher frequency of organisms with a higher potential for recovery.

CONCLUSIONS / DISCUSSION

This analysis represented a specific application of a model, which attempts to distinguish between organisms the potential for short- and long-term effects on the basis of differences in morphology, behavior and life history. The ranks and weights used in the model were developed on the premise of first principles, literature search or expert consensus (chapter 4). Further experimentation is needed to determine how these differences correlate with differences in field susceptibility, in both short and long-term.

This application of the model did distinguish between the potential for short- and long-term risk between four geographically different stream types. Overall, the model identified the two cascade streams, (Gate Creek and Calapooia Trib) as exhibiting a systematic trend toward a higher potential for short-term effects compared to the two valley streams (Beaver Creek and Camous Creek). Assemblages from the two cascade streams were more diverse, and included many organisms exhibiting high short-term effects indices (>400) compared to valley streams, including many Ephemeroptera, Plecoptera and Trichoptera genera. In comparison, the valley stream assemblages contained more organisms with lower potential for short-term effects, including Coleoptera and Diptera.

The trends were clearer within the model component of uptake compared with the exposure component. This may show that characteristics of the organisms

that govern the rate of uptake of a chemical in the environment, including morphology body size, integument permeability, respiratory strategy and food source, may be more useful in distinguishing in the potential for short-term effects between different stream assemblages than habitat association.

This type of analysis could be conducted in conjunction with biotic indices if species are identified to the appropriate level of taxonomic resolution. This analysis distinguished between community assemblages based on morphological, behavioral and life history characteristics of the species within a community. This approach provides more information on distinguishing attributes of community exposure, uptake and recovery than biotic indices, and provides a basis for ranking organisms in the potential for short- and long-term effects. This analysis could form the basis for comparative risk assessments for geographically different streams.

Chapter 6

Conclusions and Discussion

The objective of this research was to evaluate the risk of macroinvertebrate exposure to pesticides in the stream environment. Pesticides are widely used in throughout Oregon, however, very little data exists on the potential impacts of these pesticides on native stream communities. In order to increase the degree to which we can accurately assess the effects of pesticides to stream communities in both the short- and long-term, this research provided a quantification of uncertainty associated with risk assessment in four areas. These were: 1) the evaluation of the intrinsic susceptibility of organisms representative of the community of organisms representative of the region where the assessment is to occur; 2) an assessment of effects under conditions of varying duration of exposure and concentration which may be more representative of exposure in the stream environment; 3) an incorporation of the ecological attributes of the organisms in order to better assess the potential for effects in the short-and long-term in the field; and 4) an evaluation of distributions of attributes that affect exposure and uptake for community assemblages that differ taxonomically.

LABORATORY TESTING

CONTINUOUS TESTING

Single-species laboratory tests have been criticized for their lack of realism to the natural environment (Cairns, 1983; Kimball & Levin, 1985). Most regulatory tests currently utilize standardized test species, whose laboratory sensitivity may not be representative of the community where the risk assessment is to occur. In addition, the use of several species to evaluate organism sensitivity to a chemical is rare. In order to increase the applicability of laboratory test data to be predictive of effects that may occur in the field, test organisms representative of field communities were selected. Several species were tested in order to evaluate sensitivity across a range of morphology, development and function feeding

strategy from a community of organisms. The physiological susceptibility of this representative community was determined using standardized continuous, 96 h tests, which are currently used in regulatory testing. This allowed for comparisons to be made between sensitivity data obtained in this research with data in the literature.

Community sensitivity analysis (HC_5) (Kooijman, 1987) was used to establish concentrations that were protective of 95% of the theoretical community. The community sensitivity value is based on the sensitivity of test organisms selected to represent the diversity in taxonomy found in the natural environment. Therefore, the HC5 values calculated here for carbaryl and triclopyr may give a good estimation of the environment concentrations at which significant effects may occur in the field.

Sensitivity data from six representative organisms was used in the calculation of the HC_5. However, how much confidence can be placed in this estimate given the range of physiologies that exist within stream communities? Given that it is impractical to test all species within a community, this approach does provide a good estimate that is certainly better than a single species LC_{50} for one invertebrate species. However, future testing may expand our understanding of community sensitivity by testing additional representative species. This may include: 1) the random selection of test species to determine variation around the HC_5 and address bias that may have occurred due to limitations of testing in this research 2) systematic testing to capture existing variation in physiology, which may involve selecting representatives from each aquatic family. As more species are tested, the confidence we can place in community analysis such as the HC_5 increases.

Additional studies may address mechanisms in uptake, metabolism and elimination of pesticides that vary taxonomically between organisms. For example, initial studies have been conducted to determine the levels of detoxification enzymes that exists between different orders of aquatic insects (Siegfried & Young, 1993). Expanding studies in this area to address variation in sensitivity that may

exist between organisms from different Orders and families may lead to sensitivity factors that could be applied to all organisms within a community based on taxonomy.

PULSED TESTING

Continuous testing utilizes uniform exposure conditions and fixed durations of exposure (usually 96 h), that increase the probability of equilibrium between the concentration of the chemical in the water and the organism in order to maximize the probability of detecting an effect. However, studies have shown that exposure in stream systems may be variable and of a short duration (Richards & Baker, 1993; Bath et al., 1970). Therefore, the use of continuous, fixed duration testing alone to establish protective environmental concentrations may be over-protective where exposure in the field may be variable, and of a shorter duration. This research assessed the sensitivity of stream macoinvertebrates under pulsed, or shorter exposure durations in this research to explore to what extent the use of fixed durations is a built in safety factor or a design flaw, and the degree to which differences in sensitivity between the two types of testing can be quantified.

Significant differences between the sensitivity of two organisms, *Cinygma* sp. and *Calineuria californica* tested under continuous and variable exposure conditions was established in this research. Although both organisms exhibited similar sensitivity in continuous testing, there were significant differences in sensitivity to pulsed exposures. Differences in organism susceptibility to pulsed exposure may be a function of differences in uptake and elimination rates. Internal concentration, and subsequent effect, is determined through uptake and elimination of the chemical. Shorter exposure time may not allow for equilibrium between the water and the organism. Under these conditions, uptake rates become important in determining the internal concentration, or dose, the organism receives.

Variation in uptake rates that exist between organisms may help explain differences in susceptibility between organisms exposed in continuous and pulsed testing (Table 6.1). Organisms with low rates of uptake would accumulate lower concentrations of the chemical at all durations of exposure compared to those with high uptake rates. Differences of this kind may alter sensitivity rankings based on continuous testing alone, and suggests an important avenue of further research.

Additional experiments could help expand our understanding of properties influencing of uptake rates and subsequent effects. Whole body tissue residues could be measured after a range of exposure durations to determine how uptake varies between organisms. For example, ^{14}C labeled aminocarb was used to examine the effects of concentration and temperature on uptake for a stream isopod *Caecidolea*, and the clearance rate of the of residues (Richardson et al., 1983). This testing revealed the exposure period up to 12 hours incurs the most rapid uptake of the chemical, beyond which uptake falls off dramatically indicating a balance between uptake and clearance. Testing a range of organisms with different morphological and physiological characteristics could establish characteristics that are critical in determining uptake rates, such as properties of the integument, gill structures or other morphological structures that vary between organisms. Establishing differences in uptake rates as a result of morphological characteristics would establish quantitative relationships that could be used to improve database models (Chapter 4).

In addition to biological properties of the organisms, differences in uptake rates will vary with the properties of the test chemical (i.e. hydrophilic chemicals are more likely to cross a biological membrane). Testing a range of chemicals from different classes will help determine how chemical properties influence uptake rates.

Table 6.1. Characteristics of uptake and consequences for internal concentration which may help explain differences in susceptibility between organisms determined from continuous and pulsed testing.

	Internal Concentration	Differences in Toxicity Between Continuous and Pulsed Tests
High Uptake	A relatively short exposure period is required to accumulate a concentration that may result in an effect.	Difference in effects between pulsed and continuous testing not likely to be significant.
Low Uptake	A longer exposure period is required to accumulate a significant concentration to result in an effect	A significant different in effects would be seen between pulsed and continuous tests. The organism would be expected to be less sensitive to pulsed exposures.

Probit Plane Model

Results from pulsed testing were used to develop a probit plane model to predict mortality at different combinations of dose and time for *Cinygma* sp. exposed to carbaryl. This model provides a tool for decision makers in predicting effects under field conditions, where exposure duration is variable. This data may be useful in establishing protective environmental concentrations for environments where organisms may be exposed for shorter periods than are evaluated in longer duration, continuous testing.

Confidence in the model developed in this research to predict effects as a function of both concentration and duration could be improved by testing additional exposure durations between the short exposures (15, 30 and 60 min) used in pulsed testing (chapter 3) to the longer exposures used in continuous testing (96 hours) (chapter 2). Additional testing could also help identify thresholds that exist for combinations of concentration and duration of exposure. For example, a 60 minute

exposure may elicit effects equivalent to a 96 hour exposure at identical concentrations.

The model developed in this research was developed to predict toxicity as a result of carbaryl exposure to one invertebrate species, *Cinygma* sp. However, given the differences in sensitivity between *Cinygma* sp. and *Calineuria californica* established in chapter 3, a probit plane model for *Calineuria californica* may look quite different. Extending this type of testing to additional organisms that encompass the wide variation in morphology and physiology that exists in macroinvertebrate communities will improve our understanding of field susceptibility.

Parameters of the model will also vary with the properties of the test chemical. Chemicals vary in their propensity to cross biological membranes, which may alter uptake rates. In addition, chemicals vary in their mode of action and the potential for reversibility. These characteristics will invariably influence the parameters of the model, which are based on effects resulting from a variety of test durations and concentrations. For example, carbamate and organophosphorus insecticides share the same mode of action: inhibition of acetylcholenesterase in the nervous system. However, this inhibition is typically reversible in carbamate compared to organophosphorus compounds. This reversibility may lead to a higher potential for recovery after exposure. Determining how chemical properties influence combinations of exposure duration and concentration and subsequent effects will improve our knowledge of field susceptibility.

Recommendation for Risk Assessment Based on Continuous and Pulsed Testing

Current pesticide regulation is carried out by the Environmental Protection Agency's Office of Pesticide Programs. Registration and re-registration is currently based upon information available from laboratory studies, published

information, and incident data. Regulatory decisions are based on the effects, both acute and chronic, of pesticides to several aquatic species (i.e. warm water fish, cold water fish, marine fish, invertebrates, estuarine / marine mollusks, and estuarine/marine shrimp). Levels of concern are established through acute and chronic laboratory testing, which is then compared to the expected environmental concentration (EEC). For acute tests, levels of concern are as follows (SETAC, 1994):

1. If EEC > ½ LC_{50} then the aquatic risk of the pesticide is deemed to be of high concern (LC_{50} measured from the most sensitive species). This may warrant regulatory action in addition to restricted use classification.

2. If 1/10 LC50 <= EEC <=1/2 LC50, then the pesticide is considered for classification as a restricted use pesticide.

3. If EEC < 1/10 LC50, then the pesticide is considered to be a low aquatic risk, and no regulatory action will be pursued.

Based on the results obtained in continuous and pulsed tests using native invertebrates, a recommendation for an appropriate risk assessment methodology for stream systems can be made. First, lethal estimates obtained in continuous testing may trigger the need for further testing under pulsed exposure regimes, depending on how this value compares to the expected environmental concentration. This may be appropriate for LC50 values that close to the EEC, as was the case with carbaryl in this research, where additional testing under realistic exposure regimes may provides a basis for more accurately evaluating risk.

Testing under more realistic exposure regimes would include laboratory tests carried out at exposure durations similar to what would be expected in the field. For stream systems, pulsed testing methods may include shorter exposure durations (<2 hours). Due to the variation in organism response to pulsed exposure determined in this research (chapter 3), it is recommended that several species are selected for testing.

Pulsed test methods may include concentrations 10 and 100 times the LC_{50} values calculated for the organism in continuous tests. Based upon the findings of this research, recommendations for susceptibility data obtained from short exposure durations are as follows:

1. If no LC_{50} can be calculated (mortality <30%) at exposures < 60 minutes at test concentrations $\leq$ 100 times the LC_{50} values calculated in continuous tests, then the mortality is predicted to be low. However, this does not take into account possible sub-lethal effects.

2. If significant mortality is seen at exposures <60 minutes such that a LC_{50} can be calculated (mortality > 50%) at concentrations < 100 times LC_{50} values calculated in continuous tests, then the chemical is considered to pose a hazard to stream invertebrates, even at pulsed exposures. This was the pattern seen for *Cinygma* sp. exposed to carbaryl in this research (chapter 3). This gives an indication of how rapidly the chemical is take up, and the ability of an organism to recover. If LC_{50} values can be calculated at short exposures, a short exposure to the chemical will result in sufficient uptake and accumulation of the chemical to result in significant effects. The chemical may pose a risk to stream invertebrates in field, even under short exposure conditions.

However, in addition to acute thresholds, further testing needs to be completed that identifies thresholds for sub-lethal effects. Even very low concentrations of a chemical present for a short amount of time may trigger organisms to enter the drift. These organisms are transported downstream, and effectively lost to stream communities. Additional laboratory tests could identify concentrations which result in organisms entering the drift, as well as how able an affected organism is at reattaching to stream substrate downstream.

VARIATION IN THE POTENTIAL FOR SHORT- AND LONG-TERM EFFECTS

This research has shown that the inclusion of ecological characteristics allows for distinctions to be made in the potential for short- and long-term risk between different stream organisms. The analysis of the ecological characteristics

of different stream insects in this research identified organisms at an increased risk for short-and long-term effects in the field. Inclusion of these characteristics may alter laboratory-based rankings of species most affected by pesticide exposure, and establishes a basis for quantifying the uncertainty to which we can make extrapolations from the laboratory to the field. This approach has not been done other than implicitly by experts in the field, and a formal process is needed so that it can be used by other and adapted to other circumstances.

An application of this model was described in chapter 5, which explored the ability of the model to distinguish between the potential for short-and long-term effects between streams that differed physically and biologically. By analyzing characteristics of morphology, behavior and life history of a community of organisms, distinctions could be made in relative potential for short-and long-term effects for a community of organisms. Distinguishing the potential for short-and long-term effects resulting from pesticide exposure between different stream communities can be used to interpret monitoring data. For example, this approach can help identify streams that may be potentially at an increased risk for the effects of pesticides. Communities identified as having a high potential for short -or long-term effects on the basis of their make-up can be held to higher protective environmental concentrations. In addition, this type of analysis can use long-term monitoring data to identify patterns in species composition over time, which may show a loss of species with high potential for effects in both the short and long-term.

SHORT-TERM EFFECTS

First principles and data from the literature were used to establish the parameters and working hypothesis of the model. However, testing of these hypotheses is needed such that the characteristics of organisms that may increase exposure and uptake in the field be qualitatively determined. Laboratory testing

that utilizes radiolabled chemicals, for example, could determine diffusion rates associated with cuticle type and gill properties that vary among organisms. Uptake rates associated with different food sources and functional feeding groups could be assessed, such that organisms most at risk by dietary exposure could be quantitatively determined. For example, evaluating variation in uptake rates associated with different modes of feeding, such as a filter feeder, scraper or collector-gatherer would determine dietary uptake rates.

In order to determine the error associated with the current risk assessment process, which relies heavily on laboratory obtained susceptibility data, laboratory rankings of risk need to be compared to field derived rankings. Testing may include at set of species tested in both the laboratory and in the field. By comparing laboratory rankings of susceptibility with field obtained rankings, species and characteristics could be identified which result in counter-indicative results between the two.

The understanding of pesticide exposure in the stream environment would be strengthened if quantitative differences in potential for exposure associated with different stream habitats (i.e. riffle, pool, glide, backwaters, hyporheic zone) was determined. For example, the chemical concentration different stream habitats are exposed to in the field after a pesticide application could be quantitatively determined. Using a known concentration of a test chemical released into the environment, this monitoring could determine concentration profiles for different stream habitats, including riffles, glides, pools, backwaters and the hyporheic zone. In addition, within these major zones determined by water flow, variation in exposure associated with different microhabitats including the sediment, on the surface of, under and between rocks, algae and macrophytes could be examined. Obtaining concentration profiles relevant to the stream environment would help correlate laboratory tests data with effects in the field.

Chemical properties could also be included in a future model to identify habitats that may accumulate the chemical at higher rates. For example, the herbicide triclopyr has high calculated K_{ow} (octanol-water partition coefficient) of

1.2 x 10^4 (McCall & Gavit, 1986), and has been found to sorb to organic matter in the environment, such as stream leaf packs, at concentrations up to 20 times the maximum water concentration (Thompson et al., 1995; Kreutzweiser et al., 1998). The fungicide chlorothalonil has a low water solubility, and therefore may accumulate at the air / water interface or with suspended material in the water column (Davies, 1988). A model that incorporated this type of information could assign higher ranks and weights for habitats and food sources that, based on chemical properties, would be predicted to accumulate the chemical.

LONG-TERM EFFECTS

In addition to properties determining the potential for short-term effects in the field, the potential for long-term effects is rarely evaluated in risk assessment. Evaluating the potential for long-term effects in this research was hampered by the lack of information related to characteristics of the adult stage. Many of the organism characteristics that could be used to examine how organisms vary in their potential to sustain long-term effects are absent from the literature. Future work in this area would be greatly improved by research addressing how recolonization and recovery parameters differ between organisms, and how this variation may result in long-term effects when evaluated in conjunction with stream terrestrial, watershed, and landscape processes.

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