EVALUATING THE POTENTIAL CONTRIBUTION OF EPISODIC TOXICITY DATA TO ENVIRONMENTAL WATER QUALITY MANAGEMENT IN SOUTH AFRICA

Report to the
Water Research Commission

by

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The publication of this report emanates from a project titled *Evaluating the potential contribution of episodic toxicity data to environmental water quality management in South Africa* (WRC Report No. K8/851)

Executive summary

Rationale

One of the limitations identified by Roux et al. (1996) regarding the derivation of the South African Water Quality Guidelines for Aquatic Ecosystems (1996) was that they failed to reflect the effects of duration of exposure, frequency of exposure, and interval period between consecutive exposures. There is at present a Department of Water Affairs (DWA) funded project reviewing the derivation of South African Water Quality Guidelines, and consequently an opportunity exists to begin investigating the incorporation of episodic toxicity test data in managing environmental water quality. This project also provides an opportunity to assess the incorporation of episodic toxicity data in the direct toxicity assessment method proposed by DWA (Direct Estimation of Ecological Effect Potential – DEEEP).

Project aims

The approach of the project was to undertake a desktop literature review with the aim of addressing the following questions:

- What are the quality and quantity of episodic toxicity data available in the aquatic environmental water quality literature?
- What are the philosophical and practical constraints limiting their inclusion in environmental water quality management procedures and guidelines in South Africa?
- How could these data be incorporated into the current development of a risk-based approach to deriving water quality guidelines for aquatic ecosystems, and the continued refinement of DEEEP?

Report structure

The major product emanating from this project is an aquatic episodic toxicity database for utilization in environmental water quality management. Due to its size, the database could not be included as a hardcopy in this report, but can be downloaded as an Excel file from the IWR website (http://iwr.ru.ac.za/iwr/download). The report is structured as a supporting document to the database.

Chapter 1 presents the rationale for this project and its aims.

Chapter 2 details a literature review characterizing the episodic exposure of toxics, the challenges of including episodic toxicity data in environmental water quality management, and gives examples of international attempts to do this (Aim 2).

Chapter 3 explains the methods used to locate, assess and record the applicable episodic toxicity data available in the literature. A summary of the episodic toxicity data available for each chemical or stressor is provided (Aim 1).

Chapter 4 details possible approaches for incorporating episodic toxicity data into the proposed revision of the South African Water Quality Guidelines, and possible application to DEEEP (Aim 3).

Episodic exposure characteristics

Episodic exposures can vary in magnitude (chemical concentration), frequency (the number of exposure events) and duration (the time of the exposure event). The interaction of these three factors makes the prediction of the effects of episodic toxicity exposure on organisms very difficult.

Episodic stress/pollution can occur naturally, as a result of fluctuations in physico-chemical water quality that occur with changes in season, rainfall and time of day. Anthropogenic causes of episodic pollution are varied and can be the result of either accidental or deliberate releases of pollutants to water resources as a result of agricultural or industrial activities or from sewage treatment works.

In an individual organism, an effect (e.g. mortality) occurs when the specific threshold body burden of the toxicant is exceeded. This process can often be a gradual weakening of mitigation mechanisms (e.g. stress proteins) to the point where failure occurs resulting in an organism level response.

Challenges to the inclusion of episodic toxicity data in environmental water quality management

Toxicant exposure profiles can be square, sinusoidal or skewed. Different profiles can elicit varying responses from exposed organisms, and greatly affect the method used to define the toxicants exposure concentration. There is no standardized approach and some approaches that have been used include: the mean test concentration; the mean exposure concentration and; the peak concentration. A risk assessment approach which uses the area under the curve of the continuously fluctuating toxicant exposure to derive toxic exposure equivalents (akin to hazard quotients regularly used in toxicology) can also be used to define exposure concentration.

Organism responses to episodic exposures are complicated by the varying modes of action among chemicals, and the variable physiological responses to a chemical among organisms.

Approaches for determining the toxicity of episodic pollution

Generally, efforts to address episodic toxicant exposure have focused on two main approaches:

1. Experimental. The development of toxicity tests that attempt to incorporate the episodic nature of exposure to pollutants; and
2. Predictive models. The development of models that use traditional constant exposure data to predict toxicity under episodic exposure conditions.

The use of alternative toxicity tests has been hampered by the large natural variability of the endpoints they employ, leading to uncertainty over whether an effect has actually occurred or not. Although promising, the models at present often do not show a good enough fit to observed biological effects to allow for regulatory use.

Quality and quantity of episodic toxicity data available in the scientific literature

After an extensive search of available literature, the episodic toxicity data from 112 scientific papers were used to populate an episodic toxicity database. These 112 references were assessed to determine if the data they contain were of suitable quality. The database can be downloaded from the Institute for Water Research website.

Summary of collated information by chemical

Characteristics of the episodic toxicity of six metals, 39 pesticides, three physical water-parameters and the chemical ammonia were summarized. Generally, a greater toxic effect on the organism was observed when the number of pulse exposures and length of each pulse exposure were increased, and when a decrease in recovery time between pulses occurred. However, there were exceptions, most often caused by differences in the sensitivity of exposed organisms, probably as a consequence of morphological differences (Petersen et al., 2001), i.e. significant differences – species and age related – in uptake and depuration rates of specific chemicals by different organisms.

Naturally, the concentration of the toxicant or level of stress affected the toxic effect observed in the organism too (at low concentrations – below the threshold of affect level – recovery from exposure to the stressor was observed regardless of number of pulses and length of exposure), however at higher concentrations the complexity of episodic exposures (the relationship between the length of exposure and number of pulses) was observed.

The effect of repeated pulse exposures

Increasing the number of pulsed exposures to a toxicant generally resulted in increased toxicant effect to the organism, probably because of a threshold tissue burden being exceeded. Repeated pulse exposures reduced the ability of freshwater clams (*Anodonta anatina* and *Unio pictorum*) to eliminate aluminum (Pynnonen, 1990).

The survival of fathead minnows (*Pimephales promelas*) was significantly lowered when exposure to the number of copper pulses increased from one to two. In addition fish biomass was negatively correlated to the number of pulses (Diamond et al., 2005). Bearr et al. (2006) report that although fathead minnow survival was not significantly different between the single- and double-pulse treatments to copper (with 96 hr recovery time in between), addition of a third pulse (96 hr after the second pulse) resulted in a significant decrease in fish survival. A similar response was observed when Andersen et al. (2006) exposed *Daphnia magna* to dimethoate an organosphosphate insecticide. Following the first exposure pulse there was recovery from immobility for all pulse durations. However, after the second pulse, mortality occurred and increased significantly during the recovery period for pulse durations greater than 2 hr.

Even if no mortalities occur as a result of increasing the number of pulse exposures, sublethal effects are sometimes significantly affected. Experiments testing the effects of exposure frequency of ammonia (8-24 pulses of 0.2-0.4 mg/L) on brown trout (*Salmo trutta*) found no mortalities. However, lower fish weights were recorded in some instances, and growth, gill condition, organ weights, and hematocrit were all significantly affected by repeated exposures, particularly at the higher exposure frequency (Milne et al., 2000).

Effect of the duration of the pulse exposure

Increasing the length of exposure to a cadmium pulse reduced population growth rate of cladocerans (*Moina macrocopa*) and rotifers (*Brachionus calyciflorus* (Gama-Flores et al., 2006). A similar response was observed for fathead minnow (*P. promelas*) biomass which was negatively correlated to length of copper pulse exposure (Diamond et al. (2005). Bearr et al. (2006) report that when *P. promelas* fry were exposed to copper

pulses of 12 hr or longer their internal dose threshold appeared to have been exceeded, resulting in significant mortality, whereas 24 hr exposures initiated acclimation of sorts with significantly less mortality recorded.

In the case of *D. magna*, Hoang & Klaine (2008) found that daphnids exposed to a single 4-24 hr pulse of between 800-2000 µg/L selenium showed no mortality during exposure; but latent mortality during post-exposure with mortality increasing with pulse exposure duration and exposure concentration. In the case of zinc, daphnids were more sensitive to 24 hr pulses of 250-1000 µg/L (with continued mortality even in post-exposure) while 3-6 hr pulses at high concentrations resulted in no effects on daphnid survival or reproduction (Diamond et al., 2006a). Lastly, when daphnids were exposed to two 12 hr pulses of 0.5 µg/L chlorpyrifos, > 85% mortality was observed, regardless of the pulse exposure interval which varied from 0 to 3 to 7 and to 14 d in length (Naddy et al., 2000).

Effect of recovery time between pulse exposures

Longer recovery times between multiple exposure pulses led to greater survival in *D. magna* (Diamond et al., 2006a) and amphipod (*Hyalella azteca*) (Zhao & Newman, 2006) exposed to copper, and *D. magna* exposed to selenium (Hoang & Klaine, 2008) and zinc (Diamond et al., 2006a). A recovery period of at least 72 hr between pulses of organophosphate insecticide chlorpyrifos was necessary for daphnids to recover from 0.5µg/L pulse, while a longer recovery period of 96 hr was necessary for 1.0 µg/L pulse (Naddy et al., 2000). Milne et al. (2000) report that rainbow trout (*Oncorhynchus mykiss*) and brown trout (*S. trutta*) juveniles exposed to repeated pulses of potentially lethal ammonia concentrations were able to survive if enough time for recovery was allowed.

Bearr et al. (2006) report a more complex relationship between post-exposure mortality and length of recovery between pulses. Fathead minnows (*P. promelas*) exposed to 24 hr copper pulses of 30-40 µg/L had significantly higher mortality when pulses were spaced farther apart in time (to a threshold) than when pulses of the same magnitude were spaced more closely, i.e. exposures having a 48-96 hr recovery time between pulses had less effect on fish survival (i.e. increased mortality) than did treatments with shorter (12-24 hr) or longer (>120 hr) recovery times. Indeed, Diamond et al. (2006a) report an experiment in which *P. promelas's* biochemical defense system was activated by copper exposure for approximately 48-96 hr, after which it ceased if copper was removed from the media, leaving the fish susceptible to a new pulse.

Effect of organism age on toxicity of a pulse exposure

Hoang & Klaine (2007) investigated the effects of a 12 hr pulse of either arsenic, copper, selenium or zinc on *D. magna* and monitored effects after 20.5 d of recovery. They found that the 21 d mortality increased with organism age from 3-48 hr old and then decreased with age from 48-240 hr old for arsenic and selenium. For copper and zinc however, 21 d mortality increased with organism age from 3-96 hr old and then decreased with age from 96-240 hr old. No difference in 21 d growth was detected by the study for the metals tested. Reproduction was, however, affected with 21 d cumulative reproduction for arsenic decreasing with age from 3-72 hr old and then increasing with age from 72-240 hr old. For copper and zinc, 21 d cumulative reproduction decreased with age from 3-96 hr old and then increased with age from 96-240 hr old, and for selenium 21 d cumulative reproduction decreased with age from 3-48 hr old and then increased with age from 48-240 hr old.

However, Andersen et al. (2006) exposed *D. magna* aged <24 hr and 3 d old exposed to a single pulse of dimethoate, an organophosphate insecticide and found no significant difference in recovery based on age. In addition, Hosmer et al. (1998) exposed *D. magna* of varying ages (< 24 hr, 4-6 d, 8 d, 11 d) to varying concentrations of insecticide fenoxycarb and monitored effects for 21 d. There were no significant effects on survival or time to first brood of first and second generation daphnids in any age group at all exposure concentrations.

Sellin et al. (2005) investigated cellular mechanisms associated with the acclimation of *P. promelas* larvae and juveniles to copper pulse exposures. They found that 12 d old episodically exposed larvae exhibited acclimation while 8 d old larvae had significantly lower survival suggesting that at this age these fish were not acclimated.

Australian crimson-spotted rainbowfish (*Melanotaenia fluviatilis*) larvae were exposed to a 2 hr cyanazine pulse at concentrations between 1.9-43.2 mg/L and recovery monitored for further 94 hr. Five age groups were exposed (0, 3, 6, 9 and 12 d post-hatch). Toxicity was found to decrease with larval age. A considerable progressive reduction in cyanazine toxicity was noted for larvae aged 0-3 d post-hatch with no further reduction in toxicity for 6, 9 and 12 d old fish. The authors proposed this as evidence of hepatic xenobiotic metabolism, suggesting that the liver of larval rainbowfish becomes increasingly more functional with age. However, results could also be influenced by the greater respiration rates and small body surface area of smaller larvae (Reid et al. 1995).

Lastly, eggs and alevin of Kokanee and Sockeye salmon exposed to 24 hr pulse of pH 4 showed variation in sensitivity by developmental stage (Parker & McKeown, 1987), with the most sensitive stage being early embryonic development and newly-hatched alevins. The most significant effect on survival and median hatching time was noted when the eggs were episodically exposed during early development, and exposure at later stages had no apparent effect on egg survival.

Consequently, when attempting to determine the risk of a certain episodic pollution event, the specific situation needs to be investigated (i.e. the specific affected species involved and their developmental stage, the specific chemicals (not just chemical groupings) and their concentrations), and naturally, the number of pulses, length of pulse exposure time and recovery time.

Possible approaches for incorporating episodic data into the proposed risk-based approach to deriving water quality guidelines (WQGs) for South African aquatic ecosystems, and the continued refinement of DEEEP

The SA WQGs are envisaged to comprise of a three tier system:

Tier 1. Provides 'generic' guidelines values that are made available in a decision support system (DSS) and hard copy manuals. These guideline values will be conservative as the worst case scenario is assumed.

Tier 2. Allows for site specificity in specified contexts and is facilitated by the DSS, consequently there is more confidence in the derived value

Tier 3. Full risk assessment. Not facilitated by the DSS but will use information contained within the DSS information database.

As the intention of the SA WQGs is that they should, as far as practically possible, serve as a stand-alone source of information and support base for decisions for water resource

managers, the inclusion of episodic toxicity data within the DSS for use at Tier 2 and 3 assessments is catered for philosophically.

The procedure for generating the guideline values will follow a probabilistic risk assessment process. The exact procedural methods are still to be resolved, but the thinking process by which one goes about undertaking a risk assessment is also the method used in developing the guidelines and is depicted as an event tree (see Appendix A). The episodic data would contribute at the level where intake, excretion and metabolism of the chemical are considered.

Direct Estimation of Ecological Effect Potential (DEEEP) is a method for directly measuring the potential effect / toxicity / ecological hazard of complex wastewater effluents. Like direct toxicity assessment (DTA) methods being used worldwide, DEEEP uses a number of lethal and sublethal bioassays to measure ecological hazard. The current DTA testing protocols ignore duration of exposure. The information generated in the episodic toxicity database, however, shows the importance of considering the variability of exposure duration and frequency when assessing organism and ecosystem effects. Further research, beyond the scope of this report, needs to be undertaken to investigate the development of testing protocols that take account of exposure duration and frequency for application in DEEEP.

Recommendations

Further research regarding the application of episodic toxicity to environmental water quality management in South Africa should focus on:

- The refinement of models that use traditional constant exposure data to predict toxicity under episodic exposure conditions
- Investigating the development of toxicity test protocols that take account of exposure duration and frequency for application in WQG development and DEEEP
- Ensuring that provision is made for including episodic toxicity data in the revision of the SA WQGs and ongoing refinement of DEEEP by the prospective project teams involved, and
- Ensuring episodic toxicity database is frequently updated.

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Abbreviations

ChE	Cholinesterase
DEEEP	Direct estimation of ecological effect potential
DTA	Direct toxicity assessment
DWA	Department of Water Affairs (South Africa)
EC50	Effect concentration at which 50% of the test organisms experience an adverse effect
LC50	Concentration that kills 50% of the population observed. Also known as median lethal concentration
LOEC	Lowest observed effect concentration
LT50	Time at which 50% of population has experienced mortality
NOEC	No observed effect concentration
MATC	Maximum acceptable toxicant concentration
PE LC50	Pulse exposed LC50
WQG	Water quality guideline

Chapter 1 Introduction

1.1 Rationale for the project

One of the limitations identified by Roux et al. (1996) regarding the derivation of the South African Water Quality Guidelines for Aquatic Ecosystems (1996) was that they failed to reflect the effects of duration of exposure, frequency of exposure, and interval period between consecutive exposures. There is at present a Department of Water Affairs (DWA) funded project reviewing the derivation of South African Water Quality Guidelines, and consequently an opportunity exists to begin investigating the incorporation of episodic toxicity test data in managing environmental water quality. This project also provides an opportunity to assess the incorporation of episodic toxicity data in the direct toxicity assessment method proposed by DWA (Direct Estimation of Ecological Effect Potential – DEEEP).

1.2 Project aims

The project aims were identified as the following questions:

- What are the quality and quantity of episodic toxicity data available in the aquatic environmental water quality literature?
- What are the philosophical and practical constraints limiting their inclusion in environmental water quality management procedures and guidelines in South Africa?
- How could these data be incorporated into the current development of a risk-based approach to deriving water quality guidelines for aquatic ecosystems, and the continued refinement of DEEEP?

1.3 Report structure

The major product emanating from this project is an aquatic episodic toxicity database for utilization in environmental water quality management. Due to its size, the database could not be included as a hardcopy in this report, but can be downloaded as an Excel file from the IWR website (http://iwr.ru.ac.za/iwr/download). This report is structured as a supporting document to the database.

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Chapter 4 details possible approaches for incorporating episodic toxicity data into the proposed revision of the South African Water Quality Guidelines, and possible application to DEEEP (Aim 3).

Chapter 2 Literature review

Organisms in the environment are rarely exposed to environmental or anthropogenic stress on a continuous basis at constant levels. Instead, these stresses fluctuate in severity or intensity. A variety of terms have been used to describe this situation of fluctuating concentrations of exposure to stress: episodic, intermittent, pulse, plug and spike. In this report the term episodic will be used as a synonym for these different terms.

2.1 Episodic exposure characteristics

An episodic exposure can vary in:

- magnitude (the severity of the stress e.g. chemical concentration)
- frequency (the number of exposure events)
- duration (the time of the exposure event)

The interaction of these three factors makes the prediction of the effects of episodic toxicity exposure on organisms very difficult (Diamond et al., 2006a).

Sources of episodic pollution or stress

Sources of episodic stress/pollution can occur naturally. For example, fluctuations in physico-chemical water quality (temperature, pH, DO, salts etc) occur with changes in season, rainfall and time of day (McCahon and Pascoe, 1990; Muusze et al., 1998).

Causes of anthropogenic episodic pollution are varied and can be the result of either accidental or deliberate releases of pollutants to water resources. The fluctuating chemical concentrations will vary depending on the type and amount of a chemical released to the environment, the dilution rate and the potential degradation of the chemical (Butcher et al., 2006). McCahon and Pascoe (1990) list the following as the main anthropogenic sources of episodic pollution:

- Agricultural activities:
  - release of animal waste and silage liquor as a result of inadequate provision for these wastes or failure of contaminant systems
  - Fertilizer input to aquatic systems following rainfall runoff events
  - Unregulated disposal of pesticides
  - Run-off following spraying of crops with pesticides
- Sewage treatment works:
  - Inability of sewage works to effectively treat all their effluent
  - Discharge of sewer overflows during high stormwater runoff
- Industrial activities:
  - Unregulated releases of oil and chemicals as a consequence of failures of treatment processes, pipelines or storage vessels; negligence during handling and transport; and following major accidents such as fire or explosions at chemical plants and oil refineries.

The resultant episodic pollution is thus sometimes composed of a single chemical (e.g. a pesticide), but most often it is a combination of various chemicals (i.e. an effluent.).

In an individual organism, an effect (e.g. mortality) occurs when the specific threshold body burden is exceeded. This process can often be a gradual weakening of mitigation mechanisms (e.g. stress proteins) to the point where failure occurs resulting in an organism level response. Consequently, body burden is an important factor in episodic pollution and is a function of exposure period, chemical concentration and the organism's mitigation measures (i.e. a function of the difference between uptake and detoxification).

2.2 Challenges to the inclusion of episodic toxicity data in environmental water quality management

Varying toxicant exposure profiles
Toxicant exposure profiles can be square, sinusoidal or skewed (Handy, 1994). Different profiles can elicit varying responses from exposed organisms, and greatly affect the method used to define the toxicants exposure concentration.

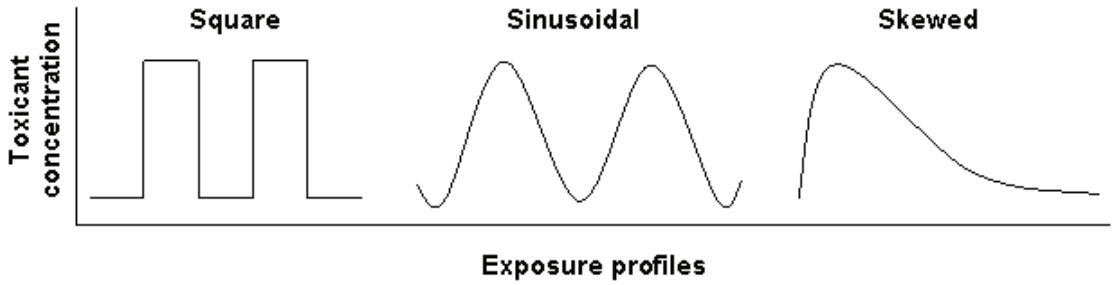


Figure 1 Simplified representations of three toxicant exposure profiles

Defining episodic toxicant concentration levels
Various methods of defining or describing the episodic exposure concentration have been utilised. These include: the mean test concentration (derived from both the positive and negative fluctuations in the exposure concentration); the mean exposure concentration (defined as the mean of the exposure concentrations only, excluding the recovery periods between repeated episodes) and; the peak concentration (the highest concentration reached in each exposure profile) (Handy, 1994). In addition, Morton et al. (2000) describe a risk assessment approach which uses the area under the curve of the continuously fluctuating toxicant exposure to derive toxic exposure equivalents (akin to hazard quotients regularly used in toxicology). Handy (1994) suggests that the definition used to describe the toxicant concentration should be matched with the appropriate toxicant exposure profile to ensure that a sensible and toxicologically useful lethality estimate is derived. Examples of appropriate and inappropriate definitions under various scenarios are provided in Handy (1994).

Routes of exposure and modes of action vary in chemicals
The responses of organisms to a toxicant are dependent on the characteristics of the chemical. As an example, a short (24 hr) exposure of high concentrations of copper or ammonia to water flea *Daphnia magna* and fish *Pimephales promelas* resulted in mortality during the pulse, but an immediate cessation of mortality during the recovery period. However, the same short exposure of zinc to the same species resulted in continued latent mortality for 4 d of the recovery period (Diamond et al., 2006a). Brent and Herricks (1998) report sublethal latent effects (immobility) for water flea

Ceriodaphnia dubia and amphipod *Hyalella azteca* exposed to short pulses of zinc or cadmium, whereas *C. dubia* exposed to phenol showed some recovery during post exposure period.

Variable responses of organisms to episodic exposure
Physiological differences in response mechanisms to toxicant stress vary among organisms. For example, Diamond et al. (2006a) reported that *D. magna* survival was improved with increased recovery times between copper pulses, whereas *P. promelas* survival was significantly lower with copper pulses in excess of 96 hours apart. These results are attributed to *D. magna's* control of copper toxicity using the outer integument which has limited ability to regulate the internal concentration of contaminants. As a result, the more closely spaced the pulses, the more likely it is that the internal chemical accumulation will exceed the threshold level, resulting in mortality. In *P. promelas* however, it is postulated that physiological response mechanisms to the copper exposure occur in the gills (e.g. induction of metallothioneins), and that the observed resistance of *P. promelas* to the copper exposure was activated for only 48-96 hours, after which it was removed when the copper was removed from the media, leaving the fish susceptible to a new pulse of copper (Diamond et al., 2006a).

2.3 Approaches for determining the toxicity of episodic pollution

In the monitoring and regulation of continuous pollution discharges, chemical specific water quality criteria/guidelines have been traditionally applied. These criteria are derived from the results of controlled laboratory tests, in which test organisms are continuously exposed to constant chemical concentrations for a specified time span. Various researchers have suggested that these criteria are not always appropriately protective when toxicant exposure in the field is episodic in nature (Handy, 1994; Brent and Herricks, 1998; Zhao and Newman, 2006).

The U.S. Environmental Protection Agency attempted to address episodic toxicant exposures by proposing a two-number criteria system (TNC). The first criterion is a concentration not to be exceeded as a 24 hour average, usually the no-observed-effects-concentration (NOEC), whereas the second is a maximum or peak permissible concentration, usually the 48 hour or 96 hour LC50, which can only be maintained for a duration such that the 24 hour average is not exceeded. Several studies designed to test the validity of the TNC concept using *Daphnia* and fish demonstrated that the TNC did not consistently protect aquatic life (Hickie et al., 1995). The TNC approach has not been widely adopted owing to the lack of a sound basis for selecting the averaging period and the high frequency of monitoring required (Hickie et al., 1995).

Generally, efforts to address episodic toxicant exposure have focused on two main approaches:

3. Experimental. The development of toxicity tests that attempt to incorporate the episodic nature of exposure to pollutants; and
4. Predictive models. The development of models that use traditional constant exposure data to predict toxicity under episodic exposure conditions.

Development of alternative toxicity test endpoints for episodic exposures
Handy (1994) describes possible alternatives to traditional toxicity test methods that typically measure toxicity by comparing organism response (e.g. mortality) to chemical

concentration. The alternative methods proposed compare mortality with some other dose-dependent response in the test organism. For example, mortality can be compared to chemical accumulation in specific tissues of the test organism. This approach relies on the assumption that the contamination of the body tissue measured is the ultimate cause of death. For example, Connolly (1985) showed that endrin and copper accumulation in the gills of fish produced linear correlations with observed mortalities and thus a lethal residue concentration could be determined from a plot of mortality versus branchial contamination. However, fish have been shown to rapidly accumulate toxins after death (Eisler and Gardner, 1973), and thus the time of death is important consideration for correct determination of tissue residue data. Handy (1994) has also proposed the use of biochemical and physiological responses as alternatives with which to compare mortality data. However, the normal range of these types of responses is often very large leading to uncertainty as to whether the measured responses are due to toxicity exposure or just natural variation.

Development of models to predict effects of fluctuating concentrations on aquatic organisms

Methods that integrate the fluctuating levels of a toxicant's concentration (such as deriving median lethal concentration i.e. LC50) have been shown to inadequately interpret the biological effects observed during these episodic exposures (Widianarko et al., 2001; Butcher et al., 2006). Consequently, much effort has been directed towards models that are capable of adequately representing the actual exposure concentration of the episodic event. Mancini (1983) and Breck (1988) created models using toxicokinetic equations to predict toxicant concentration at the site of action within the organism as a function of ambient or nominal concentration and uptake/clearance rates. Under varying ambient concentrations, body tissue residue levels of toxicants (controlled by the rates of toxicant accumulation and depuration or repair by exposed organisms) have been shown to adequately predict levels of biological response that occurred (Mancini, 1983; Hickie et al., 1995). These simple toxicokinetic models utilise data from classical bioassay tests obtained using constant toxicant exposures together with information regarding the accumulated dose of the toxicant in the organism to estimate probable effects of time varying exposures to the toxicant.

The original Mancini/Breck model thus consists of two compartments (Figure 2): the internal concentration (*I*) which is increased and/or decreased by uptake and/or depuration from the external exposure concentration (*C*). In turn, the internal concentration can cause uptake and accumulation at the site of action of a sensitive organ leading to damage (*D*). As with the internal concentration, the site-of-action concentration is potentially reduced by depuration, or the accumulated damage may be repaired (Butcher et al., 2006).

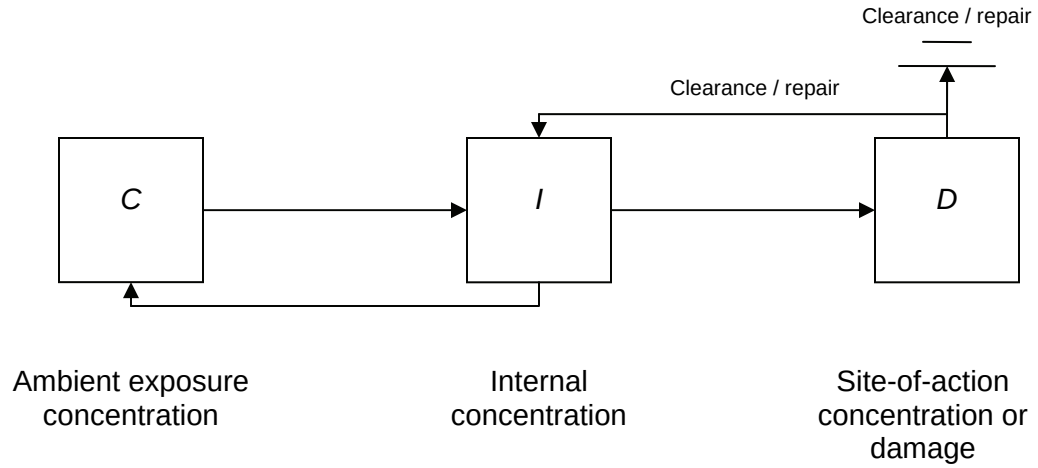


Figure 2. Simplified schematic representation of the Mancini/Breck type kinetic model (adapted from Butcher et al., 2006).

This two compartment model has been adapted and simplified. The model has been reduced to one compartment by assuming that the rates of accumulation and depuration/repair for compartment *D* were equal, effectively collapsing compartments *I* and *D* into one (Hickie et al., 1995). Butcher et al. (2006) made adaptations by using time history of response (e.g. one hourly adjustments in mortality using numerical integration) instead of end-of-test net response (e.g. LC50) as an input to the model. In addition, they made use of variable kinetic response inputs (e.g. uptake and depuration rates varied with exposure concentration). Generally, at present, the models do not show a good enough fit with observed biological effects to allow for use in a regulatory setting (Diamond et al., 2006b). The experimental data fed into the models require greater replication in order to reduce the statistical variability (Butcher et al., 2006). However, they do provide useful information for increasing our understanding of organism responses to fluctuating toxicant concentrations, which will in turn allow interpolation to ecosystem responses.

Chapter 3 Quality and quantity of episodic toxicity data available in the scientific literature

3.1 Data collection

The search for available literature was undertaken by searching the online scientific reference databases ScienceDirect, EBSCOhost and SpringerLink using the keywords "episodic or pulse* or periodic or intermittent and exposure" within the Life Sciences subject area producing 8952 hits. Archives of specific toxicology related journals have been searched independently (Environmental Toxicology and Chemistry, Australasian Journal of Ecotoxicology, etc) resulting in over 200 hits. The citations were downloaded to RefWorks database manager and titles assessed for applicability yielding 435 references with potential aquatic episodic toxicity information. The pdf files of these references were downloaded, and after further assessment 112 were found to provide relevant episodic toxicity data.

3.2 Assessment of literature

Suitable references were assessed for the quality of the aquatic toxicity data based on the method refined by Hobbs et al. (2005) for the Australian and New Zealand WQGs. Although not directly applicable to episodic toxicity data the method does give an indication of the scientific rigor or standards used to generate the data. All references assessed were found to be of acceptable quality.

3.3 Database format

The database was designed to record as much pertinent information from the episodic references as possible. Information recorded included:

- type of chemical and whether it was tested singly or in combination with another stressor (e.g. varying pH levels)
- genus, species, common name and age of the test organism
- exposure scenario (e.g.: the chemical's concentration; pulse duration and frequency; the interpulse period; post exposure recovery time; and exposure profile)
- toxicity test location, the experimental medium used and it's water chemistry (e.g. pH, water harness and any other inferred chemical information that may affect the bioavailability of the toxicant (e.g. amount of ammonia in the water – produced by the test organisms as a result of stocking density)
- biological endpoint tested (e.g. mortality or reproduction) and the effect measured (e.g. NOEC or LC50 etc)
- summary of the results and any other pertinent information such as body burden information or toxicant mode of action if stated.

This database is available on the internet as an Excel spreadsheet, based at the Institute for Water Research website (http://iwr.ru.ac.za/iwr/download/). Once downloaded, searches for specific information will be possible (e.g. type of chemical). It is expected that this information will be of use to water resource practitioners when determining site specific WQGs (Tier 2 and 3 of the proposed WQGs (DWAF, 2008)).

3.4 Summary of collated information by chemical

The information gathered from the episodic toxicity literature and uploaded to the database was reviewed. Characteristics of the episodic toxicity of six metals, 39 pesticides, three physical water-parameters and the chemical ammonia were summarized.

Metals

Aluminum

Aluminum toxicity, generally studied in association with low pH, results in the disruption of ion regulation leading to respiratory stress, inability to maintain position in the water column, and inefficient foraging (DeLonay et al., 1993; Allin, 2000).

Effects of aluminum have been tested on fishes and freshwater clams. Pynnönen (1990) studied accumulation of aluminum (concentrations of up to 900 µg/L) in various organs of freshwater clams (*Anodonta anatina* and *Unio pictorum*) and found that their gills accumulated 5 times higher concentration of aluminum than kidneys and they also required 3 times as long to eliminate it. Reduced pH was shown to exaggerate aluminium accumulation in these organs. Recovery was shown to occur with aluminium elimination if given sufficient time, although repeated pulses reduced the ability of the freshwater clams to eliminate aluminium. Pynnönen (1990) used aluminum in the form of $AlCl_3$ which previously has been reported to be less toxic due to chlorides forming complexes with metals.

Mortality and sublethal effects were measured in *Oncorhynchus mykiss aquabonita* (golden trout), exposed as eggs, alevins and swim-up larvae (DeLonay et al., 1993). Mortality in the organisms exposed to concentrations up to 300 µg/L total aluminum for 7 days, was limited to the exposure period. During pulse exposure, survival increased with age and developmental stage of the fish. Sublethal effects of inhibited locomotory activity and reduced feeding activity were, however, noted during the post-exposure period. Some sublethal effects took a number of days to recover to unexposed levels, while others did not recover by the time the organism reached 40 d posthatch (alevin locomotory activity NOEC after 19 d recovery at pH 5.5 was 50 µg/L; feeding activity NOEC for alevin after 19 d recovery at pH 5.5 was 50 µg/L and after 33 d recovery at pH 5 was 50 µg/L; NOEC for swim-up larvae after 7 d recovery at pH 5 and 5.5 was 50 µg/L).

Mortality and hypoactivity in fish was also recorded by Allin & Wilson (2000) who compared the effects of a 4 d pulse of 36 µg/L labile aluminum (equivalent to 98% total aluminum) on acclimated and non-acclimated adult trout (*Oncorhynchus mykiss* rainbow trout of 2.3-16.7 g). Mortality during and immediately following exposure was recorded for non-acclimated fish while pre-acclimated fish mortality was the same as control fish. Hypoactivity was noted for swimming behavior during and post exposure, again with pre-acclimated fish showing lower levels of effects.

Arsenic

The mode of action for arsenic is through energy inhibition via compromised adenosine triphosphatase molecules (Hoang & Klaine, 2007).

Hoang & Klaine (2007) investigated the effects of a 12 hr pulse at a concentration of 5000 µg/L arsenic on *Daphnia magna*. They found that the 21 d mortality increased with

organism age from 3-48 hr old and then decreased with age from 48-240 hr old. Compared to other tested metals, age influenced toxicity of arsenic more than toxicity of Cu and Zn. The authors state the reason for greater sensitive to arsenic on or after first moulting was that arsenic may be absorbed through the new exoskeleton of the newly moulted daphnid. No difference in 21 d growth was detected by the study. The 21 d cumulative reproduction decreased with age from 3-72 hr old and then increased with age from 72-240 hr old. The influence of organism age (≤48 hr old) on the effect of arsenic on daphnid reproduction appeared to be stronger than the effects of Cu, Zn, and Se.

Cadmium

Cadmium's mode of action is believed to be mediated through reduced growth and reproduction (see references below).

Testing for cadmium effects has been conducted on a range of organisms from rotifers and daphnids to fishes. One of the first studies on water fleas (*Ceriodaphnia dubia*) looked at immobility of neonates as an indicator for mortality and found latent effects during post-exposure period at concentrations in the range of 0.37-11.84 mg/L (Brent & Herricks, 1998). At highest cadmium concentration, 100% immobility was recorded for exposure periods as short as 30 min. Later studies looked at the population growth rate of cladocerans (*Moina macrocopa*) and rotifers (*Brachionus calyciflorus*), which was found to be reduced at concentrations as low as 0.05 mg/L and the effect increased as exposure period increased from 3 hr to 24 hr (Gama-Flores et al., 2006). The authors looked at the response of the species in a mixed culture and found that generally *M. macrocopa* outcompeted *B. calyciflorus* and completely eliminated it under conditions of high toxicant concentrations and longer exposure time. Population growth rate of *B. calyciflorus* was also studied in a later paper (Gama-Flores et al., 2007b) and comparison with copper showed that cadmium's impact was more severe on the organisms. Reduction in rotifer body size was noted for exposures of 12 hr for concentrations ≥ 0.05 mg/L, while exposures of 3 and 6 hr showed no significant difference in body size from the control group. Egg ratio was not significantly affected by cadmium exposure in this study.

Another study investigating the effects on life history parameters for *M. macrocopa* found that concentrations of 0.08-0.32 mg/L resulted in negative effects on survivorship and reproductive variables (Gama-Flores et al., 2007a). Survivorship was low even at the lowest concentration for a 3 hr exposure and high mortality was recorded for 24 hr exposures at these cadmium concentrations. Offspring production was also found to be affected by cadmium, and the animals ceased to reproduce when exposed to cadmium concentration of 0.32 mg/L for 6 hr or longer.

Cadmium effects on amphipods have been investigated by a number of studies. Experiments using neonate amphipods have shown latent effects (immobility) during post-exposure similar to water fleas with exposure of 30 min at the highest cadmium concentration studied (11.84 mg/L) resulting in 95% immobility (Brent & Herricks, 1998). Post-exposure mortality up to 14 d was also noted for amphipod individuals of 1-1.5 cm length exposed to 0.5-5 mg/L cadmium for 1-200 min (Able & Garner, 1986). Schill et al. (2003) investigated sexually mature amphipod individuals that showed various responses by sex and concentration; no mortality was found during exposure of 5 d for initial nominal concentrations of 8-125 µg/L, but a strong decrease in survival during the exposure period was noted for female gammarids exposed to concentrations of

250-2000 µg/L. Post-exposure mortality was noted for all concentrations in females particularly during 5 d following exposure. A trend towards decreasing stress protein (hsc/hsp70) levels with increasing cadmium concentrations were also found in both sexes during post-exposure. The authors refer to high pH of 8.5 and low water temperature affecting their results.

Studies looking at the effects of cadmium on fish fry have used fathead minnows and rainbow trout for testing. Brent & Herricks (1998) found latent effects of mortality (85% immobility) in fry of fathead minnows at highest cadmium concentration of 11.84 mg/L when exposed for 30 min. Diamond et al. (2005) found survival of fathead minnows (*Pimephales promelas*) related to duration, frequency and magnitude, and additionally effects on growth mirroring mortality effects. A single 6 hr exposure to 60 µg/L was significantly different from control treatment and from single and double 6 hr 40 µg/L exposures, but not different from a single 12 hr exposure to 40 µg/L. Although no delayed mortality was recorded during post-exposure by Diamond et al. (2005), higher concentrations (1 mg/L for 32 min or 10 mg/L for 10 min) resulted in post-exposure mortalities in fry of rainbow trout (*Oncorhynchus mykiss*) (Pascoe & Shazili, 1986). The latter also recorded median post-exposure lethal time (PeLT50) and found it to decrease as cadmium exposure time increased. Pascoe & Shazili (1986) also found cadmium concentration in fish fry reduced over time and suggest that cadmium was not irreversibly bound to a metallothionein-like protein. These authors also investigated effects of pretreatment on fish survival and found no clear indication of build-up of immunity in pretreated rainbow trout relative to unexposed fish.

Copper

The mode of action of copper in alga is through inhibition of nitrate uptake (Tripathi et al., 2004), while its effect in animals is through inhibition of sodium uptake in the sodium channel (Hoang & Klaine, 2007).

Effects of copper on cellular processes, growth, photosynthesis, biomass and community structure of algae have been studied by various authors. Tripathi et al. (2004) tried to understand the cellular processes in toxicity tests at exposure of 2.5 and 10 µM copper with the algal species *Scenedesmus*. They found that during exposure, $NO3^-$ uptake was inhibited more strongly than nitrate reductase (NR) activity. There was faster recovery of NR activity, an enzyme required for recovery from metal stress, in the absence of a metabolic inhibitor. When a photosynthetic inhibitor was added to the culture, no recovery of NR and $NO3^-$ uptake occurred, suggesting that photosynthesis was required for recovery from metal stress. Tripathy & Gaur (2006) took the understanding of copper toxicity in alga further through their findings of concentration-dependent inhibition of growth, photosynthesis, respiration, $NO3^-$ uptake, and nitrate reductase activity, along with reduction in protein, carbohydrate, and photosynthetic-pigment levels during exposure. Most of the processes recovered following exposure at lower concentrations. Serra et al.'s (2009) experiments with a community of periphytic algae found that multiple pulses at concentrations of 20-30 µg/L copper did not affect the algal biomass or community structure.

Diamond et al. (2005) conducted a series of experiments with daphnids (*Daphnia magna*) and flathead minnows (*Pimephales promelas*). Survival of 6 d old minnows after 6 hr single exposures to 50-75 µg/L was not significantly different to controls, but double 6 hr exposures resulted in significantly lower survival (Diamond et al., 2005). A single 12 hr pulse exposure to 50µg/L resulted in lower survival than double exposures of 6 hr at

50 µg/L. Fish biomass was negatively correlated to pulse frequency (number of pulses) and duration, but not to concentration. Generally, no delayed mortality was noted during post-exposure.

Butcher et al. (2006) conducted Mancini/Breck-type model fitting on the results of Diamond et al. (2005) and Diamond & Butcher (2006). The model explained 43%-83% of variability in survival, growth and reproduction data with lower explanatory power for relative growth data due to high variability in endpoint. The authors concluded that organism response is sensitive to prior conditions and that constant exposure experiments can underestimate the risk from intermittent exposures to the same concentration. For pulsed exposures, neither the average nor the maximum concentration alone was an adequate index of risk, which depended on the magnitude, duration, and timing of exposure pulses.

Diamond et al. (2006a) further investigated copper effects on daphnids and *Pimephales*. Daphnids (< 24 hr old) exposed to 8-48 µg/L copper for 12-24 hr were able to recover from pulsed exposures to reproduce at control or higher levels when they were given a few days recovery time; mortality effects were confined to within 48 hr of pulse termination (Diamond et al., 2006a). *Pimephales* fry (< 24 hr) exposed to 30-40 µg/L displayed 20-60% mortality in 24 hr pulsed exposures. Mortality response to the ≤24 hr pulses (single or multiple) was at or near continuous exposure 48 hr LC50 values. Longer recovery times between multiple pulses led to greater survival in daphnids, unlike *Pimephales*. This response in *Pimephales* was due to their adaptation to pulsed copper being activated for approximately 48 to 96 hr, after which it was removed if copper was removed from the media, leaving the fish susceptible to a new pulse. Growth of the *Pimephales* fry was found to be capable of rebounding from short pulse exposures.

Measurable effects on mortality have been noted for daphnids, rotifers and amphipods by other studies also. Hoang & Klaine (2007) found that the 21 d mortality to 12 hr 70 µg/L exposure increased with daphnid age from 3-96 hr old and then decreased with age from 96-240 hr old; however, no difference in 21 d growth was detectable. The 21 d cumulative reproduction decreased with age from 3-96 hr old and then increased with age from 96-240 hr old. The authors indicate that the sensitivity of *D. magna* to copper during the reproductive period arises from their greater need for sodium for their developing young. Rotifers (*Brachionus calyciflorus*) exposed to daily 0.0375-0.15 mg/L pulses for 2 weeks had reduced population growth due to the interaction of increasing copper concentration and exposure time (Gama-Flores et al., 2007b). However, copper concentration alone at the levels tested did not significantly affect the population growth rate. Significant reductions in body size and egg ratio were noted for ≥12 hr, while there was significant reduction in egg hatching success at ≥3 hr at all concentrations tested. For amphipods (*Hyalella azteca*) exposed to 12 hr pulses of 0.8-1.1 mg/L copper, higher copper concentration resulted in higher latent mortality (Zhao & Newman, 2006). Latent mortality was noted up to 60-70 hr post exposure. Recovery time (up to 72 hr) had a significant effect on the second-exposure mortality with animals exposed to longer recovery times showing mortalities close to control treatments; mean complete recovery time for $CuSO_4$ was 83 hr.

Bearr et al. (2006) conducted a range of copper toxicity experiments with *P. promelas* fry (< 24 hr) using 1-3 pulsed exposures of 5-40 µg/L with various recovery periods. Mortality was recorded immediately following pulsed exposure; pulses of 12 hr or longer seemed to result in exceedence of an internal dose threshold and significant mortality,

whereas 24 hr exposure initiated acclimation of sorts with significantly less mortality recorded. Fish biomass was also affected with a 24 hr pulse of 40 µg/L resulting in significantly less fish biomass than in controls. Recovery time between pulses had a significant effect on fish survival: a 48 hr recovery time between 24 hr 40 µg/L pulses resulted in significantly greater survival as compared to either shorter or longer recovery times. In another experiment, 24 hr copper pulses of 30-40 µg/L caused significantly higher mortality when spaced farther apart in time than when pulses of the same magnitude were spaced more closely. Exposures having a 48-96 hr recovery time between pulses had less effect on fish survival than did treatments with shorter (12-24 hr) or longer (> 120 hr) recovery times. Fish survival was not significantly different between the single- and double-pulse treatments (with 96 hr recovery time in between); however, addition of a third pulse (96 hr after the second pulse) resulted in a significant decrease in fish survival.

Sellin et al. (2005) investigated cellular mechanisms associated with the acclimation of *P. promelas* larvae and juveniles. They found that the recovery of ionoregulatory function at the gills resulted in acclimation to copper. However, in juvenile fathead minnows exposed to copper, whole-body Na^+ did not correlate well with the ability to acclimate. In their experiments, they found that 12 d old episodically exposed larvae were acclimated while 8 d old larvae had significantly lower survival than continuously exposed fish suggesting that these fish were not acclimated.

Rabago-Castro et al.'s (2006) experiments with juvenile *Ictalurus punctatus* (Channel catfish) found control fish to be 30% heavier and 10% larger than fish treated with copper sulfate and the specific growth rate was lower in treated fish for 8-11 weeks. Treated catfish had lowest feed intake and the authors concluded that growth suppression was more likely due to a decrease in feed conversion index (FCI) possibly for maintaining homeostasis instead of due to appetite suppression.

Selenium

The mode of action for Selenium is through induction of oxygen radicals (Hoang & Klaine, 2007).

Selenium toxicity effects have been studied using *Daphnia magna*. Daphnids exposed to 12 hr pulse of 1000 µg/L had 21 d mortality that increased with age for 3-48 hr old organisms, but decreased with age for 48-240 hr olds (Hoang & Klaine, 2007). Age influenced toxicity of selenium more than toxicity of Cu and Zn as selenium may be absorbed through the new exoskeleton of newly moulted daphnids, and thus Hoang & Klaine (2007) state that daphnids may be more sensitive to selenium on or after first moulting. No difference in 21 d growth was detectable and 21 d cumulative reproduction decreased with age from 3-48 hr old and then increased with age from 48-240 hr old. In later experiments, Hoang & Klaine (2008) found that < 24 hr old daphnids exposed to a single 4-24 hr pulse of 800-2000 µg/L showed no mortality during exposure; but latent mortality during post-exposure with mortality increasing with exposure duration and exposure concentration. Generally no effect on growth, time to first brood and cumulative reproduction was detected for single or multiple pulses. Exposure to 2 pulses of 800-1800 µg/L for 3-12 hr showed that pulses with no delay had higher mortality than those with recovery time up to 288 hr. Daily mortality due to first pulse was generally higher than that from the second pulse. Post-exposure cumulative mortality at 21 d was lower when the time interval between pulses was longer.

Zinc

Zinc's mode of action is through inhibition of calcium uptake at calcium channel (Hoang & Klaine, 2007). Hoang & Klaine (2007) indicate that the sensitivity of *Daphnia magna* to zinc during the reproductive period arises from their greater need for calcium for their developing young.

Effects of zinc exposure have been tested for on algae, invertebrates (daphnid, water flea and amphipod) and vertebrates (fish). Alga *Scenedesmus* sp. showed various sub-lethal effects such as concentration dependent inhibition of growth, photosynthesis, respiration, reduction in protein and carbohydrate and increase in intracellular metal levels during exposure (Tripathi & Gaur, 2006). Recovery occurred during post-exposure but the speed of recovery was concentration dependent.

Mortality (measured as immobility over 24 hr) was noted during post-exposure for neonates of waterflea and amphipod (Brent & Herricks, 1998). Increased levels of mortality and reduced levels of cumulative reproduction was recorded with age for daphnids up to 4 d old that were exposed to single pulses of 12 hr; mortality levels reduced and reproduction levels increased with age for individuals > 4-12 d age (Hoang & Klaine, 2007). Growth was not a sensitive parameter in these tests. Daphnid neonates < 24 hr old were more sensitive to 24 hr pulses of 250-1000 µg/L with continued mortality even in post-exposure (Diamond et al., 2006a). In comparison 3-6 hr pulses at high concentrations resulted in no effects on daphnid survival or reproduction. Daphnid survival was greater if recovery times were longer between pulses and they showed reproduction levels similar to control individuals after a few days post-exposure.

Fry of flathead minnows (*Pimephales promelas*) showed high (20-60%) mortality during 24 hr pulsed exposures and during post-exposure (Diamond et al., 2006a). The guppy, *Poecilia reticulata* (an introduced fish in Southeast Asia that is found in drains, canals, reservoirs) was comparatively more resistant to a much higher concentration (Widianarko et al., 2001). The guppy's internal body concentration of zinc increased over the 6 days of exposure to 1500 µg/g and then reduced exponentially during post-exposure with a half life of only 1.5 d.

Pesticides

Atradex

Atrazine is the main active ingredient in Atradex.

Littlefield-Wyer et al. (2008) exposed microbial communities to a pulse of Atradex over a range of concentrations (24.5-245 µg/L). The Atradex concentration was allowed to dilute (decay) over 8 d. Although microbial biomass decreased during the trial, at the end of 8 d microbial communities in all treated groups (except tanks spiked with 245 µg/L Atradex) had recovered and showed similar metabolic fingerprints and fatty acid methyl ester profiles to those of controls. The results indicated that exposure to Atradex with a diluted-pulse exposure profile at a concentration above 245 µg/L, may irreversibly change the structure and functional status of aquatic microbial communities.

Atrazine
Atrazine is a herbicide whose mode of action is inhibition of photosystem II (Vallotton et al., 2008a).

Vallotton et al. (2008a) report a recovery from exposure to atrazine in the alga *Scenedesmus vacuolatus* which was exposed to higher concentrations (80 to 510 µg/L) but for a shorter pulse (10-24 hr). Growth rate was reduced by over 40% by exposure to 125 µg/L of atrazine or higher. However, recovery of growth occurred following removal of atrazine within 5 hr, with growth recovery of the algae during the post-exposure period being independent of the applied concentration and duration of pulse exposure. Vallotton et al. (2008a) postulate that the fast recovery of *S. vacuolatus's* growth might be a consequence of the rapid elimination of the compounds from the cells and the complete reversibility of atrazine's mode of action.

Carbaryl
Carbaryl is a carbamite insecticide whose mode of action is inhibition of acetylcholinesterase (Ashauer et al., 2007b).

Petersen et al. (2001) exposed the invertebrates *Calineuria californica* (Plecoptera: Perlidae) and *Cinygma* sp. (Ephemeroptera: Heptageniidae) to short pulses (15, 30, 60 min) of carbaryl and monitored mortality over the remaining 96 hr. *Cinygma* sp. was considerably more sensitive than *C. californica*, with post-exposure 96 hr LC50s for the 60 min pulse exposure of 165 µg/L and 1139 µg/L respectively. *Cinygma* sp. post-exposure 96 hr LC50s for 30 min and 15 min pulses were 220 µg/L and 848 µg/L respectively. In contrast, 50% mortality was not reached for any test concentration for the 15 and 30 min exposures for *C. californica*. Whereas there was no post-exposure recovery for *Cinygma* sp that had become moribund during the pulse exposure, moribund *C. californica* individuals recovered 5 hr after the end of the pulse exposure. The authors suggest that the difference in sensitivity between the two species could be a result of significant differences in rates of uptake and time to equilibration between the two species, possibly due to morphological differences, such as gill surface area, or higher rates of metabolism or excretion.

Ashauer et al. (2007a) exposed *Gammarus pulex* to multiple 24 hr pulse exposures of carbaryl at concentrations between 16-90 µg/L. At the lower pulse concentrations (< 30 µg/L), mortality began to decelerate during the post-exposure period between pulses, however further pulse exposures increase the rate of mortality dramatically. At higher pulse exposure concentrations (> 60 µg/L) however, mortality occurs steadily during the post-exposure period with little indication of leveling out between pulses. The authors estimated that for all concentrations tested, it would take 3 d for internal damage to decline sufficiently not contribute to the effects of a subsequent pulse.

Ashauer et al. (2007b) exposed *Gammarus pulex* to sequential 24 hr pulses of either 26.5 µg/L carbaryl and then 0.494 µg/L chlorpyrifos with a 2 week recovery period in between, or chlorpyrifos first and then carbaryl. Previous exposure to chlorpyrifos led to dramatically increased mortality from the subsequent pulse of carbaryl, but not the other way round. The authors ascribe this to the fact that enzyme recovery from inhibition of acetylcholinesterase generally show faster reactivation after inhibition by carbamates (e.g., carbaryl) than that after inhibition by organophosphates (e.g., chlorpyrifos). They determined that the internal damage (term damage is used as a generic measure for the overall reduction in fitness of the organisms) following exposure to carbaryl falls below

threshold levels on day 8 due to fairly quick recovery processes, whereas the slower recovery for damage caused by chlorpyrifos results in damage levels above the threshold until day 15 when the second pulse starts.

Cartap

Mode of action is as a nicotinic acetylcholine receptor blocker (Dropdata.org 2010).

Recent findings of endocrine-disrupting effects and developmental neurotoxicity of cartap led Kim et al. (2008) to investigate the long term affects of a short pulse of cartap on Japanese medaka fish (*Oryzias latipes*) and *Daphnia magna*. For *O. latipes* exposed for 4 d and then monitored for post-exposure effects for 17 d, NOECs determined for deformities and time to hatch were 125 µg/L and 250 µg/L respectively. There was rapid recovery of *D. magna* after short-term 2 hr pulse exposure. Observations 20 d post-exposure did not show adverse effects on mortality, reproduction, brood size or population rate at the highest concentration of 1240 µg/L.

Chloramine-T

Chloramine-T (Cl-T) is used to control bacterial gill disease in salmonids. Chloramine-T is effective against many bacteria, viruses (enveloped and naked), fungi, algae, yeast, and parasites. The mode of action is thought to be through oxidative processes, quickly destroying cell material or disrupting essential cellular processes (Gaikowski et al., 2009).

Channel catfish (*Ictalurus punctalus*) and Walleye fish (*Sander vitreum*) were exposed to chloramine-T concentrations of 20, 50, 80 mg/L in 12 pulses, each of 3 hr length, over 21 d. Both species recorded no significant mortality in any treatments over the trial. The only exposure-related histological changes observed were in the spleen of the catfish with significantly greater erythrocyte swelling and necrosis occurring in fish exposed at 80 mg/L relative to exposure at 0 mg/L (i.e. NOEC = 50 mg/L). The only significant change in peripheral blood cytology was observed in walleye fingerlings exposed at 80 mg/L which had significantly fewer mature red blood cells and significantly more immature red blood cells per oil-immersion field than controls (i.e. NOEC = 50 mg/L).

Chlorpyrifos

Chlorpyrifos is an organophosphate insecticide. Mode of action is inhibition of acetylcholinesterase (Ashauer et al., 2007c).

Van der Hoeven & Gerritsen (1997) exposed both juvenile and adult *Daphnia pulex* to 1, 2 and 3 d pulses of chlorpyrifos and monitored recovery over 5, 4 and 3 d respectively. Effects of toxicity increased with increasing concentration and exposure time. The 6 d LC50s for adults and juveniles subjected to the 3 d pulse exposure were 0.55 and 0.53 µg/L respectively. Juveniles became immobilized quicker than adults. Almost no recovery was recorded during the post-exposure period, and in fact 20% of the juvenile and 30% of the adult *D. pulex* became immobile after the transfer to uncontaminated medium and eventually died, suggesting slow elimination of chlorpyrifos from the organism.

In a study using *D. magna* neonates exposed to chlorpyrifos for 1-72 hr and monitored for remaining 21 d, Naddy et al. (2000) report significantly reduced survival with exposure durations > 12 hr at 0.5 µg/L (survival curves similar to continuous exposure). When daphnids were exposed to two 12 hr pulses of 0.5 µg/L a similar response was

observed (> 85% mortality) regardless of the pulse exposure interval (0, 3, 7, 14 d). NOECs in both instances were 0.25 µg/L. Daphnids were, however, able to survive a 12 hr exposure at 0.5 µg/L if the exposure regime was separated into two 6 hr pulses with a minimum interval of 3 d, suggesting that the animals might be able to detoxify if given sufficient time. The response to the second pulse was generally more significant in terms of mortality than the first pulse. In a similar study using adult daphnids, Naddy & Klaine (2001) found exposure to a single pulse of 0.5 µg/L did not result in significant mortality. However, animals exposed to 1-3 pulses of 1.0 µg/L for 2-6 hr had significantly reduced survival (5-40% compared to 90% survival for control treatment). Daphnids receiving two 0.5 µg/L pulses of 6 hr (with 0-48 hr interval) showed decreased mobility, but over 80% of animals recovered within 7 d. A recovery period of at least 72 hr between pulses was necessary for daphnids to recover from 0.5µg/L pulse, while a longer recovery period of 96 hr was necessary for 1.0 µg/L pulse. Ashauer et al. (2007c) determined that the much more sensitive *Gammarus pulex* required 25 d for internal damage caused by exposure to chlorpyrifos to decline sufficiently not to contribute to the effects of subsequent pulses. Continued post-exposure effects of chlorpyrifos have also been reported by Jarvinen et al. (1988a) in the fathead minnow *Pimephales promelas.* Exposure to chlorpyrifos for as few as 5 hr at a concentration similar to the continuous exposure 96 hr LC50 value (122.2 µg/L) resulted in increased deformities and a reduction in growth, but did not have an effect on survival. At end of 30 d trial the NOEC for the 5 hr pulsed exposure for survival was 264.0±2.8 µg/L, for deformities was 68.55±1.35 µg/L and for mean weight 68.55±1.35 µg/L. In contrast, the NOEC for continuous exposure for deformities was 1.29±0.007 µg/L and for mean weight 3.88±0.03 µg/L.

Cyanazine

Cyanazine is a triazine herbicide used for pre- and post-emergence weed control (Reid et al., 1995). Its mode of action is inhibition of photosynthesis.

Australian crimson-spotted rainbowfish (*Melanotaenia fluviatilis*) larvae were exposed to a 2 hr cyanazine pulse at concentrations between 1.9-43.2 mg/L and recovery monitored for further 94 hr. Five age groups were exposed (0, 3, 6, 9 and 12 d post-hatch). Toxicity was found to decrease with larval age. The NOEC and LOEC values based on larval mortality for cyanazine were < 1.9 and 1.9 mg/L. A considerable progressive reduction in cyanazine toxicity was noted for larvae aged 0-3 d post-hatch with no further reduction in toxicity for 6, 9 and 12 d old fish. The authors proposed this as evidence of hepatic xenobiotic metabolism, suggesting that the liver of larval rainbowfish becomes increasingly more functional with age. However, results could also be influenced by the greater respiration rates and small body surface area of smaller larvae (Reid et al., 1995).

Cypermethrin

Cypermethrin is a pyrethroid insecticide (Kim et al., 2008) whose mode of action is as a sodium channel modulator.

Daphnia magna neonates were exposed to a 24 hr pulse of cypermethrin at a range of concentrations up to 1.9 µg/L. No adverse effects on reproduction or survival were recorded 20 d after the pulse (Kim et al., 2008). However, exposing *Oryzias latipes* (Janapense medaka fish) to a 96 hr pulse at concentrations of 8, 40, 200, 1000, 5000 µg/L cypermethrin produced a mortality NOEC of 40 µg/L and deformity NOEC of 8 µg/L (Kim et al., 2008).

DDT and dieldrin combination

DDT is a sodium channel modulator and dieldrin a chloride channel agonist (Dropdata.org 2010).

The effect of these two insecticides released from sediment after a dredging event was monitored in a range of marine and estuarine organisms. Sediment disturbance during dredging introduced a pulse of dieldrin and DDT (and its metabolites) into the surrounding ecosystem. Body burdens of fish and invertebrates increased 2- to 76-fold, depending on the species. Approximately 1.5 years after remediation, 11 of 14 organisms showed contamination comparable with or worse than the contamination that existed prior to dredging (Weston et al., 2002).

Diazinon

Diazinon is an organophosphate insecticide whose mode of action is acetylcholinesterase inhibition (Dropdata.org 2010).

Juvenile snakehead fish *Channa striata* were exposed twice to 4 d pulses of 0.016, 0.079 or 0.35 mg/L of diazinon, separated by a 2 week interval to imitate the exposure conditions in the field (Cong et al., 2009). After the 4 d exposures, the fish were moved to clean water for recovery and fish were monitored for 20 d. Results show mortality in exposed treatments did not significantly differ from the control. Brain cholinesterase (ChE) was significantly inhibited by all concentrations by the end of the 4 d pulse exposure. While there was some recovery during post-exposure periods, the brain ChE levels in fish exposed to higher diazinon concentrations were still significantly lower by the end of the trial (i.e. NOEC = 0.016 mg/L). Specific growth rate was significantly lower in fish exposed to the highest concentration (i.e. NOEC = 0.079 mg/L) (Cong et al., 2009).

Chinook salmon (*Oncorhynchus tshawytscha*) eggs and alevins that were exposed to 0.01-0.1 mg/L diazinon for 96 hr and monitored for a further 14 d hatched and developed normally and did not experience significant mortality. Metabolic effects of exposure were not observed post exposure either (Viant et al., 2006).

Dimethoate

Dimethoate is an organophosphate insecticide whose mode of action is inhibition of acetylcholinesterase (Andersen et al., 2006).

Andersen et al. (2006) exposed *Daphnia magna* of different ages to a variety of pulse scenarios. The first scenario was for animals aged < 24 hr and 3 d old exposed to a single pulse. Dimethoate pulse durations of 2.4 hr (30 mg/L) to 4.8 hr (10 mg/L) resulted in 50% immobilization of the exposed population immediately after transfer to clean media. After a recovery period of 48 hr, the estimated pulse duration to cause 50% immobilization had increased to 3.9 hr at 30 mg/L and 6.3 hr at 10 mg/L, suggesting recovery post-exposure. The results also indicated a concentration-dependent response, with smaller exposure times needed to for a given response with increasing concentrations. There was no significant difference in recovery based on age.

The second scenario involved exposure of < 24 hr *D. magna* to multiple pulses of various lengths. Following the first exposure pulse there was recovery from immobility for all pulse durations. However, after the second pulse, mortality occurred and increased significantly during the recovery period for pulse durations greater than 2 hr.

The second pulse exposure of 10 mg/L affected all daphnids in the groups receiving pulses of 4 and 6 hr durations. Immediately after transfer to clean media, the effect was mainly immobilization, because only 5% died in the group receiving the longest pulse of 6 hr. The degree of recovery was much less pronounced during the post-exposure period following the second pulse, and after 24 hr in clean media, 60 and 95% of the daphnids were immobilized or dead in the groups exposed to 4 and 6 hr pulses, respectively. In these groups, 75 and 85%, respectively, of the animals were dead 48 hr after exposure, and even in groups exposed for 1 and 2 hr, mortalities of 15 and 25%, respectively, were observed. Exposure to a second pulse at a higher concentration (20 mg/L) of dimethoate resulted in even more pronounced effects on mobility and mortality. All animals exposed to repeated pulses of 4 and 6 hr in duration were immobilized or died immediately after exposure to the second pulse, and 30, 15, and 60% of the animals in the 0.5, 1, and 2 hr groups, respectively, were affected (either dead or immobilized). Forty-eight hours after the second pulse, dead animals were found in all exposed groups, and all animals exposed to the 6 hr pulse were dead

The third scenario was to determine long-term effects of dimethoate exposure. Daphnids were exposed to single 1-3 hr, 30 mg/L pulses. Results showed significant reduction in daphnid body length, significantly fewer offspring produced, and delayed time to reach maternity for all pulse exposure durations.

Dinoseb

Dinoseb is a herbicide whose mode of action is disruption of the organism's metabolism (Viant et al., 2006).

At end of a 96 hr pulse exposure period there had been 100, 87 and 15% mortality of Chinook salmon (*Oncorhynchus tshawytscha*) alevins exposed in the 250, 100 and 50 ppb dinoseb respectively. During the post-exposure period of 14 d, latent mortality continued. *Oncorhynchus tshawytscha* eggs exposed for 96 hr all died at 750 ppb, while 24% died at 250 ppb. However there was no effect on time to hatch or normal development of survivors. After 14 d recovery period, all eyed eggs from the 250 ppb exposure failed to hatch and subsequent mortality resulted. Metabolic effects of exposure were not observed in exposed eggs or alevins at the end of the recovery time (Viant et al., 2006).

Diquat

Diquat is a herbicide whose mode of action is inhibition of photosynthesis (Cedergreen et al., 2005).

At end of post-exposure period of 7 d the relative growth rate of *Lemna minor* (duckweed) exposed to a 3 hr pulsed of diquat (concentration range 0.01-1.0 µM) had not decreased compared to non-exposed plants. The EC50 of pulse exposed plants was 100 fold higher than EC50 of continuously exposed plants.

Diuron

Diuron is a herbicide whose mode of action is inhibition of photosynthesis (Tlili et al., 2008.

Biofilms were grown in indoor microcosms that were either non-contaminated or exposed to low-level chronic contamination, and not exposed, or exposed to single or double pulses of two environmental concentrations (7 and 14 µg/L) of diuron (Tlili et al.

2008). Both single and double pulses inhibited carbon incorporation of all biofilm communities, especially of the pulsed control ones relative to the chronically exposed community. Biofilm eukaryotic communities (DGGE on 18S rDNA gene fragments) from the control microcosm were restructured by acute exposure to diuron, whereas the eukaryotic community structure of biofilms that have previously been exposed to a low concentration of diuron were not affected by the diuron pulses. PSII inhibitors can inhibit the photosynthesis of aquatic microorganisms within a few seconds, but only subsequently (d later) induce physiological stress and long-term effects on microbial communities. NOEC for ash-free dryweight of the biofilms or the biofilm sensitivity was 7 µg/L (Tlili et al., 2008).

In a study investigating the combined effects of diuron and azoxystrobin (mode of action is by inhibition of mitochondrial respiration in fungi, thus inhibiting spore germination, mycelial growth, and spore production of fungi) on the European top minnow *Phoxinus phoxinus*, Bony et al. (2008) reported a significant increase (3-5 fold) in DNA damage in exposed fish at the end of the exposure period. However, after 22 d of post-exposure recovery the DNA damage in exposed fish erythrocytes had recovered to unexposed levels.

Endosulfan

Endosulfan is an organochloride insecticide and acaricide. Mode of action is to antagonize the action of the neurotransmitter gamma-aminobutyric acid (GABA) leading to neurotoxic effects (Broomhall, 2002).

When compared with both endosulfan-exposed *Litoria citropa* tadpoles maintained at stable temperatures, and tadpoles not exposed to endosulfan, the exposure to 0.8 µg/L endosulfan for 96 hr on a variable temperature cycle increased the tadpoles' subsequent vulnerability to predatory odonates when tested 24 d later (Broomhall, 2002).

Endrin

Endrin is an organochloride pesticide whose mode of action is as a central nervous system stimulant (Jarvinen et al., 1988a). Endrin is a stereoisomer of dieldrin (chloride channel agonist).

Jarvinen et al. (1988a) reported that as exposure time of fathead minnow *Pimephales promelas* to endrin increased so did toxicity, with deformities being a more sensitive endpoint than mortality (e.g. the 96 hr LC50 and EC50 for: 1 hr pulsed exposure was > 16 µg/L and > 16 µg/L respectively; for 5 hr pulse exposure 14.9 µg/L (11.5-19.3) and 11.3 µg/L (9.8-13.1)(95% confidence limits); for 48 hr pulse exposure 6.8 µg/L (5.6-8.2) and 1.2 µg/L (1.1-1.4) and; for 96 hr continuous exposure: 0.7 µg/L (0.5-1.0) and 0.5 µg/L (0.4-0.6).

In a trial assessing recovery over longer periods, a 48 hr exposure at a concentration similar to a continuous 96 hr LC50 value was required to cause a reduction in growth. All deformed fish either died or recovered completely. At end of 30 d trial: NOEC for 24, 48, 72 hr and continuous pulse exposure for survival was 3.43±0.40, 0.62±0.09, 0.63±0.1 and 0.38±0.007 µg/L (Jarvinen et al., 1988a).

Esfenvalerate

Esfenvalerate is a pyrethroid insecticide whose mode of action is as a sodium-channel agonist (Holdway et al., 1994). Esfenvalerate is an isomer of fenvalerate.

In fish exposure trials, Holdway et al. (1994) report a 96 hr LC50 of 1.81 µg/L for juvenile *Melanotaenia fluviatilis* (Australian crimson-spotted rainbow fish) when exposed to a 1 hr pulse of between 0.1-32.6 µg/L of technical grade esfenvalerate. At low concentrations, most mortality occurred within the first 24 hr post exposure, while at higher concentrations, mortality continued for the full 95 hr post-exposure period.

Viant et al. (2006) exposed the eyed eggs and alevins of *Oncorhynchus tshawytscha* (California's Chinook salmon) to 1, 10 and 100 µg/L esfenvalerate for 96 hr and then monitored recovery for 14 d. During the post-exposure period, eyed eggs from the 10 and 100 µg/L groups hatched significantly earlier than controls and developed abnormally, generally with lack of normal swimming. The NOEC for number of days to 100% hatch was 1 µg/L. For alevins, at the end of the recovery period there was significant mortality of 100% for 10 and 100 µg/L treatments, and 95% for the 1 µg/L treatment. Metabolites were also analysed at the end of the exposure period and then 14 d after recovery. For eyed eggs, a NOEC was determined for PCr and ATP at 1 µg/L immediately after exposure, but after 14 d in clean water the only NOEC determined was for AMP at 1 µg/L. In alevins, a NOEC for ATP of 1 µg/L was determined immediately after exposure, but after 14 d in clean water no NOEC could be determined.

In a trial exposing Bluegill fish *Lepomis macrochirus* to low pulsed concentrations of esfenvalerate (0.01-0.2 µg/L) for between 11-44 hr every two weeks for a total of six exposures, Little et al. (1993) found that behavioural responses, including gross body tremors, were highly sensitive indicators of toxicity, occurring within 4 hr after exposure to concentrations ≥ 0.025 µg/L (NOEC = 0,01 µg/L). Tremor frequency declined to control value during the 2 week recovery period between pulses and there was no significant difference among treatments 21 d after the final pulse. Aggression was significantly lower among fish exposed to concentrations of 0.1 µg/L or greater (NOEC = 0.05 µg/L), however by 21 d after the last pulse exposure aggression was similar among all treatments. Although no fish survived continuous exposure to esfenvalerate at 0.2 µg/L for 30 d or 0.1 µg/L for 60 d, all fish survived the six 44 hr pulse exposures up to 0.2 µg/L.

Fairchild et al. (1992) exposed *L. macrochirus* in field mesocosms to higher concentrations of esfenvalerate (0.25, 0.67 and 1.71 µg/L), also in six pulses at two week intervals. The esfenvalerate dissipated rapidly from the water column with a half life of 10 hr. Various endpoints were measured 45 d after the last pulse, revealing that esfenvalerate concentrations of ≥ 0.67 µg/L resulted in significantly reduced survival, reproductive success and biomass (NOEC = 0.25 µg/L). In addition to *L. macrochirus*, Fairchild et al. (1992) also measured responses from the macroinvertebrate, zooplankton, macrophyte communities and primary production in the mesocosms. All concentrations significantly reduced total numbers of invertebrates during and between pulses. However, some recovery was evident by the end of the 45 d post-exposure period. The zooplankton community dynamics were affected at all concentrations. In contrast, no direct or indirect effects were observed on the macrophyte community. Finally, regarding effects on primary production, indirect effects caused decreased levels of chlorophyll a at concentrations of ≥ 0.67 µg/L (NOEC = 0.25 µg/L).

Cold & Forbes (2004) exposed *Gammarus pulex* at various stages of development to a 1 hr pulse of esfenvalerate and monitored effects during a 14 d recovery period. Exposing juveniles (3-6 mm body length)and adults (9-15 mm body length) to 0.1 and 0.3 µg/L resulted in continued mortality during the recovery period with no sign of ceasing. When copulatory adults were exposed to concentrations between 0.05-0.3 µg/L, significant mortality of newly released juveniles occurred during the recovery period with higher exposure concentrations resulting in greater mortality. In all exposed treatments, significantly fewer juveniles were produced by adults over the exposure period compared to controls. In another trial, increased mortality occurred with increasing exposure concentration when newly released juveniles (1-2 mm body length) were exposed to concentrations 0.05-0.6 µg/L. Lastly, higher exposure concentrations also resulted in a significant effect on pair reformation and number of juveniles produced in precopulatory adults (10-15 mm body length).

Forbes & Cold (2005) report that exposure to a 1 hr, 0.2 µg/L esfenvalerate pulse reduced time to emergence and number of emerging adults at the end of a 39 d recovery period. Mortality was significantly lower in the exposed group, and although there was reduced overall fecundity in exposed group due to high mortality, the egg laying or egg viability of surviving females was not affected. Overall population growth rate was significantly affected when the larvae were transferred to clean medium with contaminated sediment during post-exposure period, but not if transferred to clean medium and sediment.

Fenitrothion

Fenitrothion is a cholinesterase inhibiting organophosphorus insecticide (Scherer & McNicol, 1986)

Predatory stoneflies (*Acroneuria lycorias*) were exposed to a pulse of either 4, 8 and 12 µg/L fenitrothion and monitored for 24 hr. A delayed dose-dependent response was detected 7-12 hr after exposure of 8 and 12 µg/L with abandonment of normally preferred areas during night period. Response was still present at the end of observation time of 24 hr. The NOEC for locomotor activity and drift was 4 µg/L. The observed response fits with gradual uptake of feniotrothion and with slow increase and persistence of acetylcholinesterase inhibition.

Fenoxycarb

Fenoxycarb is an insecticide that disrupts development by inhibiting metamorphosis to the adult stage and inducing interference with the moulting of early instar larvae (Hosmer et al., 1998).

Daphnia magna of varying ages (< 24 hr, 4-6 d, 8 d, 11 d) were exposed to nominal concentrations of 0.2, 0.8, 3.2, 13, 50 µg/L and monitored for 21 d. There were no significant effects on survival or time to first brood of first and second generation daphnids in any age group at all exposure concentrations. The number of young per daphnid was significantly lower than controls only among daphnids that were < 24 h old at test initiation and exposed to the highest initial measured concentration of fenoxycarb (45 µg/L). The estimated maximum acceptable toxicant concentration (MATC) was 26 µg/L, calculated from exposure of the most sensitive age group (< 24 hr old). This represents a substantial reduction in toxicity when compared to the MATC of 0.0016 µg/L previously reported from a standard, constant-exposure study (Hosmer et al., 1998).

Fenvalerate

Fenvalerate is a pyrethroid pesticide whose mode of action is as a sodium channel modulator (Dropdata.org 2010).

Holdway et al. (1994) exposed Australian crimson-spotted rainbowfish (*Melanotaenia fluviatilis*) to technical and emulsified grade fenvalerate concentrations between 0.1-32.6 µg/L for 1 hr and monitored recovery for the remaining 95 hr. At low concentrations, most mortality occurred within the first 24 hr, while at higher concentrations, mortality continued for the full 95 hr post-exposure period. The technical grade was significantly more toxic than emulsified grade. The 1 hr pulsed exposure LC50 measured after 95 hr recovery was 12.75 µg/L for technical grade fenvalerate and 30.25 µg/L for emulsified fenvalerate.

In exposing another fish, fathead minnow *Pimephales promelas*, to technical grade fenvalerate, Jarvinen et al. (1988a) report that deformities were a more sensitive endpoint than mortality. The 1 hr pulsed exposure LC50 measured after 95 hr recovery could not be determined as exposure concentrations were too low, however the 96 hr LC50 and EC50 (deformity) were determined for 5, 24, 48 and 72 hr pulse exposures: > 5 µg/L and 2.85 µg/L (-)(95% confidence limits); 2.63 µg/L (2.34-2.95) and 2.68 µg/L (2.44-2.95); 1.87 µg/L (1.59-2.19) and 1.34 µg/L (1.27-1.42); and 1.61 µg/L (1.34-1.94) and 0.52 µg/L (0.45-0.60) respectively. It was determined that a 62 hr exposure at 0.85 µg/L (the 96 hr LC50 continuous exposure value) would be necessary for 50% deformation to occur within 96 hr. Only an extra 3 hr exposure (i.e. 65 hr pulse) to 0.85µg/L was necessary for fenvalerate to significantly affect fathead minnow survival within 30 d. However only a 48 hr exposure was necessary to cause significant growth affects within the same time period. At end of 30 d trial: NOEC for 24, 48, 72 hr pulsed exposure for survival was 1.08±0.11 µg/L; 0.88±0.20 µg/L and 0.49±0.03 µg/L respectively.

Regarding aquatic invertebrates, Wendt-Rasch et al. (1999) exposed the net-spinning caddisfly *Hydropsyche siltalai* to fenvalerate concentrations ranging from 0.25-0.5 µg/L. No significant difference was found in the number of survivors and number building nets at all concentrations. However, exposure to higher concentrations resulted in decreased symmetry of the nets and significantly large mesh openings. The NOEC for net symmetry, i.e. the average difference in area between the corresponding meshes on each side of the midline, and average mesh-opening was 0.25 µg/L. Schulz & Liess (2000) exposed another caddisfly, *Limnephilus lunatus*, to fenvalerate, but at significantly lower concentrations (0.0001-0.1 µg/L). Significant effect levels measured 240 d after a 1 hr were determined for reduced emergence success and production at 0.1 µg/L, temporal pattern of emergence at 0.001 µg/L and dry weight of adults at 0.01 µg/L. Organisms were also exposed to a 10 hr pulse and in order to compare results with concentrations being converted to a concentration-time endpoint (0.001, 0.01, and 0.1 µg/h). Thus, a NOEC for emergence success for both 1h and 10h exposure was determined to be 0.01 µg/hr. The NOEC for temporal emergence pattern after 1 hr exposure was < 0.001 µg/hr and for 10hr exposure was 0.001 µg/hr. The NOEC for dry weight of emerged adults was 0.001 µg/hr for the 1 hr exposure and 0.01 µg/hr for the 10 hr exposure.

Daphnia magna neonates (< 24 hr old) exposed to a 24 hr pulse of fenvalerate and monitored for recovery during the following 20 d produced a NOEC for survival of 0.6 µg/L (Pieters et al., 2005; Reynaldi & Liess, 2005) for fed individuals and 0.3 µg/L for a

low-food treatment (Pieters et al., 2005). The low food conditions exacerbated the effects of the fenvalerate exposure on juvenile survival and growth during the first week, resulting in a much stronger reduction in population growth compared to high food conditions. After 20 d of recovery the NOEC for population growth rate for high food condition was 0.3 µg/L and for low food condition 0.1 µg/L (Pieters et al., 2005). There was no increased sensitivity to fenvalerate at low food conditions in terms of reproduction endpoints however, and thus increased juvenile mortality is thought to be primarily responsible for the greater reduction in population growth rate. Higher mortality at low food is attributed to two causes: due to fewer resources available for defense against stressors, and/or secondly due to reduced bioavailability of fenvalerate in high food conditions due to its sorption to algae. The first reason may be more likely a the main route of fenvalerate uptake is thought to be dissipation through the surface area of daphnids instead of ingestion with algae (Pieters et al., 2005). In a longer trial, Liess et al. (2006) investigated a 24 hr fenvalerate pulse exposure effect on population abundance and structure of *D. magna* over 60 d. Abundance was reduced at 1.0 and 3.2 µg/L compared to the control. As a consequence of reproduction of the surviving individuals, abundance at 1.0 and 3.2 µg/L recovered to control levels after 12 and 17 d, respectively, but then exceeded control levels. This higher abundance decreased afterward and reached control levels again after 24 and 52 d respectively. Thus an abundance NOEC of 0.8 µg/L was determined. Population structure (size distribution) was affected at lower concentrations than abundance (≥ 0.8 µg/L). In addition, the alteration of population structure lasted for a long time, so that control levels were approached only after approximately six or seven generation times. Thus a population structure NOEC of 0.6 µg/L was determined for this long term recovery experiment.

In further studies on *D. magna* and fenvalerate, Pieters & Liess (2006a) tested the hypothesis that at low nutritional supply, *Daphnia* produce fewer but larger offspring, which are less sensitive to chemical stress. Maternal cohorts were maintained under either a high or low food treatment. Offspring were then exposed to a 24 hr pulse exposure of fenvalerate and their recovery monitored over 20 d. The NOEC for survival was 0.3 µg/L for the high maternal food treatment, and 1.0 µg/L for low maternal food treatment. Low maternal food conditions did indeed increase the offspring size at time of birth, reduce age at first reproduction and increase reproductive output, which jointly enhanced offspring fitness as estimated by the population growth rate. The reduction in population growth rate after the pulse exposure was significantly less strong in daphnids originating from low fed mothers compared to those from high fed mothers. Despite this, the NOEC for population growth rate was 0.1 µg/L irrespective of food treatment.

Lastly, Pieters & Liess (2006b) explored the effect of population development stage (in terms of food shortage and density) on fenvalerate toxicity to *D. magna*. The fenvalerate induced mortality in the 1.0-10.0 µg/L treatments causing rapid and severe declines in all size classes in both exponential (high food level/low density) and stationary (low food level/high density) phases, while no significant effects on survival were observed at 0.03-0.6 µg/L for either of the phases. Recovery from the 24 hr pulse for daphnids in the exponential growth phase started almost instantaneously, while onset of recovery showed delays in daphnids in the stationary phase. In addition, recovery periods increased with increasing fenvalerate concentration and with increasing size class.

Glyphosate
Glyphosate is a herbicide that inhibits the EPSP synthase enzyme, which leads to depletion of key amino acids that are necessary for protein synthesis and plant growth (Dropdata.org 2010).

Fed and unfed *Jordanella floridae* (Flagfish) were exposed to a 2 hr pulse of glyphosate at concentrations between 0.1-30 mg/L and assessed at end of a 94 hr recovery period. There were no mortalities recorded in the fed and unfed 2 and 4 d old fish. Fed 8 d old fish were significantly more tolerant (96 hr PE LC20 = 29.6 mg/L) than unfed 8 d olds (96 hr PE LC20 = 2.94 mg/L). The difference in flagfish toxicity to glyphosate over small changes in age, and between fed and unfed fish is evidence of the need for toxicologists to be more aware of the developmental ontogeny of exposed organisms when determining the effects of chemical exposure (Holdway & Dixon, 1988).

Imazamox
Imazamox is a herbicide that inhibits branched chain amino acid synthesis (Cedergreen et al., 2005)

Cedergreen et al. (2005) exposed duckweed *Lemna minor* to 3 hr pulse exposure of imazamox and assessed growth 4 and 7 d after exposure. At end of post-exposure period the relative growth rate EC50 of pulse exposed duckweed was approximately 10 fold higher than EC50 of continuously exposed plants.

Imidacloprid
The insecticide imidacloprid is a chlorinated analog of nicotine, the compound therefore belongs to the class of neonicotinoid insecticides, and acts on the nicotinic acetylcholine receptor; the chlorination inhibits degradation by acetylcholinesterase (Alexander et al., 2008)

Alexander et al. (2007) exposed the Heptageniidae Mayfly *Epeorus longimanus* and the Oligochaete *Lumbriculus variegatus* to a 24 hr pulse of imidacloprid (0-10 µg/L). Mayflies exposed to greater than 0.5 µg/L did not recover to control feeding rates by 96 hr post-exposure (NOEC = 0.1 µg/L). Oligochaetes exposed to greater than 5 µg/L did not recover to control feeding rates by 96 hr post-exposure either (NOEC = 1 µg/L).

Alexander et al. (2008) determined the longer term effects of abundance, emergence and development on *Epeorus* sp. and *Baetis* sp. mayflies after a 12 hr pulse exposure to imidacloprid. At end of a post-exposure period of 19.5 d, *Epeorus* sp. adult and nymph density was significantly lower at 9.1 µg/L (NOEC = 3.9 µg/L). In addition, no males emerged at this concentration (NOEC = 3.9 µg/L). Males had significantly reduced thorax length at 0.1 µg/L, the lowest toxicant concentration tested. Regarding the *Baetis* sp., at end of post-exposure period, all endpoints were similar to control values, except for males having significantly reduced head length at 0.1 µg/L (the lowest toxicant concentration tested) compared to control.

Isoproturon
Isoproturon is a herbicide whose mode of action is inhibition of photosystem II (Vallotton et al., 2008a)

Scenedesmus vacuolatus (alga) was exposed to pulses of 10-24 hr in length at concentrations of 60-320 µg/L and allowed to recover for 24 hr. Growth rate was

reduced by over 50% by exposure to 200 µg/L or higher. Recovery of growth occurred following removal of isoproturon within 5 hr, with growth recovery of the algae during the post-exposure period being independent of the applied concentration and duration of pulse exposure. The fast recovery of *S. vacuolatus's* growth might be a consequence of the rapid elimination of isoproturon compounds from the cells and the complete reversibility of it's mode of action (Vallotton et al., 2008a). The authors compared these responses of *S. vacuolatus* to isoproturon to responses measured from exposure to a similar herbicide, atrazine. Although the atrazine 10 hr pulse EC50s was more than 2.5 times higher than that isoproturon, the toxicity of both herbicides was similar after 48 hr of exposure.

Lambda-cyhalothrin

Lambda-cyhalothrin is a pyrethroid insecticide that targets the nervous system as a sodium channel modulator (Heckmann & Friberg, 2005)

Heckmann & Friberg (2005) determined the effect of a 30 min pulse of Lambda-cyhalothrin on drift and density of a macroinvertebrate community. Drift was determined 9 and 36 hr post exposure, and density up to 14 d post exposure. During exposure there was a significant difference in total drift at all concentrations compared to the control. By 9 hr post-exposure, however, there was no significant difference in total drift between exposed and control treatments. However, some specific taxa (*Gammarus* sp., simulidae and dytiscidae) were still significantly affected 3 d later at lowest concentration tested. Regarding benthic density: At 7 d post-exposure, *Gammarus* sp. exposed to 5 µg/L had significantly lower density; at 14 d post-exposure, total density in the 10.0 µg/L exposure treatment was significantly higher than the control and other treatments, caused by increased numbers of oligochaeta; structural change in the community had recovered within 2 months.

Malathion

Malathion is an organophosphate insecticide, and acetylcholinesterase inhibitor (Dropdata.org 2010).

Australian crimson-spotted rainbowfish (*Melanotaenia fluviatilis*) aged 0, 4, 8 and 12 d after hatch were exposed to a 2 hr pulse exposure of malathion at concentrations between was 15.6-72.1 mg/L. Toxicity did not differ with larval age and there was no significant difference in larval size measured at end of 96 hr, although 7 and 12 d old fish were significantly heavier. The 2 hr pulse exposure NOEC and LOEC values measured after 94 hr of recovery and based on larval mortality were < 15.6 and 15.6 mg/L (Reid et al., 1995).

Methoxychlor

Methoxychlor is an organochloride pesticide whose mode of action is as a sodium channel modulator.

The predatory stonefly *Acroneuria lycorias* was exposed to a pulse of 0.5-5 µg/L methoxychlor which diluted over 24 hr (Scherer & McNicol, 1986). Although no mortalities occurred, exposure concentrations caused abandonment of microhabitats which was associated with increased locomotor activity and drift. Responses occurred within 1-2 min after contact with the pesticide at 5 µg/L, and within 2-3 min at 1 and 2 µg/L, and persisted for several hours. The NOEC for locomotor activity and drift response was 0.5 µg/L. A rapid recovery followed the rapid response with pre-stress

distribution patterns and activity levels restored by < 10 h post-exposure. After 5 min of exposure to a 5 µg/L pulse of methoxychlor, the average body burden was 0.091 ug/g, after 6 hr exposure 0.596 ug/g and after 24 hr 0.283 ug/g.

Heming et al. (1989) exposed a number of fish to a range of methoxychlor concentrations (which were not explicitly stated in the reference) for varying short pulses and recorded mortality at end of 96 hr. Results were presented graphically as LC50s, and for all fish LC50 values increased as pulse exposure time decreased. For *Coregonus clupeaformis* (Lake whitefish) delayed mortality continued to occur during the post-exposure period in the 2 hr and 6 hr pulse treatments for up to 24 hr before ceasing. However, no delayed mortality during the post-exposure period was observed in the 24 hr pulse treatment. The results were similar for *Notropis hudsonius* (Spottail shiner), *Stizostedion vitreum* (Walleye) and *Salmo gairdneri* (Rainbow trout). Only for *Stizostedion vitreum vitreum* (White sucker fish) was there no delayed mortality during the post-exposure period.

Holdway & Dixon (1985) investigated the effect of larval age and nutritional status on the toxicity of methoxychlor to flagfish *Jordanella floridae*. There was a statistically significant impact of age on pulse exposure LC50s for fed juveniles with LC50s for 2, 4 and 8 d old larvae being 3.2, 13.5 and 38.6 mg/L. Conversely for unfed larvae there was no impact of age on LC50s. Four and 8 d old unfed fish (PE LC50 = 1.6 and 13. mg/L respectively) were significantly less tolerant than those fed (PE LC50 = 3.8 and 38.6 mg/L respectively), while for 2 d old fish food ration had no effect on LC50. Exposure to 1.29 mg/L resulted in a PE LT50 of 57 min for unfed fish, significantly reduced relative to the 144 min LT50 shown by fed fish. The 96 hr LC50 for continuously exposed adult fish was 0.29 mg/L, 10-20 times lower than the pulse exposed LC50s of juveniles.

Lastly, Holdway & Dixon (1986) investigated the effects of methoxychlor on *J. floridae* eggs. In the first experiment, 1-3 d old eggs were exposed to a 2 hr pulse of 0-5.48 mg/L and monitored for 5 d. There was significantly reduced hatching success in the 1 d old eggs exposed to ≥ 2.58 mg/L (NOEC = 1.29 mg/L). However, there was no reduced hatching success in the 2 and 3 d old exposed eggs at any concentration. In the second experiment, 4 and 8 d old larvae that had been previously exposed when either 1 or 3 d old eggs to 3.51 mg/L were again exposed to 3.51 mg/L for 2 hr and monitored for 96 hr. Results were compared with larvae that had not been pre-exposed. The 4 and 8 d old larvae that had been exposed when 1 d old eggs had significantly reduced LC50 values when exposed to 3.51 mg/L compared to those that had not been pre-exposed (0.5 against 5.02 mg/L for 4 d olds, and 0.6 against 3.04 mg/L for 8 d olds). However, 8 d old larvae hatched from 3 d old eggs previously exposed to 3.51 mg/L and subsequently exposed to 3.51 mg/L again for 2 hr did not show significantly reduced LC50 values. In the last experiment, a 2 hr pre-exposure of 1 d old eggs to 3.51 mg/L, and/or a subsequent 2 hr exposure of 8 d old juveniles to 2.58 mg/L, significantly altered whole-body levels of tryptophan, serotonin and 5-hydroxyindoleacetic acid. The authors concluded that some form of protective mechanism prevented methoxychlor from affecting the embryo by 48 hr post-fertilization. Up to 24 hr post-fertilization, however, the embryo was detrimentally affected, as evidenced by reduced hatching success and juvenile tolerance levels.

Metsulfuran-methy

Metsulfuran-methy is a herbicide that inhibits branched chain amino acid synthesis (Cedergreen et al., 2005)

Cedergreen et al. (2005) exposed duckweed *Lemna minor* to 3 hr pulse exposure of metsulfuran-methy and assessed growth 4 and 7 d after exposure. At end of the post-exposure period the relative growth rate EC50 of pulse exposed duckweed was approximately 10 fold higher than EC50 of continuously exposed plants.

Paraoxon-methyl

Paraoxon-methyl is the oxygen analogue of parathion-methyl. It is an organophosphate insecticide whose mode of action is cholinesterase inhibition (Duquesne, 2006).

Duration of exposure to paraoxon-methyl strongly altered the magnitude of effects, concentration-response relationships, and the recovery potential of *Daphnia magna* individuals (Duquesne, 2006; Duquesne et al., 2006; Duquesne & Kuster, 2010). In *D. magna* exposed to 10-1000 µg/L for 1 hr and 0.03-3.0 µg/L for 24 hr, mortality slowed post-exposure between day 3-7 and stopped from day 7 onwards. Continuously exposed daphnia continued to die during the 21 days of the trial (Duquesne et al., 2006). The LC50 values for a 24 hr exposure were 2.3, 2.1, and 2.0 µg/L at 3, 7, and 14 d post-exposure, respectively, and at 20 d post-exposure there was significantly reduced survival at ≥ 2.2 µg/L (i.e. NOEC = 1.5 µg/L) (Duquesne, 2006).

In terms of reproductive effects (measured as the decrease in the total number of neonates per surviving female) there was a significant decline for the 1 hr pulse exposure at concentrations of ≥ 100 µg/L on d 14 and 21 post-exposure (i.e. NOEC = 30 µg/L). For the 24 hr pulse exposure, the NOEC at 14 d post-exposure was 1 µg/L, but by 21 d post-exposure numbers had recovered to control levels. For the continuous exposure, there were significantly fewer neonates produced at ≥ 0.7 µg/L at both 14 and 21 d post-exposure (NOEC = 0.3 µg/L) (Duquesne et al., 2006).

For other sublethal endpoints, such as cholinesterase (ChE) inhibition, there was significant inhibition immediately after exposure to ≥ 1.0 µg/L, but by 48 hr post-exposure, ChE inhibition had recovered to 60% of controls in highest concentrations (EC50 values for ChE were 0.7 and 2.8 µg/L for exposure and recovery phases, respectively) (Duquesne, 2006). Duquesne & Kuster (2010) determined a ChE NOEC following 24 hr recovery from a 24 hr exposure to be 0.7 µg/L. Population growth rates were significantly decreased at 24 hr pulse concentrations ≥ 1.5 µg/L at 14 and 21 d of post-exposure (i.e. NOEC = 1.0 µg/L)(Duquesne, 2006). Although filtration rate, swimming behaviour and change in nitrogen abundance were significantly reduced at the end of the exposure, by 24 hr post-exposure results were similar to control) (Duquesne & Kuster, 2010). The results of this study combined with those of Duquesne (2006) suggest that until a certain level of sublethal exposure to paraoxon-methyl is reached (in this case, ≈1.0 µg/L), *D. magna* is able to overcome the transient effects of paraoxon-methyl on ChE, swimming, and feeding activities. However, above a threshold level (≈1.5 µg/L), although the parameters monitored in the current study recovered, some effects such as a persistent reduction in body size and an altered reproductive output were observed. Long-term effects on population structure and dynamics (e.g. population growth rate) could thus occur following short-term pulse exposure to paraoxon-methyl at concentrations in excess of ≈1.5 µg/L.

Pendimethalin
Pendimethalin is a herbicide that inhibits microtubule organization (Cedergreen et al., 2005).

Cedergreen et al. (2005) exposed duckweed *Lemna minor* to 3 hr pulse exposure of pendimethalin and assessed growth 4 and 7 d after exposure. At end of the post-exposure period the relative growth rate EC50 of pulse exposed duckweed was approximately 10 fold higher than EC50 of continuously exposed plants.

Pentachlorophenol (PCP)
Pentachlorophenol (PCP) is a biocide whose mode of action is believed to be inhibition of the formation of ATP by uncoupling oxidative phosphorylation (Samis et al., 1991).

Bluegill sunfish *(Lepomis macrochirus),* exposed to a continuous 22 d subchronic exposure of pentachloropbenol at concentrations of approximately 20 and 75% of the 96 hr median lethal concentration (96 hr LC50 = 240 µg/L) showed significant reductions in food conversion efficiency measured during the last 10 d of exposure (Samis et al., 1991). However, Bluegills exposed to a 3 d acute spill-mimicking exposure of pentachlorophenol at a concentration of approximately 100% of the 96 hr LC50, failed to show a significant reduction in food conversion efficiency measured during the 10 d following exposure. The authors conclude that Bluegill sunfish exposed to pentachlorophenol at continuous low-level concentrations are at a greater risk for decreased growth than those exposed to a more concentrated short-term pulse of toxicant.

Hickie et al. (1995) exposed *Pimephales promelas* (Fathead minnow) to varying exposure durations (2-96 hr) and cycles (1-15 pulses) and monitored effects after varying recovery periods. The authors reported that LC50 values decreased with increasing exposure duration until an incipient lethal level is reached near 48h of exposure, that LC50 values increased with increasing interval period between pulses, and that increasing the number of pulse cycles resulted in diminished additional contribution to toxicity, e.g. pulse LC50 reached 90% of final values within 3-5 cycles in test with 10-15 cycle exposures. The LD50 (lethal dose i.e. exceeding critical body residue) was 0.29 mmol/Kg (confident limits 0.26-0.34).

When Ashauer et al. (2007c) exposed *Gammarus pulex* to multiple pulses (3-4) of PCP at higher concentrations (≈1.5-11 mg/L), continued mortality occurred at all concentrations tested regardless of length of pulse. However, during the recovery period after each pulse there was a reduction in the rate of mortality. Authors estimated for all concentrations tested that it would take 15 d for internal damage to decline sufficiently not contribute to the effects of subsequent pulses.

Permethrin
Permethrin, being a pyrethroid insecticide, is a sodium channel modulator (Holdway & Dixon, 1988)

Abel & Garner (1986) report continued post-exposure (14 d later) mortalities for *Gammarus pulex* exposed to varying concentrations of permethrin (1-200 µg/L) during short pulse exposures (1-200 min).

For 2, 4 and 8 d old Flagfish (*Jordanella floridae*) larvae exposed to a 24 hr pulse of 0.2-2.0 mg/L permethrin, Holdway & Dixon (1988) report that age at exposure and presence/absence of food significantly modified toxicity of permethrin. Fed and Unfed 8 d olds (PE LC50 = 0.57 and 0.5 mg/L) and unfed 2 d olds (PE LC50 = 0.68 mg/L) were significantly less tolerant than 4 d old unfed fish (PE LC50 = 2.97 mg/L), which were in turn significantly less tolerant than 2 and 4 d old fed fish (PE LC50s = 5.55 and 7.91 mg/L). The theory of saltatory ontogeny was suggested as a possible reason for periods of differing susceptibility in this fish and in the larvae of the White sucker fish (*Catostomus commersoni*) which were exposed when 12, 30, and 26 d old to much lower concentrations (0.0001, 0.001, 0.01 and 0.1 mg/L). In the white sucker fish, the age at exposure and presence/absence of food significantly modified toxicity of permethrin too. Unfed fish were less tolerant than fed fish at all ages. Unfed 13 (PE LC50 = 0.002 mg/L) and 20 d olds (PE LC50 = 0.001 mg/L) were less tolerant than unfed 26 d olds (PE LC50 = 0.172 mg/L) and fed 13 d olds (PE LC50 = 0.185 mg/L), which were in turn were significantly more tolerant than 20 d old fed fish (PE LC50 = 0.010 mg/L). 26 d old fed fish were the most tolerant (PE LC50 = 3.668 mg/L).

Pirimicarb

Pirimicarb is a carbamate insecticide whose mode of action is inhibition of acetylcholinesterase (Andersen et al., 2006).

Andersen et al. (2006) undertook a range of experiments exposing *Daphnia magna* to pirimicarb. In the first experiment, two age groups (≤ 24 hr and 3 d old) were exposed to a single pulse of 40, 70 or 100 µg/L for 0.5-6 hr and immobilization measured after 38-47.5 hr of recovery. Daphnids recovered within 24 hr in clean media, with no significant difference in recovery based on age. Pulse durations of from 1.2 hr (100 µg/L) to 3.2 hr (40 µg/L) resulted in 50% immobilization of the exposed population immediately after transfer to clean media. After the post-exposure period of 48 hr, the estimated pulse duration to cause 50% immobilization had increased to 9.1 hr at 100 µg/L and 19 hr at 40 µg/L.

For *D. magna* of ≤ 24 hr in age, exposed to two pulses of 40 or 70 µg/L according to the same experimental procedure as above, the effects of the second pulse exposure to 40 µg/L were comparable to the effects of the single pulse exposure (although a few more animals died after the second pulse at the longest exposure times). A second pirimicarb pulse at 70 µg/L had a somewhat weaker effect on the mobility of the animals exposed to a 2 hr pulse (21% immobilized and 5% dead). However, the mortality in groups exposed for 4 and 6 hr increased from 0% in both groups after the first pulse to 11 and 20%, respectively, immediately after the second pulse. Fewer of the immobilized animals recovered during the post-exposure period, leaving 28 and 35%, respectively, immobilized after 48 hr, compared to 0% after the single pulse (Andersen et al., 2006).

In longer 21 d tests, *D. magna* of ≤ 24 hr old exposed to 100 µg/L for 3 hr had significantly reduced body length at the end of the post-exposure period. And when exposed to between 1-6 hr produced significantly fewer offspring, with time to maternity delayed at all exposure lengths (Andersen et al., 2006).

Propyzamide
Propyzamide is a herbicide that inhibits microtubule organization (Cedergreen et al., 2005).

Cedergreen et al. (2005) exposed duckweed *Lemna minor* to 3 hr pulse exposure of propyzamide and assessed growth 4 and 7 d after exposure. At end of the post-exposure period the relative growth rate EC50 of pulse exposed duckweed was approximately 10 fold higher than EC50 of continuously exposed plants.

S-metolachlor
S-metolachlor, a herbicide, is classified as an inhibitor of the formation of very long chain fatty acids (VLCFA) (Vallotton et al., 2008b).

Scenedesmus vacuolatus (alga) was exposed to 750 µg/L for 24 hr and recovery monitored over 48 hr. No recovery in cell reproduction was observed during first 24 hr, but reproduction rate increased during the second 24 hr. The time to recovery after the end of the exposure period took 29 hr. Reproduction rates had reached 3-4.5 at 29 hr and leveled off at 4.6-6.4 at 30 hr following chemical removal. The authors suggest that the delay in recovery following exposure to S-metolachlor could be the consequence of the irreversible covalent binding of the herbicide to the fatty acid elongase. The authors hypothesize that the time required for the replacement of inactive elongase enzymes, the synthesis of VLCFA, and the restoration of the fatty acid balance in the membranes exceeds 13 hr, because the synthesis of VLCFA is a light driven process. However, there is also the possibility that other relevant cell structures or metabolic processes recovered during the dark period, allowing the recovery of cell division just one day after chemical removal (Vallotton et al., 2008b).

Terbuthylazine
Terbuthylazine is a herbicide whose mode of action is as a photosynthetic inhibitor (Cedergreen et al., 2005).

Cedergreen et al. (2005) exposed duckweed *Lemna minor* to 3 hr pulse exposure of terbuthylazine and assessed growth 4 and 7 d after exposure. At end of post-exposure period the relative growth rate of pulse exposed duckweed had not decreased compared to non exposed plants. The EC50 of pulse exposed plants was 100 fold higher than EC50 of continuously exposed plants.

Thiacloprid
Thiacloprid is a neonicotinoid insecticide. Mode of action is interference with nicotinic acetylcholine receptor (Beketov et al., 2008).

Beketov et al. (2008) exposed freshwater macroinvertebrate community mesocosms to a pulse of 0.1, 3.2 and 100 µg/L thiacloprid and measured results over 7 months. The long-term community LOEC was determined to be 3.2 µg/L. However, stonefly (*Nemoura cinerea*) was affected at the lowest tested concentration, 70 times below the lowest known LC50. Regarding the time it took until recovery from when the effect took place, the duration depended on the life-cycle characteristics of species, but not on the toxicant concentration.

Triclopyr butoxyethyl ester (TBEE)
TBEE is a herbicide that mimics indole auxin plant growth hormones, causing uncontrolled growth and accelerated maturation in plants (Dropdata.org 2010).

Thompson et al. (1995) applied nominal concentrations of 0.8 and 2.7 µg/mL TBEE at various points along a stream and monitored periphytic algae and invertebrate responses. In-stream TBEE concentrations attenuated rapidly to 1/3rd of peak concentration in 55 to 80 min and degraded into Triclopyr acid (TRI). Post-exposure measurements of chlorophyll-*a* indicated stimulated periphyton growth since chlorophyll-*a* concentrations in the stream section below injection point were significantly higher than in the control section on certain d after application. Little or no effect on invertebrate numbers or community structure in drift and core samples was observed. The herbicide was strongly sorbed to organic material in leaf packs. Post-exposure measurements in sediment showed 10 fold less values than maximal total trichlopyr residues in stream water. The authors believe that photolytic degradation was also important, besides dilution and sorption, in the dissipation of TBEE.

Tripphenyltin hydroxide (TPTH)
Tripphenyltin hydroxide is an organotin fungicide Jarvinen et al. (1988b).

Jarvinen et al. (1988b) determined the effect of TPTH on *Pimephales promelas* (Fathead minnow). In determining lethality and behavioural effects, fish were exposed concentrations of between 2-100 µg/L for either 12, 24, 48 or 72 hr and the endpoint measured 84, 72, 48 or 24 hr later, respectively. Increasing exposure time resulted in increased mortality (e.g. the 96 hr LC50 and EC50 for 12 hr pulsed exposure were 61.8 µg/L (-) and 50.3 µg/L (43.2-58.6)(95% confidence limits); for 24 hr pulse exposure: 20.0 µg/L (17.1-23.3) and 15.8 µg/L (13.3-18.8); for 48 hr pulse exposure: 6.5 µg/L (6.0-7.1) and 5.0 µg/L (4.3-5.7); for 72hr pulse exposure: 6.0µg/L (5.1-7.1) and 3.5µg/L (-); and for 96hr continuous exposure: 7.1µg/L (5.6-8.6) and 3.7µg/L (2.9-4.5)). It was determined that a 43 hr exposure at the 96 hr LC50 continuous exposure value was necessary for 50% change in behaviour in fish.

Fathead minnow growth and survival after 27-29 d recovery from pulse exposures of 24, 48 and 72 hr to concentrations between 0.15-27.3 µg/L were equally sensitive to TPTH. At end of 30 d trial the NOEC for 24 hr pulsed exposure for survival and mean weight was 5.0±0.0 µg/L; NOEC for 48 hr pulsed exposure for survival and mean weight was 5.7±0.0 µg/L; NOEC for 72 hr pulsed exposure for survival and mean weight was 3.0±0.0 µg/L. The NOEC for continuous exposure for survival was 1.2±0.2 µg/L and LOEC for mean weight 0.6±0.2 µg/L. Increased adverse effects on survival and growth occur at a similar exposure concentration with increased exposure duration, perhaps indicating that TPTH effects are cumulative (Jarvinen et al., 1988b).

Velpar L
Velpar L, is a herbicide, whose active ingredient (triazine compound hexazinone) acts by inhibiting photosynthesis (Schneider et al., 1995)

Periphyton and macroinvertebrates in artificial streams were exposed to nominal concentrations of 200 µg/L (actual values of 145-432 µg/L) for 24 hr and monitored for the following 13 d recovery period. Periphyton chlorophyl-*a* productivity reduced by 80%, but returned to control levels within 24 hr post-exposure and 24 hr after beginning of treatment (i.e. 22 hr post-exposure) periphyton productivity was higher in treated

channels than in control. The 4 hr EC50 value was 3.6 µg/L for chlorophyl-*a* productivity. No differences were noted for periphyton biomass and macroinvertebrate drift and total invertebrates, mean biomass, density and length of benthic invertebrates. The authors state that the mobility and persistence of hexazinone in soil and water is the reason for potential problem with Velpar L.

Physical water-parameters

pH

Episodic acid stress has been associated with a failure to regulate Na^+ and Cl^- levels in blood or haemolymph. In molting invertebrates with permeable exoskeleton, it can lead to sodium loss followed by death.

Recent studies have investigated the episodic effects of pH on macroinvertebrates using transplantation experiments in the field. *Baetis rhodani* was transplanted from a circumneutral stream to an acidic stream by Kowalik & Ormerod (2006). *Baetis* survival in the circumneutral streams (pH > 5.7, generally pH ~7.5) remained high during both low and high flow. By comparison, mortality increased during episodic exposure, but only during high flow (average pH 3.8-3.9 versus pH 5.5-5.8 during low flows). Lepori & Ormond (2005) exposed *B. alpinus* to pH 5.5-5.7 for 2-4 d pulse and did not find significant mortality either during the exposure or over 7 d post exposure. This lack of significant mortality indicated that acid episodes of short duration are unlikely to cause irreversible damage to *B. alpinus*. The authors state that these findings support the idea that acid episodes might not eradicate sensitive invertebrate species, but instead make their presence variable over time in the affected streams. This could have long term implications in that variable populations can become extinct, especially if their densities are low. The study found high mortality in molting nymphs which the authors suggest is due to sodium loss during molting when the exoskeletons are more permeable. Felten et al. (2006) conducted transplantation experiments with *Gammarus fossarum* in the field and found that their survival was significantly lower after 24 hr exposure to pH 4.5 and after 72 hr exposure to pH 5.5, although post-exposure mortality was similar to control levels. The levels of chloride and sodium ions in haemolymph were significantly lower at the end of exposure for both pH levels, but they returned to control levels after 24 hours post exposure. Although laboratory experiments showed fast physiological recovery, the authors caution that field populations of *Gammarus fossarum* that are exposed to episodic acid situations show strong mortality effects if pulses are continually repeated.

Macedo-Sousa et al. (2008) compared the effects of acid mine drainage using river water with pH of 3.5 on *Choroterpes picteti* (Leptophlebiidae), *Hydropsyche pellucidula* (Hydropsychidae) and *Echinogammarus meriodionalis* (Gammaridae). The gammarid was the most sensitive species in terms of mortality and behavioural endpoints with early warning response of increased locomotion, followed by increased ventilation during exposure. The authors note that amphipods have been found to be the most acid sensitive organisms by other studies also, and hardness and pH (which affect the metal toxicity) of the water from the 3 rivers where the organisms were collected did not explain the differences in sensitivity of the tested organisms. All animals were dead at the end of the exposure period. *Choroterpes picteti* was less sensitive than *E. meridionalis*, but more sensitive than *Hydropsche*, in terms of mortality and behavioural endpoints. Early warning response in *Choroterpes* was increased locomotion, along with decreased ventilation activity during exposure and post-exposure and 25% of the

exposed organisms died. No early warning responses were detected for *Hydropsyche pellucidula*, the least sensitive of the tested organisms, and no mortality was noted.

Various life stages of salmon, trout and minnows have been tested for lethal and sub-lethal effects of low pH. Eggs and alevin of Kokanee and Sockeye salmon exposed to 24 hr pulse of pH 4 showed variation in sensitivity by developmental stage (Parker & McKeown, 1987), with the most sensitive stage being early embryonic development and newly-hatched alevins. The most significant effect on survival and median hatching time was noted when the eggs were episodically exposed during early development, and exposure at later stages had no apparent effect on egg survival. However, lethargy during exposure was noted in episodically exposed hatched embryos. Low pH resulted in significant decreases in the total percentage hatch for eggs along with lower efficiency of yolk to tissue conversion in unhatched embryos. The authors suggest that these effects may be due to inhibition of enzyme systems involved in embryonic development. Cleveland et al. (1991) exposed yolksac and feeding larvae of brook trout to 4 pulses of pH 4.0-6.5, and found significant mortality at pH levels below 4.5, with most mortality after the first pulse. When individuals were concurrently exposed to aluminum, significant mortalities occurred at all pH levels ≤ 6.5. Growth and body ion concentrations were reduced significantly at all pH levels, with or without aluminum exposure. Larval development and swimming behaviour were affected at pH levels of ≤ 6.1, and concurrent Al exposure increased the level of effects.

Finstad et al. (2007) investigated lethal and sublethal effects in post-smolts of Atlantic salmon over a 42 d post-exposure period. The lice-per-smolt density decreased in the order of high-acid (pH 5.67) 3 d exposure group > high acid 10 d group > moderate-acid (pH 5.97) 10 d group > control. Mortality was significantly higher than controls in the lice infected groups (high-acid 10 d > moderate-acid 10 d > high-acid 3 d > reference groups). Plasma chloride levels were significantly elevated in the lice infected high-acid and moderate-acid 10 d groups.

Lemly & Smith (1987) found complete elimination of feeding response at pH ≤ 6 in fathead minnow adults; however, feeding stimulus was restored after a post-exposure period of 24 hr at pH 8. The authors state that the toxic effect probably resulted in mechanical and chemical inhibition, and not destruction, of epithelial receptor cells. They suggest that field populations of fathead minnows subjected to reduced pH of 6 would have reduced food intake, fecundity and long-term survival.

Dissolved oxygen

The mode of action for low dissolved oxygen (DO) is through decreased enzyme activities and metabolic rate.

Effects of low dissolved oxygen were tested with juvenile and adult males of *Gammarus pulex* (0.5-2 mg/L for 24 hr) and *Asellus aquaticus* (0.25-1 mg/L for 24 hr) (Maltby, 1995). Over 40% of juveniles and 100% adults of *Gammarus pulex* exposed to 1 mg/L died after 24 hr exposure. In comparison, the waterlouse *A. aquaticus* was less susceptible to 24 hr pulses of DO concentrations than *G. pulex*. Percentage survival for both juveniles and adults of *A. aquaticus* was close to 100% at 1 mg/L oxygen. In fact, over 80% of juveniles of *A. aquatics* were alive at the lowest concentration. Juveniles of both species were more resistant then adults. Post-exposure mortality was significantly reduced for gammarids at 1.0 mg/L oxygen; however, at concentrations ≥ 1.5 mg/L there was no difference in survival of juvenile gammarids between the end of 24 hr exposure

and the end of 6 d post-exposure period. Post-exposure mortality was not statistically different from controls for juvenile *A. aquaticus*. The authors found that the haemolymph of the waterlouse had greater haemocyanin and greater oxygen carrying capacity than gammarids, which might be partly responsible for its higher tolerance of low oxygen levels.

Field experiments on the simulated effects of farm waste effluents (low DO) on *Gammarus pulex* and other macroinvertebrates were conducted by McCahon et al. (1991). The study induced pulsed exposure of declining oxygen concentration down to 1.8 mg/L for 2-6 hr that resulted in significant invertebrate mortalities of up to 100% for parasitized *G. pulex* and the stonefly *Dicocras cephalotes*. The presence of the parasite *Pomphorhynchus laevis* possibly reduced the resistance of *G. pulex* to low DO stress. The authors found that the disruption of precopula in *G. pulex* was a good indicator of reduced dissolved oxygen stress, and the pairs separated at DO concentration below 8 mg/L. After 11 hr post-exposure, animals re-formed into pairs but with a much higher number of separated pairs compared to control.

Wilmore & Storey (1997) studied anoxia effects in red-eared slider turtles (*Trachemys scripta elegans*). Anoxia exposure of 20 hr led to selected decreases in enzyme activities in some of the organs studied, including the brain, liver and kidney. The study quantified three different products of lipid peroxidation as indicators of oxidative stress, with different ones indicating damage at different stages of the peroxidation process. During post-exposure recovery period of 24 hr, most of the changes induced by anoxia were reversed although there was still suppression of brain enzyme activities.

Some animals that live in stressed conditions are hyopoxia tolerant, such as *Astronotus ocellatus*, a cichlid found in the Amazon basin that is tolerant of varying physical and chemical conditions during the year. These cichlids were exposed to stepwise declining pO_2 from 80% to 0% (Muusze et al., 1998), and they survived more than 16 hr of severe hypoxia and 4 hr of complete anoxia at 28°C. A significant decrease in standard metabolic rate started at 20% air saturation, whereas a significant change of blood lactate did not start until 6% air saturation in water. The findings of constant cortisol and low glucose levels in the blood plasma of the fish, led the authors to the conclusion that the hypoxia was not stressful to the cichlid.

Other fish species show various levels of tolerance to low DO. Seager et al. (2000) exposed rainbow trout (*Oncorhynchus mykiss*), roach (*Rutilus rutilus*) and brown trout (*Salmo trutta*) to 1-24 hr pulses of low DO (0.7-5.5 mg/L). Rainbow trout mortality was generally during exposure period and no significant post-exposure mortality was noted. NOEC for rainbow trout mortality was 1.5 mg/L at 1 hr exposure, 2.7 mg/L at 6 hr exposure, and 3.5 mg/L at 24 hr exposure. The authors suggest the existence of a narrow threshold range that separates DO values resulting in high mortality from a safe range of DO values. This threshold is believed to be associated with a critical threshold concentration that is necessary for essential metabolic processes, and it appeared to be correlated with exposure duration. Roach fish were more tolerant of lower DO values than trout, with minimal mortality. NOEC for roach mortality was 1.3 mg/L for 24 hr exposure. No mortality was observed for brown trout exposed to 4.0 and 5.5 mg/L followed by an acute exposure of 1.5 mg/L. Significant differences were found in spleen weight (weight increased as percentage of body weight; NOEC of 5.5 mg/L for one or two exposures a week), and kidney weight as % of body weight (decreased weight in 5.5 mg/L treatment) and also in haemoglobin levels (increased levels in treatment versus

control, although within normal range for farmed trout), but the study found no effect on growth rate and no post-exposure effects. There was also no evidence of immunity to low DO exposure. The study found concentration to be a more important factor than frequency of exposure for the tested range of parameters.

Sediment

Pulses of sediment are believed to reduce foraging efficiency through reduced visibility in foraging fishes and physiological stress. In addition, sediment results in depleted food resources, thus leading to reduced growth. Sediments may also contain high concentrations of toxic trace metals that can negatively affect population growth rates.

Strand & Merritt (1997) studied the effects of sediment pulses (turbidity of 23 nephelometric turbidity units (NTU) through 16 daily pulses) on two net-spinning caddisflies. No effect was detected on relative growth rate and final mass of *Hydropsyche betteni* and *Ceratopsyche sparna*. However, *C. sparna* had higher mortality compared to *H. betteni* in the microcosms. The authors caution about applying the laboratory results to field situations as larvae in field can avoid habitats of increased sedimentation. Instead they suggest that other changed habitat parameters associated with agricultural land use, such as temperature changes and/or nitrogen and phosphate enrichment, might be responsible for reduced populations of *C. sparna* in these streams.

Shaw & Richardson (2001) exposed fry of rainbow trout (*Oncorhynchus mykiss*) to 10 pulses of various duration lengths (0.5-6 hr) over 19 d. Following the pulse on d 9, the total abundance and family richness of benthic invertebrates declined with increasing sediment pulse duration. A greater proportion of chironomidae were found in drift which had an increased number of invertebrates, but reduced family richness. The study found negative correlation between trout length and mass gain with pulse duration, particularly as the experiment proceeded. No difference in drift and benthos total abundance from control was found for 4 hr pulse treatments on d 9 and for 5 hr pulse treatments on d 19. No effect of sediment pulses of 5 hr was found on fish length gain and of 6 hr pulses on fish mass gain at the end of the experiment. NOEC for fish survival could not be determined from the longest sediment pulse tested. The authors propose two mechanisms resulting in reduction in trout growth: reduced foraging efficiency, and reduction in or altered invertebrate food resource.

Ammonia

Ammonia toxicity can result in damage to gills and liver in fish which could lead to suffocation and increased probability of disease.

Juvenile and adult males of *Gammarus pulex* and *Asellus aquaticus* subjected to 24 hr ammonia (NH_3) pulses (2-16 mg/L) showed varying responses (Maltby, 1995). *Gammarus pulex* was more susceptible to elevated ammonia concentrations than *A. aquaticus* and the juveniles of both species were more resistant than the adults. Post-exposure mortality for both species was not statistically different from controls. McCahon et al. (1991) subjected *G. pulex* and other stream macroinvertebrates to pulses of 4.7-7 mg/L unionized ammonia in field treatments and found that although no mortalities were recorded, behavioural responses of contraction (planarid *Polycelis tenuis*) and migration from water (mollusc *Physa fontinalis*) were observed during pulse exposure of 4.7 mg/L ammonia; animals recovered after 6 hr post-exposure. Feeding rates of *G. pulex* were reduced during exposure, but recovered during post-exposure, although not to pre-exposure values. Increased ammonia of 5.3-7 mg/L (and pH 9.0) for 24 hr resulted in

mortality of 53-75% for *G. pulex*. Field treatments of ammonia were 2-7 mg/L resulted in 87-100% disruption of precopula in *G. pulex*, and some recovery was recorded during post-exposure. The authors found that the disruption of precopula was a sensitive and rapid indicator of stress.

Diamond et al. (2006a) compared effects of ammonia (as ammonium chloride) pulses on < 24 hr old *Daphnia magna* and *Pimephales promelas* (fathead minnow). Daphnids subjected to two 24 hr pulses of 50-100 mg/L rebounded to reproduce at control or higher levels given a few days recovery time. Fathead minnow fry and daphnids experienced 20-60% mortality in 24 hr pulsed exposures. Following a single 24 hr pulse of 50 mg/L, daphnid and fish mortality was ~30%, and it occurred within 48 hr of termination of pulse exposure. Effects on mortality, daphnid reproduction and on fish growth to single or multiple ≤ 24 hr pulses were only noted at or near continuous exposure 48 hr LC50 values. Growth of fish fry was capable of rebounding from short pulse exposures.

Milne et al. (2000) investigated the effects of 0.2-1.5 mg/L NH_3-N on rainbow and brown trout juveniles. Rainbow trout (*Oncorhynchus mykiss*) survived lowest exposure concentration (0.2-0.24 mg/L) with no post-exposure mortality in 6 hr and 24 hr treatments. All rainbow trout died in 6 hr exposure of 0.75 mg/L (NOEC of 0.42 mg/L) and 24 hr exposure of 0.82 mg/L (NOEC of 0.43 mg/L). *Oncorhynchus mykiss* exposed to 1 hr pulse of 0.85 mg/L had 80% survival, while those exposed to 1.3 mg/L had 90% mortality during exposure and within 20 min post-exposure period. Experiments testing the effects of exposure frequency (8-24 pulses of 0.2-0.4 mg/L) on brown trout (*Salmo trutta*) found no mortalities. However, lower fish weights were recorded in some instances, and growth, gill condition, organ weights, and hematocrit were all significantly affected by repeated exposures, particularly at the higher exposure frequency. Median period of survival (ET50) was significantly higher for three pulses of 0.4 mg/L compared to control, suggesting dose-related acclimation. Fish were able to survive potentially lethal ammonia concentrations if enough time for recovery was allowed; however, physiological processes controlling growth might be affected. Histopathology indicated gill damage in repeated pulse experiments, which could lead to increased possibility of disease and suffocation. Liver weight reduction was also noted in these experiments possibly due to reduced liver glycogen.

Chapter 4 Possible approaches for incorporating episodic data into the proposed risk-based approach to deriving water quality guidelines (WQGs) for South African aquatic ecosystems, and the continued refinement of DEEEP

4.1 Background

In the 1980s, WQGs moved towards becoming ultra-conservative trigger values, with the aim of reducing chemical concentrations to levels that eliminate any effects to the environment. This approach, because of funding and capacity limitations is not practically feasible in developing countries. Instead, the option for developing countries is to get closer to realism, i.e. a risk approach to get a better understanding of the real effect of a chemical on the environment – i.e. moderately precautionary while still adhering to the precautionary principle.

4.2 Structure of the revised WQGs for South Africa

The proposed WQGs for South Africa (DWAF, 2008) will be more than just a table of numbers that define concentration ranges within which specified biological effects can be expected for specific chemicals. Citing from DWAF (2008), the guidelines will include all of the following:

- The numerical values that define ranges within which specified effects, or degrees of effect, can be expected;
- The narrative description of those effects (e.g. lethality in the test organism population; changes in community structure);
- The description of the sampling, sample preparation, and analytical procedures upon which the numerical values are based;
- The general information on the constituents (like chemical, physical, and toxicological properties) – the so-called "hazard description";
- The general information on such issues as occurrence in the environment and general behaviour (like typical fate and transport); and
- Mitigation (treatment) options that provide guidance on what can be done about specific problems.

The guidelines will therefore include all the information that may be useful to the reader, particularly in supporting informed decision making relating to water resource management. An important aspect of the revised guidelines will be site specific applicability. This is in recognition of the fact that the physical attributes of our water resources (e.g. sediment load, mineral composition) can differ considerably across the country and this can have a significant affect on biological available concentrations of some chemicals.

The primary tool facilitating the determination and use of the guidelines should be a software decision support system (DSS). This should be complemented with a set of hard copy manuals that at least present generic values and supporting information (DWAF, 2008, pg 42). A more detailed description of the DSS is presented in DWAF (2008).

The WQGs are envisaged to comprise of a three tier system:

Tier 1. Provides 'generic' guidelines values that are made available in the DSS and hard copy manuals. These guideline values will be conservative as the worst case scenario is assumed.
Tier 2. Allows for site specificity in specified contexts and is facilitated by the DSS, consequently there is more confidence in the derived value
Tier 3. Full risk assessment. Not facilitated by the DSS but will use information contained within the DSS information database.

The procedure for generating the guideline values will follow a probabilistic risk assessment process. The exact procedural methods are still to be resolved, but the approach by which one goes about undertaking a risk assessment is also the method used in developing the guidelines and is depicted as an event tree (see Appendix A). The episodic data would contribute at the level where intake, excretion and metabolism of the chemical is considered. The approach by which one goes about setting goals for the management of the resource (i.e. chemical concentrations that will result in a certain level of protection for the ecosystem) is the fault tree (see Appendix B). The guideline derivation process can take place at all three tiers, and associated with the move down the tiers is a loss in confidence in the resultant guideline value (due to the use of more generic information and less site specific data) and thus the need to be more conservative (through use of conservative data, perhaps inclusion of safety factors and/or reducing what is considered acceptable risk to the resource).

As the intention of the SA WQGs is that they should, as far as practically possible, serve as a stand-alone source of information and support base for decisions for water resource managers (DWAF, 2008), the inclusion of episodic toxicity data within the DSS for use at Tier 2 and 3 assessments is catered for philosophically. The practicalities of data inclusion require considerable more investigation.

4.3 Application of episodic toxicity approaches to DEEEP

Direct Estimation of Ecological Effect Potential (DEEEP) is a method for directly measuring the potential effect / toxicity / ecological hazard of complex wastewater effluents. Like direct toxicity assessment (DTA) methods being used worldwide, DEEEP uses a number of lethal and sublethal bioassays to measure ecological hazard. The current DTA testing protocols have specific durations of exposure (e.g. 24 hr or 96 hr etc). The information generated in the episodic toxicity database, however, shows the importance of considering the variability of exposure duration and frequency when assessing organism and ecosystem effects. Investigations into incorporating exposure duration and frequency into DTAs are still in their infancy. Chevre et al. (2007) suggest the use of two criteria – one based on the long-term effect and the other that protects organisms against pulses of pollution. These criteria were developed using methods that estimate effects of time varying concentrations i.e. duration is added to the classical concentration-response curve. However, Diamond et al. (2006b) report that a kinetic model (developed from common wastewater contaminants and various pulsed scenarios) showed that chronic water quality criteria and effluent permit limits expressed as 4 or 30 d average concentrations may be either under- or over-protective depending on the pattern of exposure, and conclude that better information is needed on the effluent variability in order to accurately determine hazard. Further research, beyond the scope of this report, needs to be undertaken to investigate the development of testing protocols that take account of exposure duration and frequency for application in DEEEP.

Chapter 5 References

ABEL PD & GARNER SM (1986) Comparisons of mean survival times and median lethal exposure times for *Gammarus pulex* exposed to cadmium, permethrin and cyanide. *Water Research* **20** 579-582.

ALEXANDER AC, CULP JM, LIBER K & CESSNA AJ (2007) Effects of insecticide exposure on feeding inhibition in mayflies and oligochaetes. *Environmental Toxicology & Chemistry* **26** 1726-1732.

ALEXANDER AC, HEARD KS & CULP JM (2008) Emergent body size of mayfly survivors. *Freshwater Biology* **53** 171-180.

ALLIN CJ & WILSON RW (2000) Effects of pre-acclimation to aluminium on the physiology and swimming behaviour of juvenile rainbow trout (*Oncorhynchus mykiss)* during a pulsed exposure. *Aquatic toxicology* **51** 213-224.

ANDERSEN TH, TJØRNHØJ R, WOLLENBERGER L, SLOTHUUS T & BAUN A (2006) Acute and chronic effects of pulse exposure of *Daphnia magna* to Dimethoate and Pirimicarb. *Environmental Toxicology & Chemistry* **25** 1187-1195.

ASHAUER R, BOXALL ABA & BROWN CD (2007) Modeling combined effects of pulsed exposure to carbaryl and chlorpyrifos on *Gammarus pulex. Environmental Science & Technology* **41** 5535-5541.

ASHAUER R, BOXALL ABA & BROWN CD (2007c) New ecotoxicological model to simulate survival of aquatic invertebrates after exposure to fluctuating and sequential pulses of pesticides. *Environmental Science & Technology* **41** 1480-1486.

ASHAUER R, BOXALL ABA & BROWN CD (2007a) Simulating toxicity of carbaryl to *Gammarus pulex* after sequential pulsed exposure. *Environmental Science & Technology* **41** 5528-5534.

BEARR JS, DIAMOND J, LATIMER H & BOWERSOX M (2006) Effects of pulsed copper exposures on early life-stage *Pimephales promelas*. *Environmental Toxicology & Chemistry* **25** 1376-1382.

BEKETOV MA, SCHÄFER RB, MARWITZ A, PASCHKE A & LIESS M (2008) Long-term stream invertebrate community alterations induced by the insecticide thiacloprid: Effect concentrations and recovery dynamics. *Science of the Total Environment* **405** 96-108.

BONY S, GILLET C, BOUCHEZ A, MARGOUM C & DEVAUX A (2008) Genotoxic pressure of vineyard pesticides in fish: Field and mesocosm surveys. *Aquatic Toxicology* **89** 197-203.

BRECK JE (1988) Relationships among models for acute toxicity effects: Applications to fluctuating concentrations. *Environmental Toxicology and Chemistry* **7** 775-778.

BRENT RN & HERRICKS EE (1998) Postexposure effects of brief cadmium, zinc, and phenol exposures on freshwater organisms. *Environmental Toxicology and Chemistry* **17** 2091-2099.

BROOMHALL S (2002) The effects of endosulfan and variable water temperature on survivorship and subsequent vulnerability to predation in *Litoria citropa* tadpoles. *Aquatic Toxicology* **61** 243-250.

BUTCHER J, DIAMOND J, BEARR J, LATIMER H, KLAINE SJ, HOANG T & BOWERSOX M (2006) Toxicity models of pulsed copper exposure to *Pimephales promelas* and *Daphnia magna*. *Environmental Toxicology and Chemistry* **25** 2541-2550.

CEDERGREEN N, ANDERSEN L, OLESEN CF, SPLIID HH & STREIBIG JC (2005) Does the effect of herbicide pulse exposure on aquatic plants depend on K_{ow} or mode of action? *Aquatic Toxicology* **71** 261-271.

CHANG LW, TOTH GP, GORDON DA, GRAHAM DW, MEIER JR, KNAPP CW, DENOYELLES Jr. FJ, CAMPBELL S & LATTIER DL (2005) Responses of molecular indicators of exposure in mesocosms: common carp (*Cyprinus carpio*) exposed to the herbicides alachlor and atrazine. *Environmental Toxicology & Chemistry* **24** 190-197.

CHE`VRE N, VALLOTTON N & ROSSI L (2007) Risk Assessment of Urban Runoff Pollution in Rivers: How to Deal with Time- Varying Concentrations? *Sixth International Conference on Sustainable Techniques and Strategies in Urban Water Management (NOVATECH)*, Lyon, France, **3** 1367-1374.

CLEVELAND L, LITTLE EE, INGERSOLL CG, WIEDMEYER RH & HUNN JB (1991) Sensitivity of brook trout to low pH, low and elevated aluminum concentrations during laboratory pulse exposures. *Aquatic Toxicology* **19** 303-317.

COLD A & FORBES VE (2004) Consequences of a short pulse of pesticide exposure for survival and reproduction of *Gammarus pulex*. *Aquatic Toxicology* **67** 287-299.

CONG NV, PHUONG NT & BAYLEY M (2009) Effects of repeated exposure of diazinon on cholinesterase activity and growth in snakehead fish (*Channa striata*). *Ecotoxicology and Environmental Safety* **72** 699-703.

CONNOLLY JP (1985) Predicting single-species toxicity in natural water systems. *Environmental Toxicology & Chemistry* **4** 573-582.

DELONAY AJ, LITTLE EE, WOODWARD DF, BRUMBAUGH WG, FARAG AM & RABENI CF (1993) Sensitivity of early-life-stage golden trout to low pH and elevated aluminum. *Environmental Toxicology and Chemistry* **12** 1223-1232.

DIAMOND J, BOWERSOX M, LATIMER H, BARBOUR C, BEARR J & BUTCHER J (2005) Effects of pulsed contaminant exposures on early life stages of the fathead minnow. *Archives of Environmental Contamination and Toxicology* **49** 511-519.

DIAMOND JM, BUTCHER JB & KLAINE SJ (2006b) Factoring frequency, magnitude, and duration in NPDES stormwater permit conditions. Water Environment Research Foundation (WERF). WERF stock no. 02WSM3. Virginia, USA.

DIAMOND JM, KLAINE SJ & BUTCHER JB (2006a) Implications of pulsed chemical exposures for aquatic life criteria and wastewater permit limits. *Environmental Science and Technology* **40** 5132-5138.

DUQUESNE S (2006) Effects of an organophosphate on *Daphnia magna* at suborganismal and organismal levels: Implications for population dynamics. *Ecotoxicology and Environmental Safety* **65**145-150.

DUQUESNE S, REYNALDI S & LIESS M (2006) Effects of the organophosphate paraoxon-methyl on survival and reproduction of *Daphnia magna*: Importance of exposure duration and recovery. *Environmental Toxicology & Chemistry* **25** 1196-1199.

DUQUESNE S AND KUSTER E (2010) Biochemical, metabolic, and behavioural responses and recovery of *Daphnia magna* after exposure to an organophosphate. *Ecotoxicology and Environmental Safety* **73** 353-359.

DWAF (DEPARTMENT OF WATER AFFAIRS AND FORESTRY) (2008) *Development of SA risk-based water quality guidelines: Phase 1. Needs assessment & philosophy*. Report No N/0000/SRQ0107. ISBN: 978-0-621-38380-5. Resource Quality Services, Department of Water Affairs and Forestry, Pretoria, South Africa.

EISLER R & GARDNER GR (1973) Acute toxicity to an estuarine teleost of mixtures of cadmium, copper and zinc salts. *Journal of Fish Biology* **5** 131-142.

FAIRCHILD JF, LA POINT TW, ZAJICEK JL, NELSON MK, DWYER FJ & LOVELY PA (1992) Population-, community- and ecosystem-level responses of aquatic mesocosms to pulsed doses of a pyrethroid insecticide. *Environmental Toxicology and Chemistry* **11** 115-129.

FELTEN V, BAUDOIN JM & GUÉROLD F (2006) Physiological recovery from episodic acid stress does not mean population recovery of *Gammarus fossarum*. *Chemosphere* **65** 988-998.

FINSTAD B, KROGLUND F, STRAND R, STEFANSSON SO, BJØRN PA, ROSSELAND BO, NILSEN TO & SALBU B (2007) Salmon lice or suboptimal water quality — Reasons for reduced postsmolt survival? *Aquaculture* **273** 374-383.

FORBES VE & COLD A (2005) Effects of the pyrethroid esfenvalerate on life-cycle traits and population dynamics of *Chironomus riparius* — Importance of exposure scenario. *Environmental Toxicology & Chemistry* **24** 78-86.

GAIKOWSKI MP, DENSMORE CL & BLAZER VS (2009) Histopathology of repeated, intermittent exposure of chloramine-T to walleye (*Sander vitreum*) and (*Ictalurus punctalus*) channel catfish. *Aquaculture* **287** 28-34.

GAMA-FLORES JL, SARMA SSS & NANDINI S (2006) Effect of cadmium level and exposure time on the competition between zooplankton species *Moina macrocopa* (Cladocera) and *Brachionus calyciforus* (Rotifera). *Journal of Environmental Science and Health Part A* **41** 1057-1070.

GAMA-FLORES JL, SARMA SSS & NANDINI S (2007a) Exposure time-dependent cadmium toxicity to *Moina macrocopa* (Cladocera): a life table demographic study. *Aquatic Ecology* **41** 639-648.

GAMA-FLORES JL, CASTELLANOS-PAEZ ME, SARMA SSS & NANDINI S (2007b) Effect of pulsed exposure to heavy metals (copper and cadmium) on some population variables of *Brachionus calyciflorus* Pallas (Rotifera: Brachionidae: Monogononta. *Hydrobiologia* **593** 201-208.

HANDY RD (1994) Intermittent exposure to aquatic pollutants: assessment, toxicity and sublethal responses in fish and invertebrates. *Comparative Biochemistry and Physiology* **107C** 171-184.

HECKMANN LH & FRIBERG N (2005) Macroinvertebrate community response to pulse exposure with the insecticide lambda-cyhalothrin using in-stream mesocosms. *Environmental Toxicology and Chemistry* **24** 582-590.

HEMING TA, SHARMA A & KUMAR Y (1989) Time-toxicity relationships in fish exposed to the organochlorine pesticide methoxychlor. *Environmental Toxicology and Chemistry* **8** 923-932.

HICKIE BE, MCCARTY LS & DIXON DG (1995) A residue-based toxicokinetic model for pulse-exposure toxicity in aquatic systems. *Environmental Toxicology and Chemistry* **14** 2187-2197.

HOANG TC & KLAINE SJ (2007) Influence of organism age on metal toxicity to *Daphnia magna*. *Environmental Toxicology and Chemistry* **26** 1198-1204.

HOANG TC & KLAINE SJ (2008) Characterizing the toxicity of pulsed selenium exposure to *Daphnia magna*. *Chemosphere* **71** 429-438.

HOBBS DA, WARNE MSTJ & MARKICH SJ (2005) Evaluation of criteria used to assess the quality of aquatic toxicity data. *Integrated Environmental Assessment and Management* **1** 174-180.

HOLDWAY DA & DIXON DG (1985) Acute toxicity of pulse-dosed methoxychlor to juvenile American flagfish (*Jordanella floridae* Goode and Bean) as modified by age and food availability. *Aquatic Toxicology* **6** 243-250.

HOLDWAY DA & DIXON DG (1986) Effects of methoxychlor exposure of flagfish eggs (*Jordanella floridae*) on hatchability, juvenile methoxychlor tolerance and whole-body levels of tryptophan, serotonin and 5-hydroxyindoleacetic acid. *Water Research* **20** 893-897.

HOLDWAY DA & DIXON DG (1988) Acute toxicity of permethrin or glyphosate pulse exposure to larval white sucker (*Catostomus commersoni*) and juvenile flagfish (*Jordanella floridae*) as modified by age and ration level. *Environmental Toxicology and Chemistry* **7** 63-68.

HOLDWAY DA, BARRY MJ, LOGAN DC, ROBERTSON D, YOUNG V & AHOKAS JT (1994) Toxicity of pulse-exposed fenvalerate and esfenvalerate to larval Australian crimson-spotted rainbow fish (*Melanotaenia fluviatilis*). *Aquatic Toxicology* **28** 169-187.

HOSMER AJ, WARD TJ & WARREN LW (1998) Chronic toxicity of pulse-dosed fenoxycarb to *Daphnia magna* exposed to environmentally realistic concentrations. *Environmental Toxicology & Chemistry* **17** 1860-1866.

JARVINEN AW, TANNER DK & KLINE ER (1988a) Toxicity of chlorpyrifos, endrin, or fenvalerate to fathead minnows following episodic or continuous exposure. *Ecotoxicology and Environmental Safety* **15** 78-95.

JARVINEN AW, TANNER DK, KLINE ER & KNUTH ML (1988b) Acute and chronic toxicity of triphenyltin hydroxide to fathead minnows (*Pimephales promelas*) following brief or continuous exposure. *Environmental Pollution* **52** 289-301.

KIM Y, JUNG J, OH S & CHOI K (2008) Aquatic toxicity of cartap and cypermethrin to different life stages of Daphnia magna and Oryzias latipes. Journal of Environmental Science & Health, Part B -- Pesticides, Food Contaminants, & Agricultural Wastes **43** 56-64.

KOWALIK RA & ORMEROD SJ (2006) Intensive sampling and transplantation experiments reveal continued effects of episodic acidification on sensitive stream invertebrates. Freshwater Biology **51** 180-191.

LEMLEY AD & SMITH RJF (1987) Effects of chronic exposure to acidified water on chemoreception of feeding stimuli in fathead minnows (*Pimephales promelas*): Mechanisms and ecological implications. *Environmental Toxicology and Chemistry* **6** 225-238.

LEPORI F & ORMEROD SJ (2005) Effects of spring acid episodes on macroinvertebrates revealed by population data and in situ toxicity tests. *Freshwater Biology* **50** 1568-1577.

LIESS M, PIETERS BJ & DUQUESNE S (2006) Long-term signal of population disturbance after pulse exposure to an insecticide: Rapid recovery of abundance, persistent alteration of structure. *Environmental Toxicology & Chemistry* **25** 1326-1331.

LITTLE EE, DWYER FJ, FAIRCHILD JF, DELONAY AJ & ZAJICEK JL (1993) Survival of bluegill and their behavioral responses during continuous and pulsed exposures to esfenvalerate, a pyrethroid insecticide. *Environmental Toxicology and Chemistry* **12** 871-878.

LITTLEFIELD-WYER JG, BROOKS P & KATOULI M (2008) Application of biochemical fingerprinting and fatty acid methyl ester profiling to assess the effect of the pesticide Atradex on aquatic microbial communities. *Environmental Pollution* **153** 393-400.

MACEDO-SOUSA JA, GERHARDT A, BRETT CMA, NOGUEIRA AJA & SOARES AMVM (2008) Behavioural responses of indigenous benthic invertebrates (*Echinogammarus meridionalis*, *Hydropsyche pellucidula* and *Choroterpes picteti*) to a pulse of acid mine drainage: A laboratorial study. *Environmental Pollution* **156** 966-973.

MALTBY L (1995) Sensitivity of the crustaceans *Gammarus pulex* (L.) and *Asellus aquaticus* (L.) to short-term exposure to hypoxia and unionized ammonia: Observations and possible mechanisms. *Water Research* **29** 781-787.

MANCINI JL (1983) A method for calculating effects, on aquatic organisms, of time varying concentrations. *Water Research* **17** 1355-1362.

MCCAHON CP & PASCOE D (1990) Episodic pollution: causes, toxicological effects and ecological significance. *Functional Ecology* **4** 375-383.

MCCAHON CP, POULTON MJ, THOMAS PC, XU Q, PASCOE D & TURNER C (1991) Lethal and sub-lethal toxicity of field simulated farm waste episodes to several freshwater invertebrate species. *Water Research* **25** 661-671.

MILNE I, SEAGER J, MALLETT M & SIMS I (2000) Effects of short-term pulsed ammonia exposure on fish. *Environmental Toxicology & Chemistry* **19** 2929-2936.

MORTON MG, DICKSON KL, WALLER WT, ACEVEDO MF, MAYER JR FL & ABLAN M (2000) Methodology for the evaluation of cumulative episodic exposure to chemical stressors in aquatic risk assessment. *Environmental Toxicology and Chemistry* **19** 1213-1221.

MUUSZE B, MARCON J, VAN DEN THILLART G & ALMEIDA-VAL V (1998) Hypoxia tolerance of Amazon fish. Respirometry and energy metabolism of the cichlid *Astronotus ocellatus. Comparative Biochemistry and Physiology Part A* **120** 151-156.

NADDY RB, JOHNSON KA & KLAINE SJ (2000) Response of *Daphnia magna* to pulsed exposures of chlorpyrifos. *Environmental Toxicology & Chemistry* **19** 423-431.

NADDY RB & KLAINE SJ (2001) Effect of pulse frequency and interval on the toxicity of chlorpyrifos to *Daphnia magna. Chemosphere* **45** 497-506.

PARKER DB & MCKEOWN BA (1987) The effects of low pH on egg and alevin survival of kokanee and sockeye salmon, *Oncorhynchus nerka*. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology* **87** 259-268.

PASCOE D & SHAZILI NAM (1986) Episodic pollution – a comparison of brief and continuous exposure of rainbow trout to cadmium. *Ecotoxicology and Environmental Safety* **12** 189-198.

PIETERS BJ & LIESS M (2006b) Population developmental stage determines the recovery potential of *Daphnia magna* populations after Fenvalerate application. *Environmental Science & Technology* **40** 6157-6162.

PIETERS BJ & LIESS M (2006a) Maternal nutritional state determines the sensitivity of *Daphnia magna* offspring to short-term Fenvalerate exposure. *Aquatic Toxicology* **76** 268-277.

PIETERS BJ, PASCHKE A, REYNALDI S, KRAAK MHS, ADMIRAAL W & LIESS M (2005) Influence of food limitation on the effects of fenvalerate pulse exposure on the life history and population growth rate of *Daphnia magna*. *Environmental Toxicology and Chemistry* **24** 2254-2259.

PYNNONEN K (1990) Aluminium accumulation and distribution in the freshwater clams (Unionidae). *Comparative Biochemistry and Physiology* **97C** 111-117.

RÁBAGO-CASTRO JL, SANCHEZ JG, PÉREZ-CASTAÑEDA R & GONZÁLEZ-GONZÁLEZ A (2006) Effects of the prophylactic use of Romet®-30 and copper sulfate on growth, condition and feeding indices in Channel catfish (*Ictalurus punctatus*). *Aquaculture* **253** 343-349.

REID HP, AHOKAS JT & HOLDWAY DA (1995) Use of cyanazine and malathion pulse-exposure toxicity to estimate the age of onset of functional liver metabolism in larval Australian crimson-spotted rainbowfish (*Melanotaenia fluviatilis*). *Water Research* **29** 2010-2013.

REYNALDI S & LIESS M (2005) Influence of duration of exposure to the pyrethroid fenvalerate on sublethal responses and recovery of *Daphnia magna* Straus. *Environmental Toxicology and Chemistry* **24** 1160-1164.

ROUX DJ, JOOSTE SHJ & MACKAY HM (1996) Substance-specific water quality criteria for the protection of South African freshwater ecosystems: Methods for derivation and initial results for some inorganic toxic substances. *South African Journal of Science* **92** 198-206.

SAMIS AJW, COLGAN PW & JOHANSEN PH (1991) A comparison of the effects of subchronic and spill-mimicking pentachlorophenol exposures on growth of bluegill sunfish (*Lepomis macrochirus*). *Aquatic Toxicology* **19** 231-240.

SCHERER E & MCNICOL RE (1986) Behavioural responses of stream-dwelling *Acroneuria lycorias* (Ins., Plecopt.) larvae to methoxychlor and fenitrothion. *Aquatic Toxicology* **8** 251-263.

SCHNEIDER J, MORIN A & PICK FR (1995) The response of biota in experimental stream channels to a 24-hour exposure to the herbicide Velpar L®. *Environmental Toxicology and Chemistry* **14** 1607-1613.

SCHILL RO, GORLITZ H & KOHLER H-R (2003) Laboratory simulation of a mining accident: acute toxicity, hsc/hsp 70 response, and recovery from stress in *Gammarus fossarum* (Crustacea, Amphipoda) exposed to a pulse of cadmium. *BioMetals* **16** 391-401.

SEAGAR J, MILNE I, MALLETT M & SIMS I (2000) Effects of short-term oxygen depletion on fish. *Environmental Toxicology & Chemistry* **19** 2937-2942.

SELLIN MK, TATE-BOLDT E & KOLOK AS (2005) Acclimation to Cu in fathead minnows: Does age influence the response? *Aquatic Toxicology* **74** 97-109.

SERRA A, CORCOLL N & GUASCH H (2009) Copper accumulation and toxicity in fluvial periphyton: The influence of exposure history. *Chemosphere* **74** 633-641.

SHAW EA & RICHARDSON JS (2001) Direct and indirect effects of sediment pulse duration on stream invertebrate assemblages and rainbow trout (*Oncorhynchus mykiss*) growth and survival. *Canadian Journal of Fisheries & Aquatic Sciences* **58** 2213-2221.

STRAND RM & MERRITT RW (1997) Effects of episodic sedimentation on the net-spinning caddisflies *Hydropsyche betteni* and *Ceratopsyche sparna* (Trichoptera: Hydropsychidae). *Environmental Pollution* **98** 129-134.

THOMPSON DG, KREUTZWEISER DP, CAPEIX SS, THOMAS DR, STAZNIK B & VIINIKKA T (1995) Fate and effects of triclopyr ester in a first-order forest stream. *Environmental Toxicology and Chemistry* **14** 1307-1317.

TLILI A, DORIGO U, MONTUELLE B, MARGOUM C, CARLUER N, GOUY V, BOUCHEZ A & BÉRARD A (2008) Responses of chronically contaminated biofilms to short pulses of diuron: An experimental study simulating flooding events in a small river. *Aquatic Toxicology* **87** 252-263.

TRIPATHI BN, MEHTA SK & GAUR JP (2004) Recovery of nitrate uptake and assimilation in *Scenedesmus* sp. previously exposed to elevated levels of Cu and Zn. *Journal of Plant Physiology* **161** 543-549.

TRIPATHI BN & GAUR JP (2006) Physiological behaviour of *Scenedesmus* sp. during exposure to elevated levels of Cu and Zn and after withdrawal of metal stress. *Protoplasma* **229** 1-9.

VAN DER HOEVEN N & GERRITSEN AAM (1997) Effects of chlorpyrifos on individuals and populations of *Daphnia pulex* in the laboratory and field. *Environmental Toxicology & Chemistry* **16** 2438-2447.

VALLOTTON N, EGGEN RIL, ESCHER BI, KRAYENBÜH J & CHÈVRE N (2008a) Effect of pulse herbicidal exposure on *Scenedesmus vacuolatus*: a comparison of two photosystem II inhibitors. *Environmental Toxicology & Chemistry* **27** 1399-1407.

VALLOTTON N, MOSER D, EGGEN RIL, JUNGHANS M & CHÈVRE N (2008b) S-metolachlor pulse exposure on the alga *Scenedesmus vacuolatus*: Effects during exposure and the subsequent recovery. *Chemosphere* **73** 395-400.

VIANT MR, PINCETICH CA & TJEERDEMA RS (2006) Metabolic effects of dinoseb, diazinon and esfenvalerate in eyed eggs and alevins of Chinook salmon (*Oncorhynchus tshawytscha*) determined by 1H NMR metabolomics. *Aquatic Toxicology* **77** 359-371.

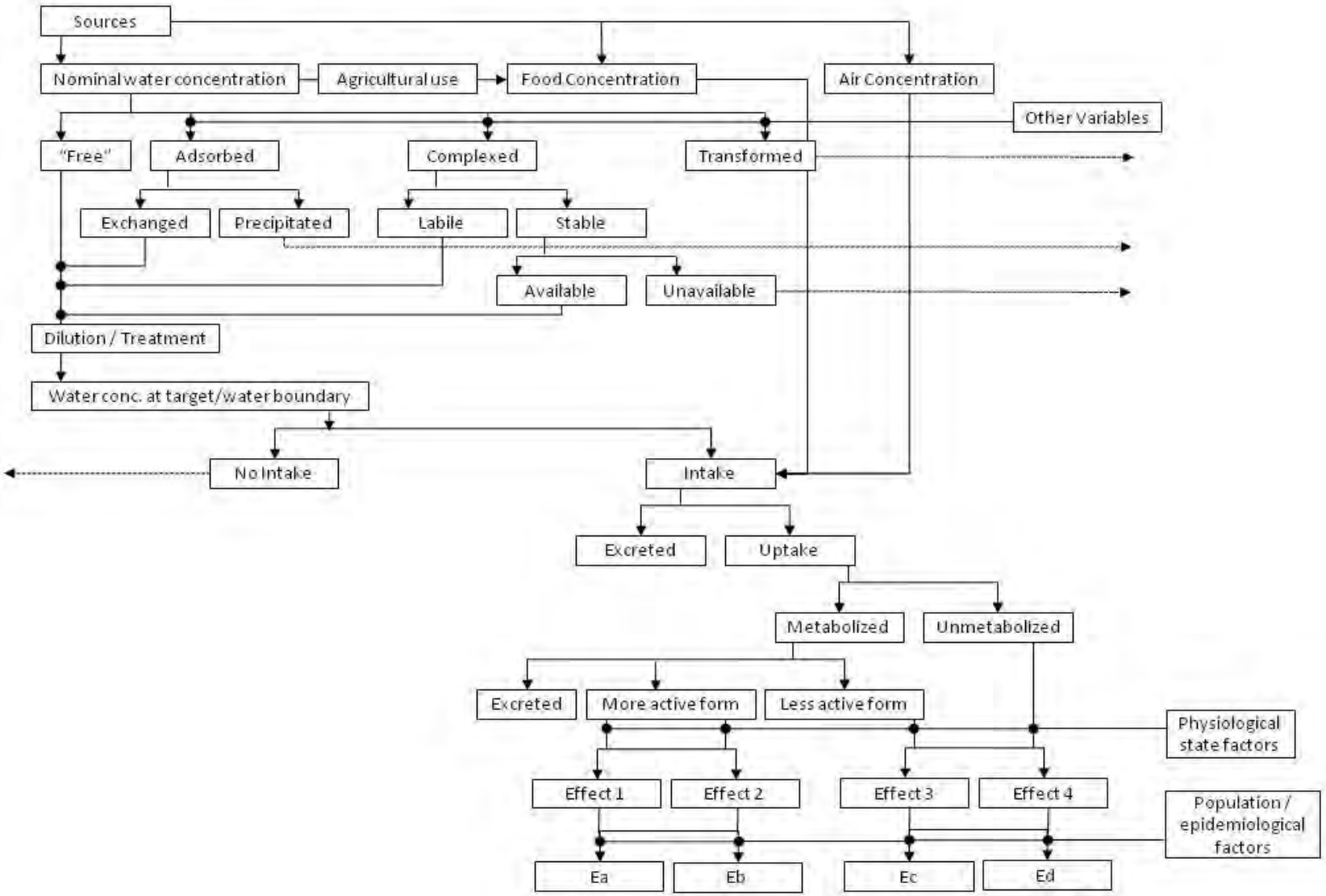
WENDT-RASCH L, VOUGHT L & WOIN P (1998) Effects of fenvalerate on the net-spinning behaviour of *Hydropsych siltalai* (Döhler) (Trichoptera: Hydropsychidae). *Hydrobiologia* **382** 53-61.

WESTON DP, JARMAN WM, CABANA G, BACON CE & JACOBSON LA (2002) An evaluation of the success of dredging as remediation at a DDT-contaminated site in San Francisco Bay, California, USA. *Environmental Toxicology and Chemistry* **21** 2216-2224.

WIDIANARKO B, KUNTORO FXS, VAN GESTEL CAM & VAN STRAALEN NM (2001) Toxicokinetics and toxicity of zinc under time-varying exposure in the guppy (*Poecilia reticulata*). *Environmental Toxicology and Chemistry* **20** 763-768.

ZHAO Y & NEWMAN MC (2006) Effects of exposure duration and recovery time during pulsed exposures. *Environmental Toxicology and Chemistry* **25** 1298-1304.

Appendix A: Risk process presented as an event tree
(Source: Sebastian Jooste, Resource Quality Services, Department of Water Affairs)



Appendix B: Risk process presented as a fault tree
(Source: Sebastian Jooste, Resource Quality Services, Department of Water Affairs)