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Literature reviews on ecotoxicology of chemicals with special focus on plant protection products. Reference: CFT/EFSA/PPR/2008/01

Lot 5: Evidence of potential long term effects in (aquatic and terrestrial) invertebrates after short term pulsed exposure

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Chapter 1: General Introduction

This final report constitutes the final deliverable for the assignment contracted by the European Food Safety Authority (EFSA) to Exponent International Limited to perform a literature review on ecotoxicology of chemicals with a special focus on plant protection products on the "Evidence of potential long term effects in (aquatic and terrestrial) invertebrates after short term pulsed exposure" (Lot 5 of CFT/EFSA/PPR/2008/01).

The literature search method is detailed in Chapter 4 of this report, while the results are presented in Chapter 2 (details) and Chapter 3 (overview tables).

Chapter 2: Literature review / assessment

2.1 Introduction

Applications of chemical pesticides are generally timed to coincide with specific crop growth phases or stages in pest life cycles when the target is most sensitive to the pesticide. Timing applications of plant protection products in this way, often results in pulsed exposures to terrestrial and aquatic habitats, due to repeat applications to control the same pest target or to control sequentially occurring pest targets. Two examples of repeat applications which may lead to pulsed exposure are described below:

- **Example 1:** Repeat application of a product containing the same active substance to control different insect pests at different growth stages of the crop. Chlorpyrifos (an organophosphate); applied to winter wheat from mid-January to March (up to crop growth stage BBCH 39, 'flag-leaf visible') at 750 g a.s./ha, to control Wheat bulb fly (*Delia coarctata,* Diptera: Anthomyiidae). Then applied to winter wheat from early-mid June (crop growth stage BBCH 51-59, 'ear emergence to the beginning of flowering') at 450 g a.s./ha, to control Orange wheat blossom midge (*Sitodiplosis mosellana*, Diptera: Cecidomyiidae).

- **Example 2:** Repeat application of a product containing the same active substance to control the same insect pest at different growth stages of the crop. Deltamethrin (a pyrethroid); applied to apples from mid-June at 21 day intervals (maximum of 3 applications per season) at 8.75 g a.s./ha, to control Summer fruit tortrix moth (*Adoxophyes orana*, Lepidoptera: Tortricidae). The active substance is only active on the larval stage, so repeat applications are necessary to control new hatchings from previously laid eggs.

Aquatic systems may also experience pulsed pesticide exposures, via rain and run-off events. These exposures are associated with differing soil and climatic conditions; for instance, drainage exposure may occur from heavy clay soils in Northern Europe which are artificially drained whilst run-off exposure may occur on sloping ground following high intensity rainfall events in Southern Europe. Pulsed exposures from both routes are simulated using the FOCUS surface water scenarios, although only the peak concentration is currently used in Regulatory assessments in the EU.

Lotic (flowing) systems, given their hydrology, are unlikely to experience chronic exposures to pesticides. Rather, these pulses are likely to be extremely brief, especially for less mobile invertebrates that resist entry into the drift. Even in lentic (static) systems with little to no flow or clean water input, rapid dissipation or breakdown of plant protection products will ultimately result in short-term, pulsed exposures. Consequently, non-target invertebrates are significantly more likely to experience exposure to plant protection products as single or multiple short-term pulses with variable lengths of intervening recovery time than as chronic, long-term exposures.

Pulsed exposures can cause significant damage to both aquatic and terrestrial invertebrate communities, if they are of sufficient duration and magnitude to elicit long-lasting damage. Furthermore, there is significant evidence that susceptibility to plant protection products can vary greatly over the invertebrate life cycle. Hence, the timing of pulsed exposures in relation to invertebrate life cycles is critical, as pulsed exposures that coincide with sensitive life stages

are more likely to elicit greater population-level effects and prolong the onset of recovery. Additionally, certain species-specific life history strategies (e.g. specific behaviours or morphologies) may increase pesticide uptake or effects, and these strategies are often linked to specific life stages (e.g. precopulatory behaviour of *Gammarus,* case construction by caddisfly larvae).

For the purposes of this literature review, static exposures lasting less than three days were considered to be short-term in duration. Some amount of degradation, dissipation, or adsorption is expected during static exposures, depending on the chemical behaviour and fate of the compound, suggesting that concentrations high enough to elicit toxicity may exist for durations less than the length of exposure reported in literature. However, short-term exposure that coincides with sensitive life stages or life history strategies (e.g. *Gammarus pulex* pre-copulatory behaviour) could result in greater than expected long-term effects. Long-term effects considered here include variables that affect population growth rate (e.g. reproductive output, time to first reproduction, reproductive behaviours, among others), delayed mortality occurring after cessation of exposure, and impacts on normal growth and development that could impact survival and/or reproduction. Community effects seen in mesocosm studies, including shifts in taxa composition, decreases in abundance, and reductions in specific invertebrate functional feeding groups, were also considered as long-term effects of exposure.

2.2 Aquatic

2.2.1 Organochlorine Insecticides

Organochlorine (chlorinated hydrocarbons) insecticides are characterized by their stability under variable environmental conditions and subsequent long-lasting toxic effect on invertebrates. Organochlorine compounds inhibit signal transmission by the peripheral invertebrate nervous system (Matsumura, 1975). Symptoms of organochlorine toxicity include tremors, uncoordinated movements and paralysis (Stenersen, 2004). While organochlorine compounds are proven potent insecticides, concerns regarding their resistance to environmental breakdown

and persistence in food webs have caused them to be widely abandoned as plant protection products. However, for the purpose of comparison, the potential long-term effects of organochlorine insecticides on invertebrates are included here.

Because exposure to organochlorines has been demonstrated to cause significant physiological effects, this suggests that invertebrate metabolism or food acquisition may be affected by low-level exposures. For example, Hartgers, *et al* (1999) observed that short-term sublethal exposure to lindane (an organochlorine insecticide) repressed gut clearance rates in young *Daphnia magna* (Diplostraca: Daphniidae), suggesting that the organochlorine compound can interfere with food throughput rates. A 2-hour exposure to 241 µg/L lindane reduced the clearance rate of polystyrene beads from the gastrointestinal tract by an average of 66%, and a 24-hour exposure to the same concentration completely arrested clearance of beads (Hartgers, *et al.* 1999). Following exposure, *D. magna* were transferred to clean media where feeding and digestion recovered to control rates within the next 24 hours. Considering this decreased energy acquisition, there is a potential that exposure to lindane at certain developmental periods may result in abnormal, decreased, or delayed growth as a result of decreased food intake, although this potential long-term effect was not specifically investigated by Hartgers, *et al.* (1999).

Given the documented behavioural impacts following organochlorine exposure, the effects of sub-lethal pulses of lindane concentrations on *Gammarus pulex* (Amphipoda: Gammaridae) reproductive behaviours may be considered a potentially sensitive endpoint, with clear ramifications on population growth. Single pulsed lindane exposures of 1.0 or 2.0 mg/L for 10 to 20 minutes resulted in the separation of precopulatory male-female pairs (Malbouisson, *et al.* 1994). Following exposure, *G. pulex* pairs were transferred to clean media and observed for 144 hours. Disruption of precopulatory behaviour continued during the first hours of depuration. Not only did short-term lindane exposure significantly depress and/or delay *G. pulex* reproduction, but also indirectly led to increased female mortality through male cannibalism of their partners following separation. Consequently, organochlorine-mediated

disruption of behaviours essential to successful reproduction can comprise a long-term impact on invertebrate population growth.

Larval exposure to sediment-associated lindane has been shown to also impact the long-term morphological and reproductive development of *Chironomus riparius* (Diptera: Chironomidae). Hirthe, et al (2001) exposed fourth instar *C. riparius* larvae to sediment lindane concentrations of 0.5, 0.75 and 1 mg/kg for 48 hours, followed by depuration in clean sediment until emergence. This exposure regime resulted in a 5 to 6% decrease of male wing length versus control following emergence, while female wing length was reduced by 6% following 48 hour exposure to 1 mg lindane/kg sediment. Emergence to adult was delayed by 2-3 days following larval exposures of 0.5, 0.75 and 1 mg/kg lindane, and egg production was reduced to 5, 4, and 2 egg ropes, respectively, versus 8 egg clutches deposited by control females (Hirthe, *et al.* 2001). Although larvae were allowed to depurate for more than 10 days in clean water/sediment, there was no recovery from exposure, and significant impacts on adult fitness were evident. Wing size is not only a proxy for adult weight in *Chironomus spp.*, but reduced wing size may also limit dispersal abilities. Reduced adult weight and mobility could significantly diminish reproductive success.

Short-term, pulsed organochlorine exposures have been shown to significantly alter macroinvertebrate assemblages and community structure in artificial stream mesocosm systems. Hose et al (2003) conducted pulsed exposures in mesocosms by introducing the insecticide into the system and blocking flow; after the appropriate exposure time, flow was re-established with clean water and invertebrate communities were observed for 108 hours post-exposure. Following a single 12-hour pulse of 50 μg/L endosulfan, abundances of invertebrate taxa were significantly reduced: mayfly species *Jappa kutera* (Ephemeroptera: Leptophlebiidae) were reduced by 80% after 12 hours in clean flow and the caddisfly *Ecnomus sp.* (Trichoptera: Ecnomidae) were reduced by 100% after 36 hours in clean flow. A 48-hour pulsed exposure of 5 μg/L or 25 μ/L endosulfan also resulted in reduced abundance of Leptophebiidae mayfly species, *Austrophleboides sp.* (reduced by 100% after 48 hours in clean flow) and *J. kutera*

(reduced by 100% after 48 hours in clean flow) (Hose, *et al.* 2003). Although recovery of these taxa was observed following pulsed exposure, algal blooms were evident prior to invertebrate community recovery. This is a strong indication that organochlorine exposure significantly impacted grazing invertebrate communities.

These effects on invertebrate assemblages are likely to occur as a result of both directly toxic effects and the behavioural impacts documented to occur following organochlorine exposure. While natural invertebrate drift is essential for species dispersion, catastrophic drift, such as that caused by exposure to neurotoxins, can result in the depletion of local invertebrate communities, including those species resistant to drift under pristine conditions, which ultimately can lead to altered stream production in the affected areas. Wallace, *et al.* (1989) observed massive invertebrate drift after exposing a headwater stream to a 4-hour pulse of 10 mg/L methoxychlor. Measured one-day pre-treatment, drift was initially 40 individuals, and was comprised largely of copepods, Chironomid larvae, and oligochaetes. In contrast, drift abundance during the 24 hours following pulsed exposure was estimated to exceed 450,000 individuals. Abundance was most severely reduced in collector-gatherer taxa, whereas biomass of shredder invertebrates was the most significantly reduced (Wallace, *et al.* 1989). The fact that organochlorine exposure affected invertebrate feeding guilds differently indicates that nutrient processing and carbon cycling may be significantly altered. In fact, the authors noted an increase in headwater leaf pack following methoxychlor pulse, a logical result of a significant reduction in leaf-shredding invertebrate biomass.

Ultimately, based on the literature mentioned in the previous paragraphs, both laboratory and field studies indicate that the long-term effects of organochlorine insecticides on aquatic invertebrates can be linked primarily to direct effects on behavioural homeostasis. Catastrophic drift following single pulsed organochlorine exposure has been demonstrated to deplete standing populations of invertebrates and alter stream nutrient cycling. At the individual level, exposure to a short-term pulse of an organochlorine was shown to disrupt behaviours essential to reproduction, and although not demonstrated herein, may interfere with other essential

behaviours. Additionally, organochlorine exposure was shown to depress energy acquisition and metabolism in *D. magna*, which is likely to reduce later development and reproduction. It has also been demonstrated that, organochlorines in sediments are bioavailable for sediment-dwelling invertebrates, and that short-term exposures of larvae to contaminated sediments can result in altered adult morphology and fitness.

2.2.2 Organophosphate Insecticides

Organophosphate (OP) insecticides are nerve poisons that exert toxicity through the irreparable inhibition of acetylcholinesterase (AChE). When exposed to an OP, affected invertebrates accumulate acetylcholine (Stenersen, 2004). This results in an initial period of restlessness and hyperactivity, followed by eventual convulsions, paralysis, and death (Matsumura, 1975). Additionally, the irreversible nature of the OP-AChE interaction indicates that physiological recovery from toxic effects is likely to be protracted. In the last 10 to 15 years, the use of several organophosphate compounds has been restricted as a result of concerns regarding toxicity to non-target birds and mammals, as well as to human applicators.

Given the irreversible mode-of-action and the fact that OP exposure can cause significant behavioural aberrations, exposed invertebrates may be more susceptible to multiple low-level pulsed exposures than a single high concentration exposure, if the cumulative AChE inhibition following a series of OP pulses is greater. Exposure to either 32 μg/L malathion or 55 μg/L parathion for 2 hours resulted in immobilization of *C. riparius* larvae, as measured by inability of larvae to complete normal "figure-eight" motions (normal mobius-shaped movements made by Chironomid larvae in response to prodding (Kallander, *et al.* 1997). If the 2-hour pulse was separated into two 1 hour pulses with intervening recovery times in clean media of 2, 6, 12, or 24 hours for malathion and 2 or 24 hours for parathion, no significant difference in behavioural effects was observed for larvae exposed to the organophosphate compounds. The fact that two 1 hour pulses were as toxic as a constant 2 hour exposure suggests that insufficient recovery occurred during the intervening time (2 – 24 hours), and that, recovery time between pulses is

critical in determining the magnitude of toxic effects observed in invertebrates. Ashauer, *et al.* (2007a) found that *G. pulex* mortality increased exponentially peaking at approximately 100 percent at day 20 when exposed to four 24-hour pulses of 0.5 µg/L chlorpyrifos with 4 days between pulses. In contrast, *G. pulex* were also exposed to chlorpyrifos for a 28 day chronic exposure period, during which concentrations fluctuated between 0.025 and 0.05 µg/L chlorpyrifos. This exposure scenario resulted in a cumulative *G. pulex* mortality of 40 percent, which suggests that pulsed exposures to organophosphates may be more toxic than chronic low-concentration exposures (Ashauer, *et al.* 2007a). When *G. pulex* were exposed to a carbamate insecticide (carbaryl) following a pulsed exposure to an OP (chlorpyrifos), the resulting toxicity was 25% greater than when the order of the pulse was reversed (e.g. carbaryl, then chlorpyrifos) (Ashauer, *et al.* 2007b). This indicates that exposure to an irreversible AChE inhibitor (OP) can sensitize invertebrates to future AChE inhibitors, if there is insufficient recovery time between exposures. Consequently, multiple pulse exposure to organophosphates is more likely to elicit long-term toxic effects in aquatic invertebrates, if insufficient recovery time separates the pulses.

Duquesne, et al. (2006) investigated delayed mortality following short-term pulsed exposures to paraoxon-methyl by adapting the OECD 21-day *D. magna* toxicity test methodology to include single pulsed exposures of either 1-hour or 24-hours in duration with the remaining bioassay duration spent in clean media. Approximately fifty percent delayed latent mortality by day 14 was caused by either a 1-hour exposure to 194 µg/L or a 24-hour exposure to 1.98 µg/L paraoxon-methyl (Duquesne, *et al.* 2006). After 14 days recovery, total reproductive output and reproductive output per female *D. magna* were reduced after 1-hour exposures of greater than 100 µg/L paraoxon-methyl and 24-hour exposures of greater than 1.5 µg/L paraoxon-methyl. Reproductive output similar to that of control animals was observed for all treatment groups at day 21, indicating that recovery from toxic effects had occurred by this date. Similarly, female lobsters exposed to three to four successive pulses of 10 µg/L azamethiphos were 50 percent less likely to spawn eight weeks after the final exposure (Burridge, *et al.* 2008). Azamethiphos exposures were one hour in duration and administered biweekly, so that spawning effects were

observed 6 weeks following the final pulsed exposure. Although sea water temperature and vitellogenesis appear to increase lobsters' sensitivity to azamethiphos, life stage seems to be an especially critical driver of organophosphate sensitivity.

A single 3 hour pulsed exposure to 30 mg/L dimethoate reduced *D. magna* length by an average of 9%, 21 days following exposure, when bioassays were performed according to ISO 6341 methods (Andersen, *et al.* 2006). These exposures also resulted in an increased time to first reproduction by 1-2 days and a 25% decrease in reproductive output. The authors surmised that long-term effects were a consequence of dimethoate-triggered paralysis and a resultant cessation of feeding. Successive pulses of dimethoate were found to significantly increase toxicity, indicating that previous exposures served to sensitise *D. magna* through additive AChE inhibition. Most critically, however, short-term pulses of dimethoate (e.g. less than 4 hours in duration) were demonstrated to produce eventual reproductive damage (Andersen, *et al.* 2006). However, Naddy, et al (2000) demonstrated that the inclusion of sufficient recovery time between two pulses of chlorpyrifos (e.g. 3 days between two 6hr pulses of 0.5 µg/L versus a single 12hr 0.5 µg/L) did improve the survival rates of *D. magna*.

Not only aqueous-phase, but sediment-bound OP contamination is capable of eliciting a toxic effect in aquatic invertebrates. Sediment chlorpyrifos concentrations of 0.1 mg/kg significantly inhibited burrowing by *C. riparius* larvae to 68% of that observed in the controls, and a 48-hour pulsed larval exposure to this sediment concentration caused decreased male emergence and reduced adult female dry weight (Callaghan, *et al.* 2001).

Experiments using artificial stream mesocosms indicate that pulsed OP exposure does not equally affect all resident lotic invertebrate taxa. Pusey, et al. (1994) established artificial stream systems with flowing water 171 days prior to exposure, at which time a constant concentration of chlorpyrifos was added to the system for 6 hours, to give 5 µg/L chlorpyrifos. This 6-hour pulse regime resulted in significantly reduced densities of *Ferrissia spp.* mussels, *Nanocladius spp.* (Diptera: Chironomidae), and *Cladotanytarsus bilinearis* (Diptera:

Chironomidae) (two-way nested ANOVA, $p<0.05$); in response to the reduced competition, *Procladius paludicola* (Diptera: Chironomidae) abundance increased followed chlorpyrifos exposure (Pusey, *et al.* 1994). Community recovery was evident 21 days post-exposure, but the authors noted that the rate of recovery would depend on the richness of the exposed system and the immigration rate.

In summary, the magnitude of effects caused by short-term organophosphate exposure is strongly dependent on duration, concentration and recovery time, given the irreversibility of the binding of these chemicals to AChE. This toxic action is also responsible for high rates of delayed mortality as damage evident at the cellular level immediately after exposure may manifest in later mortality. Reduced growth and lowered reproductive output were also evident in organophosphate-exposed invertebrates in laboratory studies, and sediment-associated organophosphate compounds have been demonstrated to elicit the same effects in invertebrates following a short-term exposure. Community metrics, are likely to recover following short pulses of organophosphate exposures, particularly if the intervening recovery time is sufficient to reverse the physiological impacts of AChE inhibition. It is likely that multiple pulsed exposures would prolong the effects of exposure due to incomplete recoveries.

2.2.3 Carbamate Insecticides

Carbamate insecticides are also AChE inhibitors, as potent as OP insecticides and often exhibiting the same environmental fate and similar biological uptake and kinetics. However, the AChE-carbamate interaction is reversible (Matsumura, 1975). Therefore, although the mode-of-action of OP and carbamate insecticides is identical, the efficacy and speed of detoxification differs between the two compound classes, which could have significant impacts on recovery rate and the evidence of long-term effects of short-term or pulsed exposures. The impact of AChE-insecticide interaction reversibility on the duration and magnitude of toxic effects has been demonstrated by Ashauer, *et al.* (2007b): higher toxicity was observed in *G. pulex* exposed to a pulse of organophosphate and then a pulse of carbamate than when they were exposed to a

pulse of carbamate followed by an organophosphate pulse (Ashauer, *et al.* 2007b). Consequently, recovery from carbamate exposure and the likelihood for long-term effects is most likely related to the reversibility of the AChE inhibition.

The benefit of a carbamate toxicity recovery period appears to depend on the target organism. *C. riparius* larvae exposed to carbaryl exhibited less severe effects following two 1-hour exposures with an intervening recovery period of either 2 or 6 hours, as compared with a single 2-hour exposure (Kallander, *et al.* 1997). However, no mitigating effect of recovery time was observed in *Aedes aegypti* (Diptera: Culicidae) mosquito larvae exposed to carbofuran or carbaryl (Parsons and Surgeoner, 1991). This suggests that the importance of recovery time in ameliorating the toxic effects of carbamates is highly dependent on the species exposed. Similarly, *Cinygmula spp*. mayfly nymphs (Ephemeroptera: Heptageniidae) exposed to single extremely short-term carbaryl pulses (e.g. 15 – 60 minutes) did not recover from initial behavioural symptoms, which included paralysis and moribundity, when removed to clean media following exposure (Peterson, *et al.* 2001). In addition, behavioural symptoms in *Cinygmula spp*. eventually led to mortality, whereas *Calineuria californica* stonefly nymphs (Plecoptera: Perlidae) had recovered from identical exposures after 5 hours in clean water. Consequently, *C. californica* nymphs were determined to be 1,000 times less sensitive to carbaryl than *Cinygmula spp.* nymphs, and this was hypothesized by the authors to result from differences in carbaryl uptake/equilibration rates between the two species.

Carbaryl exposure was also shown to induce the growth of *Daphnia spp*. morphological defences. Exposure of second-instars from two *D. pulex* varieties to 15 to 25 µg/L carbaryl for 8 to 14 hours resulted in neckspine growth several days later (Hanazato and Dodson, 1993). These pulsed carbaryl exposures also induced high helmet formation and reduced growth in *D. retrocurva* by approximately 6 to 16%, whereas 8 - 14 hour single pulsed exposure to 5 µg/L carbaryl reduced *D. galeata* growth by approximately 9%. Single carbaryl pulses of similar duration ranging from 5 - 20 µg/L causes *D. lumholtzi* to develop longer (approximate 11 to 20% increase in length) and higher helmets that were retained until the fifth instar (Hanazato

and Dodson, 1993). Development of morphological defences requires energy diversion from other purposes, and if chemically induced, represents a misallocation of energetic resources. Most significant, however, was the fact that a coinciding exposure to carbaryl and *Chaoborus spp.* predator pheromones produced a synergistic effect on *Daphnia spp.* defence morphology development. This indicates that pulsed exposures of carbaryl under field conditions would likely result in greater impacts on *Daphnia spp.* morphology than described here.

U.S. EPA/FIFRA methods were used to elucidate the long-term impacts of fenoxycarb on *D. magna* growth and reproduction over 21 days (Hosmer, *et al.* 1998). Initial concentrations of fenoxycarb ranged from 0.2 to 50 µg/L and these concentrations were reduced by 50 percent every 10 hours to mimic the natural degradation of the parent compound. Exposure of < 24-hour neonates to the highest fenoxycarb concentration resulted in reproductive output reduced by over 50% as compared to control (Hosmer, *et al.* 1998).

Population structure and life stages at the time of exposure can influence both the duration and type of long-term effects caused by short-term pulsed carbamate exposure. First instar *D. ambigua* exposed to a 10-hour pulse of 5 µg/L carbaryl experienced significantly higher mortality (approximately 70%) as opposed to other life stages exposed (egg, embryo, second instar, or third instar with approximate mortality of 0 to 20%) (Hanazato, 1991). However, individuals exposed during the embryonic stage exhibited decreased growth as late as the fifth instar stage. More significantly, reproduction was delayed until the fifth instar (as compared to the fourth instar in controls) in those *D. ambigua* exposed as embryos, first instars, second instars, or third instars. This led the author to conclude that *D. ambigua* populations with high available food resources would be most susceptible to long-term carbaryl effects, because these tend to be dominated by early instar individuals (Hanazato, 1991).

In summary, recovery time for carbamates appears to be highly dependent on the invertebrate species exposed; potency of the carbamate compound is also species-specific, but the physiological reasons for this are not known. Like organophosphates, however, pulsed

carbamate exposures were demonstrated to reduce growth and reproductive output in invertebrates. Furthermore, carbamate exposure was also shown to result in significantly altered *Daphnia spp.* morphologies that suggest a potentially significant depletion of energy reserves.

2.2.4 Synthetic Pyrethroid Insecticides

Pyrethroid insecticides are chemically synthesized analogs of naturally produced pyrethrum compounds extracted from chrysanthemum flowers (Stenersen, 2004). The mode of toxic action is the blockage of sodium channels within nerves causing repetitive discharges and eventual depolarization which results in the following symptom progression: excitation, convulsions and tremors, paralysis, and death (Matsumura, 1975). In aquatic environment, pyrethroid insecticides exhibit strong hydrophobicity, dissipating from the water column and adsorbing to organic material often within hours of introduction to the aquatic system. Consequently, short-term pulses characterise most aquatic exposure regimes.

Given the severity of behavioural effects observed in invertebrates exposed to pyrethroids, delayed mortality may be a significant long-term endpoint of exposure for this class of insecticides. Although there was no mortality observed during a 24-hour pulsed exposure to 0.2 to 0.8 μg/L cypermethrin, survival of exposed *Acartia tonsa* copepods (Calanoida: Acartiidae) was 15 to 20 percent of controls when evaluated 144 hours following exposure (Medina, *et al.* 2004). Male *A. tonsa* were especially sensitive to cypermethrin, with 1-hour exposures to 0.7 and 2.2 μg/L, resulting in mortalities of greater than 50 percent at the end of the 144 hour observation time. Approximately 75 percent of female copepods, on the other hand, survived these exposures. The authors concluded that the significant delayed mortality was a strong indication that substantial and long-lasting damage can be caused by extremely short-term exposures to cypermethrin (Medina, *et al.* 2004). Specific formulation of pyrethroid compounds may contribute to delayed mortality. Micro-encapsulated permethrin was more toxic than non-encapsulated permethrin to *A. aegypti* when applied as multiple pulses, most likely because the slow release from the encapsulated permethrin pellets allowed for increased uptake by foraging

larvae (Parsons and Surgeoner, 1991). Significant delayed mortality was also observed following exposure to 0.1 or 0.3 μg/L esfenvalerate in field collected *G. pulex* juveniles (average lifespan reduced by 44% and 85%, respectively) and non-reproductive adults (average lifespan reduced by 1% and 6%, respectively), but this effect was not evident until seven days after exposure (Cold and Forbes, 2004).

Copulatory behaviour may also be significantly impaired by pulsed exposure to pyrethroids. Paired adults separated in response to short-term esfenvalerate exposure and subsequent pair reformation required six times the duration that separated control individuals required (e.g. 12 days as opposed to 2 days for non-exposed *G. pulex*) (Cold and Forbes, 2004). Most significantly, this esfenvalerate-induced delay in pair reformation ultimately resulted in a significantly decreased reproductive output. Pre-copulatory behaviours in *G. pulex* were also disrupted when males and females were exposed to a 3-hour pulse of 0.35 μg/L lambda-cyhalothrin (Heckmann, *et al.* 2005). While behavioural recovery was evident 96 hours following this exposure, higher concentrations required a longer recovery period of up to 15 days. Since there are seasonal constraints on conditions appropriate for *G. pulex* reproduction, especially in temperature climates, disruption of pre-copulatory behaviours may significantly lower reproductive output (Heckmann, *et al.* 2005).

Schulz and Liess (2000) investigated the long-term impacts of 1-hour and 10-hour fenvalerate exposures using field-collected *Limnephilus lunatus* caddisfly larvae (Trichoptera: Limnephilidae). To compare the relative importance of pulse duration, exposure concentrations were converted from μg/L to μg/hour. Even when equivalent concentrations were compared, *L. lunatus* were observed to be more significantly affected by 1-hour pulses than by 10-hour pulses of fenvalerate (Schulz and Liess 2000). Single short-term exposures (1 hour) to 0.001, 0.01, 0.1 or 1 μg/L fenvalerate during the second, third, and fourth larval instars were also shown to produce significant long-term, population-level impacts (Schulz and Liess 2001b). Both reduced emergence (at 0.1 and 1.0 μg/L fenvalerate, ANOVA $p<0.5$) and adult weight, as well as altered emergence timing (approximately 42, 55, 53 and 47% fewer emergences,

respectively, versus control) were evident over 200 days post-exposure. Sediment-associated fenvalerate also altered emergence timing and reduced dry weight at concentrations as low as 20 µg/kg, suggesting that dissipation from the water column and adsorption to sediment does not eliminate bioavailability of the compound to *L. lunatus* larvae (Schulz and Liess 2001b).

Short-term fenvalerate exposures have been shown to significantly alter *D. magna* population structures. Stable *D. magna* populations were exposed to fenvalerate for 24 hours at a range of concentrations between 0.03 and 10 µg/L and observed for 61 days. Individual life stages reacted independently to exposure and recovered differently because of the variable life stage responses, re-establishment of stable population demographics required over 60 days and six to seven generations of *D. magna*. The authors theorized that observed increases in smaller age class abundance occurred as a result of a reduction in the larger animals, which increased food availability. However, under field conditions, increased food resources may be consumed by other, less sensitive organisms, potentially exacerbating population-level effects on sensitive *D. magna* (Liess, *et al.* 2006).

DEBtox modelling is recommended by the OECD as a statistical method for the analysis of ecotoxicological data (OECD 2006). This approach incorporates principles of the Dynamic Budget Theory, including variables such as feeding rate, metabolic rate, and growth, to predict sensitive endpoints, such as decrease in growth, time to first reproduction, and reproductive output. DEBtox modelling of fenvalerate exposure effects in *D. magna* suggested that feeding behaviours were suppressed at low, sublethal levels, which led to an eventual decrease in growth (Pieters, *et al.* 2006). In a similar vein, a 3-hour pulsed exposure to 0.001, 0.01, or 0.1 µg/L cypermethrin suppressed oxygen consumption and increased ammonia excretion in freshwater *Trichodactylus borellianus* (Decapoda) crabs (Veronica and Collins, 2003). This provides further evidence that pyrethroid exposure could alter metabolic rates of exposed invertebrates. There is also evidence that environmental conditions of the parental generation can impact offspring sensitivity to pyrethroids. Female *D. magna* reared with high food availability produced neonates markedly more sensitive to fenvalerate pulses than neonates

produced by females with restricted access to food. The two neonate stocks were exposed to 24-hour fenvalerate pulses of 0.03 to 10 µg/L according to the 21 day *D. magna* OECD test methodology and then observed for 21 days. Significantly increased mortality occurred following pulses of 0.6, 1.0, 3.2, and 10.0 µg/L fenvalerate (40, 65, 100 and 100%, respectively), but only in those individuals reared from females provided with high food. Low maternal food stock *D. magna* individuals were less sensitive, with only the two highest fenvalerate exposures elicited significant mortality (85 and 100%, respectively). As a result, population growth rates were calculated to be more significantly reduced by fenvalerate exposures to *D. magna* produced by females supplied with high food availability (Pieters and Liess, 2006).

Often, field conditions can either enhance or ameliorate the effects of pyrethroid exposures. Beketov and Liess (2005) exposed field collected *Cloeon dipterum* mayfly nymphs (Ephemeroptera: Baetidae) a range of esfenvalerate concentrations (0.001 to 100 µg/L), for 1 hour in a laboratory setting, before transfer to clean systems with variable food availability. The resulting intensity of resource competition exacerbated the toxic effects of esfenvalerate exposure, and further resulted in lowered reproductive output (Beketov and Liess, 2005). *D. magna* neonates were exposed to single short-term (24-hour) pulses of fenvalerate and provided with either low or high food conditions for 21 days post-exposure to determine the conjunctive impacts of food and pyrethroid stress on long-term endpoints (Pieters, *et al.* 2005). Low food availability intensified effects of short term fenvalerate exposure, with reproductive output and growth significantly reduced following fenvalerate exposure as low as 0.3 µg/L (30% fewer broods produced and a 13% reduced body length). These long-term endpoints may have developed as a result of decreased food incorporation following pyrethroid intoxication. For instance, *D. magna* assimilated significantly less ^{15}N-marked algae after 24-hour exposure to 0.3 µg/L fenvalerate, and subsequent filtering rates were also depressed by 18% (Reynaldi, *et al.* 2006). Although recovery of feeding behaviours was evident 2 days post-exposure, reproductive and growth rates were still significantly impacted (respective reductions of 35 and 18%).

Although dissipation of pyrethroids in aquatic systems is expected to be rapid, mesocosm studies suggest that there still is sufficient time in the aqueous phase to cause significant long-term effects on community structure. Fairchild, *et al* (1992) exposed mesocosm systems to single pulses of esfenvalerate every two weeks for three months, and then allowed to recover for an additional two months. The aqueous half-life of esfenvalerate in these systems was calculated to be approximately 10 hours. Benthic macroinvertebrate and zooplankton abundance was reduced following exposure regimes with esfenvalerate concentrations as low as 0.25 µg/L; Ephemeroptera, Gastropoda, and Diptera taxa were the most sensitive benthic macroinvertebrate taxa to esfenvalerate (Fairchild, *et al.* 1992). Copepod abundance markedly decreased 2 days after each esfenvalerate pulse, but recovered 9 days following exposure, and prior to the next pulsed exposure. Subsequent pulses, therefore, had no cumulative effect on copepod abundance, but some gastropod taxa had not recovered 2 months after the final esfenvalerate pulse. Esfenvalerate pulses also altered community dynamics, with distinct algal blooms occurring after exposures, most likely in response to depressed grazing from reduced macroinvertebrate populations. Most significantly, authors reported that 48-hour LC_{50} *D. magna* bioassays conducted according to ASTM standards concurrently to mesocosm exposures were not predictive of community-level impacts (Fairchild, *et al.* 1992). Although both mesocosm invertebrate communities and *D. magna* populations were sensitive to low concentration esfenvalerate exposure, authors surmised that the laboratory studies might underestimate field sensitivity, and stressed that single-species laboratory studies cannot predict alteration in community responses (e.g. algal blooms).

Lauridsen and Friberg (2005) linked pyrethroid-triggered behavioural effects to catastrophic drift in lotic invertebrate communities. Artificial stream mesocosm systems were constructed and separated into 4 replicate subsections; consistent flow through each channel was achieved with a pump and reservoir system. Pulses were administered through addition of an insecticide directly into the water column. Up to 22 hours after exposure, 1-hour pulses of 0.01, 0.1 and 1.0 µg/L lambda-cyhalothrin significantly increased the drift rates of *G. pulex* (100% drifted at all three exposures), *Baetis rhodani* (Ephemeroptera: Baetidae) (about 65, 70 and 85% of standing

stock), and *Leuctra spp.* (Plecoptera: Leuctridae) (about 55, 85 and 95%, respectively, of standing stock). Drifting behaviour was characterized by decreased mobility, although direct mortality was low following exposure (Lauridsen and Friberg, 2005).

Evidence suggests that although pyrethroids adhere strongly and quickly to particulates, they can still exert toxic effects in invertebrate communities. Schulz and Liess (2001a) exposed invertebrate communities contained in stream mesocosms to 1-hour pulses of 13.6, 136.5 and 1365 µg/kg of sediment-associated fenvalerate, which result in significantly increased drift rates. *G. pulex* and *Hydropsyche angustipennis* (Trichoptera: Hydropsychidae) drifted significantly following the lowest exposure, while drift rates of *Anabolia nervosa* (Trichoptera: Limnephilidae), *Plectrocnemia conspersa* (Trichoptera: Polycentropodidae), *Limnephilus lunatus* (Trichoptera: Limnephilidae), and *Tipula maxima* (Diptera: Tipulidae) increased after exposure to the two highest particulate fenvalerate concentrations only (Schulz and Liess, 2001a). Abundances of *G. pulex* and *T. maxima* were significantly reduced 93 days following the highest exposure (approximately 31% reduction in both cases), and the emergence rate of *L. lunatus* was reduced by 26% (at 136.5 µg/kg fenvalerate) and 44% (at 1365 µg/kg fenvalerate).

Following exposure to two pulses of esfenvalerate (at day 0 and day 30) ranging from 0.1 to5.0 µg/L, the macroinvertebrate and microinvertberate communities of littoral enclosures (constructed in a natural pond with three sides to include shoreline ecosystem) were observed for 70 days to determine long-term effects on abundance (Lozano, *et al.* 1992). Copepod numbers were significantly suppressed following the first pulse (either 0.01, 0.08, 0.2, 1.0 or 5 µg/L esfenvalerate, resulting in 25 to 60% reduction following the lowest three concentrations, and 100% mortality after the two highest concentrations) and none of the exposed population had recovered to control levels within the 70 days after the second pulsed exposure. *Hyalella azteca* (Amphipoda: Hyalellidae) abundances were reduced to nearly zero less than 5 days after the first pulsed exposure to all esfenvalerate concentrations and did not recover during the duration of the experiment (Lozano, *et al.* 1992). Abundances of certain insect taxa, including Chironomidae, *Caenis* spp. (Ephemeroptera: Caenidae), and Coenagrionidae (Odonata), were

also significantly reduced by a single esfenvalerate pulse of 1.0 µg/L or less, although these populations exhibited signs of recovery within the 70-day time frame.

In summary, like organochlorine toxicity, many of the effects observed in invertebrates exposed to synthetic pyrethroids can be traced to disruption of behavioural homeostasis. A significant increase in invertebrate drift was observed following pulsed pyrethroid exposures, and individual behaviours, such as *G. pulex* pre-copulatory behaviour, were suppressed following short-term exposures. Also, pyrethroids have been shown to be highly bioavailable and toxic to invertebrates even when adsorbed to organic matter; in fact, pulses of sediment-associated pyrethroids elicited similar effects as aqueous-phase compounds in exposed invertebrate communities, although greater concentrations were required to cause a similar effect. Most significantly, 1-hour exposures to field-relevant pyrethroid concentrations can cause reproductive and developmental effects more than 200 days following exposure (Schulz and Liess, 2000). This strongly indicates that short-term exposures to this class of plant protection products will likely result in significant long-term impacts to aquatic invertebrate communities.

2.2.5 Metals

Inorganic insecticides, such as metals and metal-based compounds, are relatively non-specific to invertebrates as compared with the organic insecticide compounds. More importantly, these compounds are significantly less toxic to pest invertebrates, which necessitates the use of large quantities to control pests. As such, inorganic compounds have largely been replaced with the more toxic organic compounds (Matsumura, 1975). However, as persistent general environmental pollutants, both historical and current use inorganic metal formulations should be examined for potential long-term effects on invertebrate communities from short term pulsed exposures.

Early life stage exposure to aqueous phase arsenic altered reproduction of *D. magna* exposed according to U.S. EPA methodology (U.S. EPA 2002, Hoang, *et al.* 2007). Both 24-hour exposures of 4,000 and 5,000 µg/L and a 6-hour exposure to 6,000 µg/L arsenic to neonates

delayed time to first reproduction and reduced total reproductive output. Additionally, the inclusion of recovery times of 24, 96, or 168 hours between a double-pulsed exposure of arsenic (6, 12, or 24 hour pulses of 3000ppm or 4000ppm, and 3, 6, or 9 hours of 5000ppm) had no ameliorating effect on the magnitude of response (Hoang, *et al.* 2007). Hence, recovery from pulsed arsenic exposure is likely to require more time than recovery from other compounds. Significantly delayed mortality was observed following *D. magna* exposure to aqueous selenium in bioassays conducted under U.S. EPA methodologies (U.S. EPA 2002). In these bioassays, 20-day delayed mortality was increased after 12- to 24-hour pulses of 800 μg/L, 9- to 12-hour pulses of 1,200 μg/L, 6- to 14-hour pulses of 1,600 μg/L, and 5- to 8-hour pulses of 2,000 μg/L selenium (Hoang and Klaine, 2008). This implies that under a pulsed exposure regime, selenium-exposed *D. magna* are unable to metabolise and eliminate the metal efficiently, increasing the risk of long-term effects.

Ultimately, significant delayed mortality has been documented in invertebrates following aqueous pulsed exposure, although at significantly higher concentrations than required to elicit similar effects with organic plant protection products. Due to the high concentrations required to protect against pests and the likelihood of environmental and human health risk from the use of these compounds, metal-based plant protection products are rarely used. As such, it is unlikely that the concentrations described here would regularly occur in surface waters.

2.2.6 Biorationals

This class of plant protection products includes compounds exhibiting variable modes-of-action, but linked as a result of 1) their derivation from natural sources such as pathogens, plants, or target invertebrates, and 2) toxic actions that interfere with invertebrate biochemical or endocrine homeostasis (versus neurotoxic actions) (Stenersen, 2004). These compounds are frequently used as a component of Integrated Pest Management programs. Biorational insecticides include insect growth regulators (hormone mimics that alter metamorphosis or exoskeleton development), plant extracts with antifeedant or repellant properties, and pathogen-

derived compounds, such as *Bacillus thuringiensis* endotoxin, that are specifically toxic to invertebrates.

A juvenile hormone mimic, methoprene, was demonstrated to bioaccumulate in select tissues of the larvae of the American lobster, *Homarus americanus* (Decapoda: Nephropidae). Eyestalk, epithelial, gonadal, and hepatopancreas tissue all exhibited increased concentrations of methoprene following a 3-day exposure to 25 or 50 µg/L (Walker, *et al.* 2005). This accumulation could result in significant and long-lasting tissue damage in essential invertebrate organs.

The toxicity of imidacloprid, a neonicotinoid insecticide used extensively as a systemic insecticide for the control of phytophagous invertebrates, was also evaluated for toxicity to aquatic invertebrates (Stoughton, *et al.* 2008). A 96-hour exposure to 3.5 µg/L imidacloprid significantly reduced *H. azteca* dry weight 10 days after exposure (approximately 50% of controls); however, by day 28 dry weight had recovered to approximate control levels. This was a considerably more sensitive long-term endpoint of imidacloprid exposure than delayed mortality, which was only significant 28 days following exposure to 11.5 µg/L imidacloprid (approximately 60% mortality versus < 5% for control) (Stoughton, *et al.* 2008).

Like other insecticidal compounds, recovery time may be an important determinant of the long-term effect of Neem-derivatives such as Margosan-O on invertebrates. *D. magna* exposed to 4 or 12 hour pulses of 840 mg/L Margosan-O exhibited decreased mortality when the time between pulses was increased to 7 days from 4 days (Scott and Kaushik, 1998).

When applied to stream mesocosms, short-term (5-hour) pulses of azadirachtin, an insecticidal extract of the Neem tree (*Azadirachta indica*), had significant effects on invertebrate behaviour and survival (Kreutzweiser, *et al.* 1999). Drift of *Isogenoides sp.* (Plecoptera: Perlodidae) was observed to increase following a 5-hour pulse of 0.84 mg/L azadirachtin with approximately 64% of individuals engaged in drifting. Survival of *Isonychia bicolor/rufa* (Ephemeroptera:

Isonychiidae) and *Hydropsyche bifida/recurvata* (Trichoptera: Hydropsychidae) were depressed at this exposure concentration (71.1% survival in both species) (Kreutzweiser, *et al.* 1999). However, the authors stipulated that few effects were observed following azadirachtin contamination of artificial stream systems, concluding that there would be little to no impact of Neem-derivatives on aquatic invertebrate communities.

Other biorational pesticides may impact aquatic macroinvertebrate communities, although the timing of effects is likely to differ from other plant protection compounds. For instance, the effects of a single diflubenzuron exposure in a stream system were not observed until moulting began 2–3 weeks following the application (Reinert, *et al.* 2002). This delay in effects may have occurred as a result of the insect growth regulator (IGR) mode-of-action or the chemical properties of diflubenzuron. Extremely hydrophobic and resistant to breakdown, diflubenzuron is likely to remain toxicologically reactive and adhered to organic matter, and invertebrates that feed on organic material may continue to ingest diflubenzuron long after the pulsed aqueous exposure has ended.

In summary, the long duration separating exposure and eventual effects for some biorationals (specifically insect growth regulators) suggests that long-term effects would be expected following short-term exposures of significant magnitude. In fact, such delays in response are the desired mode-of-action for the products. Additionally, evidence of bioaccumulation in specific invertebrate tissues indicates that damage may be targeted to discrete physiological systems, and may explain the delayed effects.

2.2.7 Fungicides and Herbicides

Although these compounds are intended to be selectively toxic towards plants and pathogenic fungi, herbicides and fungicides may cause long-term impacts on aquatic invertebrate populations. Pulsed exposure to algaecides or herbicides, for instance, may alter available food resources, suppressing phytophagous invertebrate growth. Fungicides may elicit unexpected

toxic effects apart from designated mode-of-action, or may interfere with chitin synthesis, a biochemical process present in both fungi and arthropods (Stenersen, 2004).

BULAB 6002, an algaecide (active ingredient: poly[oxyethylene-(dimethyl-iminio) ethylene-(dimethyl-iminio) ethylenedichloride]), was determined to be directly toxic to *Dreissena polymorpha* (Veneroida: Dreissenidae) zebra mussels when they were exposed to concentrations between 4 and 8 mg/L (Martin, *et al.* 1993). However, toxicity significantly increased as water temperature increased, indicating that exposure seasonality will extensively alter potential toxicity of the compound. Since only a simple toxicity bioassay was performed, it is unknown whether the subsequent decrease in phytoplankton and periphyton abundance would further damage *D. polymorpha* or other mussel populations.

Schneider, et al (1995) examined the impact of Velpar L, a hexazinone herbicide, on stream processes when applied to stream mesocosms as pulsed exposures. Although a 24-hour pulse of 200 μg/L Velpar L caused a significant decrease in periphyton productivity, this was rapidly reversed. There were no direct toxic effects of Velpar L on resident invertebrates, nor subsequent secondary effects of periphyton productivity on invertebrate drift, abundance, taxa richness, biomass, or length (Schneider, *et al.* 1995). Similarly, linuron was applied to mesocosm systems to determine if altered ecosystem processes could lead to long-term impacts on the invertebrate community. Three pulsed exposures of 10 μg/L linuron (one per month for 3 months) had an adverse effect on periphyton abundance and phytoplankton taxa richness, and briefly reduced the abundance of *Keratella quadrata* (Ploima: Brachionidae) rotifers and slightly reduced macroinvertebrate taxa richness (Van Geest, *et al.* 1999). It was not determined if the impacts on aquatic invertebrates were secondary effects of change to primary producer communities or if linuron had some direct toxicological effect on invertebrates.

Distinguishing between direct toxic effects of fungicides and herbicides and effects elicited through an alteration of primary producer or fungal communities is difficult. Given that modes of action of these compounds tend to target specific plant or fungal biological processes, it is

unlikely that these compounds would cause significant direct invertebrate toxicity comparable to that caused by insecticides. However, secondary effects from reduced food resources or decreased saprotrophic processing are a likely source of alterations of aquatic invertebrate communities following pulsed exposures.

2.3 Terrestrial

Given that the plant protection products of concern are largely used to control agricultural pests, terrestrial invertebrates are especially at risk of exposure to these compounds. Cuticular exposure to a spray event, as is investigated in much of the pertinent research, is the most field-relevant short-term exposure regime. Whereas aquatic research focuses on the response of all sensitive invertebrates to pesticides of concern, the bulk of terrestrial pesticide toxicity research concentrates on the effects to beneficial invertebrates, such as honeybees, natural enemies of plant pests and earthworms.

2.3.1 Organophosphate and Carbamate Insecticides

Acetylcholinesterase (AChE) inhibitors (such as organophosphates and carbamates) are potent insecticides commonly used to control agricultural pests such as cutworms, rootworms, aphids, and other phytophagous insects. When exposed to an organophosphate (OP) or carbamate affected invertebrates are unable to break down acetylcholine, which accumulates and elicits the toxic effects (Stenersen, 2004). AChE toxicity causes restlessness and hyperactivity, followed by eventual convulsions, paralysis, and death (Matsumura, 1975).

Exposure to diazinon (an OP) treated soils was demonstrated to significantly impact the food assimilation of saprotrophic isopods (Vink, ***et al.*** 1995). *Porcellionides pruinosus* (Isopoda: Porcellionidae) individuals were exposed to soils treated with a single application of diazinon to give 0.51, 1.10 or 2.37 μg/g soil. After 5 weeks, isopod protein content was reduced at the two highest exposures, while all three exposures significantly reduced glycogen concentrations

(Vink, *et al.* 1995). This suggests that diazinon exposures negatively impact isopod energy reserves and, although not specifically evaluated, may reduce reproductive capacity.

Important natural enemies of several pest invertebrates, parasitic wasps are often exposed to plant protection products at the same time as their hosts, and the potential long-term impacts on parasitoid populations hold additional consequences for pest control efforts. When *Trybliographa rapae* parasitoids (Hymenoptera: Figitidae) were subjected to a single cuticular exposure of 300 ng chlorfenvinphos /insect (another OP), individuals exhibited reduced longevity (approximately 56% reduction for males and 48% reduction for females) (Alix, *et al.* 2001). A reduction of parasitoid lifespan could ultimately result in decreased reproductive output and diminished parasitism. A concurrent decrease in potential female fecundity was also observed in the days following chlorfenvinphos exposure, with fewer eggs dissected from the ovaries of exposed females at days 5 (17% reduction), 10 (10% reduction), 15 (21% reduction), and 20 (18% reduction) post-exposure (Alix, *et al.* 2001). Experiments with multiple organophosphate and carbamate compounds indicate that brown lacewing populations are also potentially at risk from short-term, field-relevant exposures to plant protection products: *Micromus tasmaniae* (Neuroptera: Hemerobiidae) larvae reared on Petri dishes applied with 0.002 μg/cm azinphos-methyl (an OP) exhibited a reduced oviposition period ($p<0.05$ ANOVA) following emergence. (Rumpf, *et al.* 1998).

Generally, aphid mummies (i.e. dried husks of dead, parasitized aphids) are assumed to provide protection for host parasitoid larvae against plant protection products. However, single 24-hour pulsed exposures of mummies containing *Aphidius uzbekistanicus* larvae (Hymenoptera: Brachonidae) to the OP dimethoate at a concentration equivalent to 400 g a.s./ha reduced adult parasitoid longevity by more than 90%, and resulted in the decrease of successful aphid infection to 0, as did a 10-minute exposure of adults to the same concentration (Krespi, *et al.* 1991).

Significant reproductive effects following exposure to AChE inhibitors were also demonstrated to occur in German cockroaches (*Blattella germanica*, Blattodae: Blattellidae). Adults that survived a single cuticular exposure to an LC_{50} concentration (0.801 µg/insect for males and 1.743 µg/insect for females) of the carbamate propoxur produced significantly fewer oothecae (egg masses) when mated (Lee, *et al.* 1998). Females exposed to propoxur LC_{50} were notably more likely to abort first oothecae regardless of male exposure. Male and female longevity following cuticular LC_{30} and LC_{50} propoxur was reduced by 20 to 30 days, and during this time exposed females were less likely to respond to the courting behaviours of males ($p<0.05$, Scheffe's S procedure).

For the parasitoid *Aphidius ervi* (Hymenoptera: Brachonidae), increased exposure to surfaces treated with triazamate (an AChE inhibitor) was related to decreased time spent in aphid-odorized areas during an investigation of the effects of short-term, realistic insecticide exposures on host odour recognition (Desneux, *et al.* 2004). For parasitoids, inability to locate host insects results in reduced reproductive output. However, exposure to pirimicarb (carbamate) had no effect on *A. ervi* odour response to host aphids, even at near lethal doses (up to 62.34 ng/cm of exposed surface) (Desneux, *et al.* 2004).

In terms of community-level AChE long-term effects, field tests in commercial apple orchards indicate that chlorpyrifos applications can result in significant reductions of beneficial arthropods. Cross and Berrie (1996) exposed field populations of *Typhlodromus pyri* (Acarina: Phytoseiidae, a predatory mite utilised in Integrated Pest Management programs) to repeated agricultural spray exposures every three weeks May to August to mixtures or single compounds of organophosphate and fungicide compounds. The purportedly organophosphate-resistant *T. pyri*, was shown to be sensitive to multiple sprays of 960 g a.s./ha chlorpyrifos (an OP) with reduced numbers observed weeks following the final spray event. Additionally, mixtures of sprays containing chlorpyrifos and either 3600g a.s./ha mancozeb (an ethylene(bis) dithiocarbamate fungicide) or 1100g a.s./ha thiophanate-methyl (a benzimidazole fungicide) exhibited a synergistic toxic action in *T. pyri*. These results suggest that 1) multiple pulses of

organophosphates can be toxic to even highly tolerant invertebrate species, and 2) organophosphates applied in conjunction with other plant protection products can produce unforeseen synergistic toxicity.

As with aquatic invertebrates, terrestrial invertebrates exposed to acetylcholinesterase (AChE) inhibitors (organophosphates and carbamates) experienced delayed growth, and decreased reproductive output and longevity. Multiple short-term pulses of AChE inhibitors also resulted in significant reductions in invertebrate community abundances. Exposure to certain AChE inhibitors was also demonstrated to inhibit odour learning and response behaviours of Hymenopteran species, which can affect host-finding and reproduction in parasitoids and foraging efficiency in honeybees.

2.3.2 Synthetic Pyrethroids

Synthetic pyrethroid insecticides are commonly used to control a wide range of invertebrate pests, such as bugs, flies, moths, beetles, and other insects, and are used in the agricultural production of tree fruit, vegetable crops, cereals and ornamental crops. These compounds alter the transmission of signal transmissions along nerves by blocking sodium channels. The resulting repetitive discharges and nerve depolarization causes excitation, convulsions, and tremors, paralysis, and death (Matsumura, 1975). In particular, synthetic pyrethroids cause distinct behavioural responses, including "knockdown", a prolonged period of severe paralysis.

Cuticular exposure to a single dose of 2.5 and 4.5 ng deltamethrin/insect induced a significant hypothermia behavioural response in worker honeybees (Vandame and Belzunces, 1998). Affected insects avoided flight and clung to vegetation, and in severe cases, these symptoms can last several hours and are likely to result in worker bee death. Similarly, a single low-level cuticular exposure to permethrin generated abnormal behavioural responses that could adversely impact long-term hive viability and productivity (Cox and Wilson, 1984). Worker bees exposed to 0.001 μg permethrin/bee were observed to engage in excessive self-cleaning, rotating, and trembling dance, all of which resulted in significantly less time foraging (65% fewer trips out of

hive). Following exposure to 0.009 μg permethrin/bee, tagged honeybees were determined to be less likely to return to the hive on the day of exposure, and none of the exposed bees were observed within the hive the following morning. Researchers hypothesize that these observed permethrin effects (e.g. disorientation, decreased foraging, and mortality) could eventually result in reduced egg laying, brood rearing, temperature regulation, and cripple the hive, if a sufficient number of workers were affected.

Single cuticular exposures to the LD_{30} or LD_{50} of deltamethrin (0.0217 and 0.0257 µg/insect, respectively) reduced surviving *B. germanica* female life span by approximately 50 to 80 days and male life span by approximately 35 to 40 days (Lee, *et al.* 1998). The higher deltamethrin exposure also reduced female reproductive output to 14 to 30 percent of that of non-exposed females. Exposed females were also more likely to abort first oothecal productions.

However, realistic field exposures to plant protection products are likely to involve multiple spray (pulse) events over the course of the growing season. Li, et al. (1992) investigated the impacts of this type of exposure regime on the apple leaf miner *Phyllonorycter blancardella* (Lepidoptera: Gracillariidae) and its natural enemy parasitoids *Pholetesor ornigis* (Hymenoptera: Brachonidae) and Chalcididae *spp.* (Hymenoptera), using three applications of either 2.5g a.s./ha or 6.25g a.s./ha lambda-cyhalothrin once a month for 3 months. Abundances of *P. ornigis* eggs and larvae were significantly reduced at all lambda-cyhalothrin exposures, but recovered later that season to control levels. Additionally, abundances of *P. blancardella* eggs were similarly diminished following two lambda-cyhalothrin spray events, but recovered to greater than control levels four weeks following the second pulse exposure (Li, *et al.* 1992). The authors hypothesized that reductions in eggs may have been caused by parental avoidance of contaminated leaves, and that mortality of young, sap-feeding *P. ornigis* larvae may have resulted in delayed population growth. Parasitoid abundances followed a similar pattern, but it is impossible to ascribe this to toxic effects or reductions in host population.

Ultimately, synthetic pyrethroid compounds significantly alter normal invertebrate behaviour, and this basic effect can potentially lead to several long-term effects on population viability. Honeybee behavioural responses, in particular, are critical given that repressed foraging behaviours may result in severe debilitation of the hive and a reduction of reproductive output. Reduced reproduction and longevity were evident in other invertebrate species as well. Most significant, however, was that some of these responses occurred after doses as low as 0.009 µg/individual, indicating that long-term effects are likely to occur at field-relevant exposures to synthetic pyrethroids.

2.3.3 Biorationals

Because these compounds are frequently applied in conjunction with Integrated Pest Management, beneficial insects are very likely to be repeatedly exposed to biorational insecticides. Although biorational insecticides are generally considered to be less toxic to parasitoids and natural enemies than chemically synthesized carbamates, organophophates, and pyrethroids, only a few studies have focused on elucidating the long-term population-level impacts of exposure to these compounds.

Benzoylphenol urea insect growth regulators inhibit arthropod synthesis of chitin, a compound essential to exoskeleton integrity. The fact that chitin is synthesized by arthropods and fungi, but not plants, birds, or mammals, indicates that chitin biosynthesis inhibitors are highly selective plant protection products. However, given the specific mode-of-action, the efficacy and potency of this class of compounds may be highly dependent on life stage at time of exposure. Coppen and Jepson (1996) fed moulting second-instar *Schistocerca gregaria* (Orthoptera: Acrididae) nymphs barley buds treated with diflubenzuron, hexaflumuron or teflubenzuron. One-time dietary exposures of either 60 μg/insect, 30 μg/insect, or 0.25 μg/insect, respectively, resulted in delayed development and a significant increase in delayed mortality versus non-exposed insects in the four days following feeding. However, when these doses were divided into single daily feedings over either two or four consecutive

days, the developmental effects and delayed mortality were increased (Coppen and Jepson, 1996). The authors hypothesized that prolonging the time of exposure or the number of pulsed dietary exposures increases the likelihood that exposure to the chitin biosynthesis inhibitor coincides with a sensitive developmental stage.

Similarly, dietary exposures to Neem oil and other azadirachtin-containing compounds were demonstrated to cause reduced growth, decreased feeding rates, and increased latent mortality in two beneficial insect species. First instar *Episyrphus balteatus* larvae (Diptera: Syrphidae) fed on azadirachtin-treated aphids (Neem kernel water extract spray containing 3.7 to 30 mg a.s./L) for one day experienced nearly 100% mortality when reared to adulthood; an unspecified portion of the mortality response occurred during pupation (Ahmad, *et al.* 2003). A similar response was observed in first-instar *Coccinella septempunctata* (Coleoptera: Coccinellidae), with a single day of feeding on azadirachtin-treated aphids (NeemAzal-T/S spray of 40 mg a.s./L or Neem oil spray of 1.2 mg a.s./L) resulting in 100% larval mortality by the third instar. When second instar *C. septempunctata* larvae were fed exposed aphids over a two-day period, reduced developmental time (average reduction per formulation was 31% and 28%, respectively) versus controls was observed. For *Chrysoperla carnea* (Neuroptera: Chrysopidae) first instar larvae, developmental time was increased following two days of dietary exposure to azadirachtin-treated aphids (NeemAzal-T/S spray of 40 mg a.s/L or Neem oil spray of 1.2 mg a.s./L); larval developmental time was increased by an average of 2.2 days, while pupal developmental period was lengthened by an average of one day (Ahmad, *et al*. 2003).

Delayed effects are common in invertebrates exposed to biorational insecticides. Colorado potato beetle larvae (*Leptinotarsa decemlineata*, Coleoptera: Chrysomelidae) reared for 24 hours on leaves coated with 62 to 500 ppm *Bacillus thuringiensis* (BT) endotoxin solution experienced significant delayed mortality in the pupal stage (approximately 25 to 70%) (Costa, *et al.* 2000). More crucially, short-term dietary exposures to BT endotoxin (24hr) produced more significant long-term effects than chronic dietary exposures, even though the calcuated quantity of BT consumed was imilar for both acute and chronic exposures. Short-term

exposures extended developmental time in the prepupal, pupal, and adult stages, and altered the calculated population growth rate and total reproductive output (Costa, *et al.* 2000, $p<0.05$, ANOVA).

Similarly, brown lacewing (*M. tasmaniae*) larvae reared on surfaces contaminated with either 0.005 μg fenoxycarb/cm, 0.07 μg diflubenzuron/cm or 7.44 μg tebufenozide/cm exhibited significant responses to these insect growth regulators (Rumpf, *et al.* 1998). Fenoxycarb exposure reduced adult life spans to approximately 63% of control life span, lowered reproductive output per female by half. Oviposition period was significantly reduced following larval exposure to 0.007 μg/cm fenoxycarb ($p<0.001$, ANOVA). Larval diflubenzuron exposure resulted in a higher adult female/male ratio (64.9% females as compared to 53.0% females comprising control populations), increased pre-oviposition period by an average of 12 days, decreased oviposition period by an average of 10.8 days, and reduced the number of eggs produced by females by approximately 50% (Rumpf, *et al.* 1998). Interestingly, the most significant effect of tebufenozide was evident in offspring fitness. Whereas reproductive output was not significantly altered in *M. tasmaniae* exposed to tebufenozide as larvae, peak egg production in the second generation was reduced by approximately 30% when offspring were reared to adulthood.

Furthermore, short-term Neem-derived insecticide exposures designed to mimic field spray events were determined to cause significant long-term, population-level effects in the seven-spotted lady beetle *C. septempunctata.* Single spray exposures of 100 ppm Neemix inhibited *C. septempunctata* larval feeding, suppressed successful pupal development, and produced significant numbers of grossly deformed and moribund adults (Banken and Stark, 1998, $p<0.05$, ANOVA). Adults exposed to the same Neemix concentration displayed reduced oviposition, while exposure to 600 ppm Neemix resulted in a total inhibition of reproduction. In addition, Neem oil spray was demonstrated to prevent successful hatching of not only *C. septempunctata* eggs, but also the eggs of the green lacewing *C. carnea*, suggesting that Neem compounds may exert a non-specific toxicity to invertebrate eggs (Ahmad, *et al.* 2003). Soil applications of

Neem also inhibited *Diaeretiella rapae* (Hymenoptera: Brachonidae) parasitism rates of resident aphids and further reduced emergence of F1 adults from parasitized aphid mummies.

In summary, the same type of delayed effects of biorationals observed in exposed aquatic invertebrates were also observed in terrestrial invertebrates. Common long-term effects of exposure include abnormal development, altered sex ratios, reduced reproductive output, and decreased offspring fitness. There is also evidence that life stages generally considered resistant to conventional plant protection products, such as eggs, may be sensitive to biorational compounds. Interestingly, there is evidence that multiple pulses of biorationals can elicit greater effects than a single exposure of greater concentration, possibly because a multiple pulse exposure regime is more likely to coincide with a sensitive invertebrate developmental period.

2.3.4 Fungicides and Herbicides

Herbicide and fungicides are applied to control weeds and fungus species. However, repeated short-term pulsed exposures may cause long-term impacts on terrestrial invertebrate populations. In terrestrial systems, pulsed exposure to herbicides, for instance, may reduce available refugia for non-target invertebrates, and fungicide applications could alter the degradation of organic matter in soils. Additionally, as chitin synthesis inhibitors, fungicide exposures could result in both direct sub-lethal and lethal effects in terrestrial invertebrates if sufficient concentrations are applied (Stenersen, 2004).

Substrate applications of benomyl (a systemic fungicide) ranging from 15 to 15,000 µg a.s./g substrate had no apparent effect on saprotrophic *P. pruinosus* isopod metabolism or development (Vink, *et al.* 1995), providing little evidence of direct toxicity of benomyl to isopods. However, there is evidence that certain fungicides may produce a synergistic toxic effect in exposed invertebrates when applied as a part of a complex mixture.

Repeated pulsed exposure to the fungicides mancozeb or thiophanate-methyl in combination with either carbaryl or chlorpyrifos insecticides produced a greater than additive toxic effect as

gauged by the abundance of apple orchard predatory mites (*T. pyri*) (Cross and Berrie, 1996). There is additional evidence that multiple pulsed exposures to sulphur-based fungicides can alter *T. pyri* abundances. Six applications of sulphur 80WP at 0.8 percent concentration (350 L/ha spray rate) over a period of weeks were required to significantly alter abundance versus controls (Blumel, *et al.* 1997, $p<0.05$, ANOVA). This exposure regime was determined to significantly impact *T. pyri* abundances the following year as well, with application rates of only 0.4 percent (350 L/ha spray rate). Consequently, effects on terrestrial invertebrate communities were evident only following multiple pulsed exposures of fungicides. As with aquatic invertebrates, this suggests that effects observed following invertebrate exposure to fungicides are likely to occur through community-level shift, rather than direct toxicity to resident invertebrates.

2.4 Critical Appraisal

The literature reviewed indicates that non-target invertebrate populations may be impaired by pulsed exposures of plant protection products. The timing of the applications of these products, in an effort to control pest targets, is likely to result in a series of pulses entering nearby aquatic and terrestrial habitats. The ultimate impact of individual compounds on non-target invertebrate species will likely be determined by 1) pesticide mode-of-action, 2) potency and concentration of pulse, 3) chemical properties (e.g., rate of dissipation and breakdown), 4) frequency and duration of pulses and 5) species and life stage of invertebrates exposed. The research described within this review helps clarify the interaction between these variables.

A variety of long-term, population-level impacts were reported as responses to short-term pulsed exposure to plant protection products. Reproductive effects, including depressed reproductive output, reduced reproductive time, and disruption of behaviours essential to mating, were common responses, as was delayed or latent mortality (e.g. occurring some time after termination of exposure). In addition, impacts on normal growth, such as reduced dry weight, abnormal morphology (e.g., smaller wing size, altered defensive structures), and

increased incidence of deformity, also appeared to be general responses to pulsed exposures of certain chemical classes. Aquatic mesocosm studies also indicated a wide variety of community-level impacts following a short-term pulse of plant protection products, including long-term alterations of taxa composition, reduced abundance, and modified ecological function (as evidenced by algal blooms in certain cases). In many cases, only single pulses were required to elicit significant long-term impacts. In other situations, initial pulse exposures serve to sensitise exposed invertebrates to future exposure to plant protection products.

Organophosphates, in particular, appear to sensitise invertebrates with increasing number of pulsed exposures, and a series of pulse exposures may in some cases be more toxic than chronic exposures. This phenomenon is likely a result of the irreversible toxic mode-of-action on invertebrate acteylcholinesterase; thus, an additive response is expected. A single 24-hour exposure to 1.98 µg/L paraoxon-methyl resulted in 50% *D. magna* delayed mortality within the 14 days following the exposure, whereas a 3 hour pulsed exposure to 30 mg/L dimethoate reduced *D. magna* growth and delayed reproduction. Organophosphate exposure also reduced longevity of *T. rapae* parasitoids, potentially affecting reproductive output and control of aphid pests. Plus, short-term chlorfenvinphos exposure reduced *T. rapae* adult lifespan and reproductive output.

Interestingly, the same effects (reduced adult longevity and reproductive output) were observed in *B. germanica* cockroaches exposed to the carbamate; propoxur, and *M. tasmaniae* green lacewings exposed to the carbamate; fenoxycarb. Short-term fenoxycarb exposure also diminished *D. magna* reproductive output. This suggests that short-term exposure to AChE inhibitors (organophosphates and carbamates) is likely to reduce the lifespan and reproductive output of exposed invertebrates.

Exposure to both organochlorine and synthetic pyrethroid insecticide pulses was characterized by significant behavioural effects, which were demonstrated to result in diminished invertebrate communities, disrupted reproduction, and reduced feeding. Given the similarity of the modes-

of-action (disruption of sodium channel function in peripheral nervous system), it is unsurprising that similar invertebrate responses were observed (Soderlund and Bloomquist 1989). Short-term organochlorine (lindane) exposures were demonstrated to disrupt *G. pulex* reproduction, and also alter *C. riparus* adult morphology, emergence, and reproductive success. Pulsed endosulfan exposure resulted in the selective reduction of certain mayfly and caddisfly species abundance, and ultimately caused a series of algal blooms as a result of invertebrate community disruption. Most significantly, introduction of a short term (4 hour) methoxychlor pulse into a natural stream system resulted in massive catastrophic drift and severely reduced the standing populations of both collector-gatherer and shredder feeding guild invertebrates.

Both aquatic and terrestrial invertebrates experienced significant behavioural impacts following synthetic pyrethroid exposure. *G. pulex* pre-copulatory behaviour was disrupted by a 3 hour pulse of lambda-cyhalothrin with no evidence of recovery up to 15 days post-exposure. Foraging honeybees exposed to low levels of permethrin exhibited excessive self-cleaning behaviour, rotations, and trembling dance, and failed to locate hive following foraging. Exposure to low levels of deltamethrin induced hypothermic response behaviours in honeybees.

The literature reviewed herein also suggest that, population level-recovery can be prolonged following synthetic pyrethroid exposure. When a stably structured *D. magna* population was exposed to a short-term fenvalerate pulse, re-establishment of a stable population structure required six to seven generations and over three months of recovery time. Additionally, *C. dipterum* mayfly larvae exhibited significantly decreased ability to compete for limited food resources following a 1 hour esfenvalerate exposure, which ultimately resulted in reduced reproductive output.

Most significantly, pulsed exposures as short as 1 hour in duration were demonstrated to adversely affect adult caddisfly emergence and timing of emergence up to 200 days following exposure (as second instar larvae). An examination of the importance of pulse-duration (variable pulse durations with equivalent concentrations of fenvalerate) indicated that shorter

pulses of higher concentrations elicit significantly greater long-term effects than longer pulses of lower concentration fenvalerate. Given these findings, short-term pulses of synthetic pyrethroids appear to be very likely to elicit significant long-term effects in some aquatic and terrestrial invertebrate species.

The adverse effects of biorational insecticides, particularly insect growth regulators, are likely to be highly dependent on the timing of exposure in relation to the exposed invertebrate species' life cycles. For example, the negative effects of a single diflubenzuron pulse in a stream invertebrate abundance were not apparent until seasonal emergence commenced two to three weeks following the pulse. Similarly, locusts exposed to several low dietary pulses of either diflubenzuron, hexaflumuron, or teflubenzuron exhibited greater delayed mortality than those exposed to a single high dietary dose of the IGRs. This suggests that multiple pulses of IGRs may exert greater effects than a single high concentration pulse, since a succession of several exposures is more liable to coincide with their sensitive developmental stages, thereby resulting in the targeted effect. In addition, delayed effects are commonly observed in invertebrates exposed to biorational insecticides, including abnormal development, altered sex ratios, reduced reproductive output, and decreased offspring fitness. Larval *L. decemlineata* (Colorado potato beetle) exposed to *Bacillus thuringiensis* experienced up to 70% pupal mortality, and Neem exposure similarly suppressed pupal development in *C. septempunctata*, producing deformed pupae and adults.

Although significant, it is unclear how depressed reproductive output, delayed mortality, and disrupted behavioural homeostasis would affect long-term population viability. In fact, population-level recovery from long-term effects will likely be species-specific, dependant on life history strategies, including generation time, life span, reproductive output and other similar variables. Also, species-specific complex behaviours, metamorphic processes, and specialised morphologies may alter both the propensity for exposure and the magnitude of long-term effects. Therefore, it is difficult to judge the length of time required for the recovery of all impacted species. However, if pulsed exposures are repeated annually, as is the case with many

plant protection products, certain sensitive species could exhibit permanently depressed populations.

Ultimately, life stage at exposure and the life cycle of exposed invertebrates is likely to greatly influence the effects of plant protection products; for example, pulsed exposures that coincide with sensitive invertebrate life stages will result in more significant population effects, which in turn results in protracted recovery. Hence, understanding the relationship between the timing of pulsed exposures and resident invertebrate life cycles is critical. In addition, particular species-specific life history strategies may increase susceptibility to plant protection products, either by enhancing exposure or effects. Sometimes these strategies are linked to specific life stages. Consequently, the complex interaction between duration and magnitude of pesticide pulse, invertebrate life stage at time of exposure, and the vulnerability of life history strategies influences both the propensity for long-term, population-level effects and the ability to recover from these.

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Chapter 3: Overview Tables

3.1 Version 1 (in order of environmental compartment, followed by pesticide class and name)

Environ-mental Compartment	Pesticide class	Pesticide name	Species tested	Life stage tested	Number and frequency of pulses	Detected effects	Potential long-term effects	Reference
Aquatic	Organochlorine	Endosulfan	Macro-invertebrate assemblages	Stable population structures	Single static exposures of 12hr or 24hr, followed by initiation of flow	Certain caddisfly and mayfly species abundances were reduced at 12 hr of 50 ppb or 48hr of 5-25 ppb ($p<0.05$, CANOCO). Recovery was noted, preceded by algal blooms	Possible temporary alteration of community function	Hose, et al 2003
Aquatic	Organochlorine	Lindane	*D. magna*	<24hr old neonates	Single 2hr, 6hr or 24hr pulse exposure	Reduced clearance rate at all exposures to 241 ppb ($p<0.05$, ANOVA)	Fast recovery, no foreseeable long-term impacts	Hartgers, et al, 1999
Aquatic	Organochlorine	Lindane	*C. riparius*	Larvae	Single 48hr exposure to contaminated sediment	Male wing length and adult emergence reduced at >0.5 ppm lindane; reduced ovipositioning >0.75 ppm ($p<0.05$, ANOVA)	Possible reduction in reproductive output	Hirthe, et al , 2000
Aquatic	Organochlorine	Lindane	*G. pulex*	Pre-copulatory	2-20min single pulse exposure	10 and 20min pulses of 1 and 2 ppm significantly inhibited pre-copula behavior ($p<0.05$, Mann-Whitney test)	Interruption of pre-copula caused increased male cannibalism on females (altered sex abundances and potentially decreased reproductive output)	Malbouisson, et al, 1994

Environ-mental Compartment	Pesticide class	Pesticide name	Species tested	Life stage tested	Number and frequency of pulses	Detected effects	Potential long-term effects	Reference
Aquatic	Organochlorine	Methoxychlor	Stream macro-invertebrates	Stable population structures	Single 4h flow though pulse exposure	Exposure to 10ppm caused massive drift, biomass loss, and significant reduction in standing macroinvertebrate abundances	Significant alteration in invertebrate community structure and function (losses of certain functional feeding groups)	Wallace, et al, 1989
Aquatic	Organophosphate	Azamethiphos	*Homarus americanus*	Female adults	3-4 1hr pulses	Spring time exposure to pulses of 10 ppb caused significant disorientation, paralysis, mortality and decreased spawning ($p<0.05$, z-test or $\chi 2$ analysis)	Reduced reproductive output	Burridge, et al, 2008
Aquatic	Organophosphate	Azamethiphos	*Mytilus edulis*	Adult	Single 1hr or 24hr pulse	Exposure to 100 ppb for 24hr significantly reduced phagocytic activity ($p<0.05$, ANOVA)	Unclear, possible increased disease susceptibility	Canty, et al, 2007
Aquatic	Organophosphate	Chlorpyrifos	*Chironomus riparius*	Larvae	48hr pulsed sediment exposure	Exposure to 0.1 ppm caused reduced burrowing behavior, decreased male emergence, and reduced adult female weight. AChE was significantly inhibited by exposure to 0.01 ppm ($p<0.05$, ANOVA, or $\chi 2$ analysis)	Potentially reduced reproductive output.	Callaghan, et al, 2001

Environ-mental Compartment	Pesticide class	Pesticide name	Species tested	Life stage tested	Number and frequency of pulses	Detected effects	Potential long-term effects	Reference
Aquatic	Organophosphate	Chlorpyrifos	*D. magna*	<24hr old neonate	Single pulse exposure; double pulse exposures with intervening recovery time	Survival was the most sensitive endpoint with significant decreases occurring following pulses of 0.5ppb of 12 hrs or less ($p< 0.05$, ANOVA)	Increased recovery time mediated effects of chlorpyrifos	Naddy, et al, 2000
Aquatic	Organophosphate	Chlorpyrifos	Mesocosm invertebrates	Stable population structure	Single 6hr flow-through pulse exposure	6hr exposure to 5ppb reduced abundances of *Ferrissia* sp., *Nanocladius* sp., *Cladotanytarsus bilinearis*, and an unidentified nematode; *Procladius paludicola* abundance increased ($p<0.05$, ANOVA)	Recovery was evident by day 21, but resilience of invertebrate community would depend on richness and timing of exposure relative to reproductive period.	Pusey, et al 1994
Aquatic	Organophosphate	Paraoxon-methyl	*D. magna 0*	<24hr old neonates	Single 1hr or 24hr exposure	Single 1hr exposure to 100 or 1000 ppb significantly reduced 21d reproduction; 24hr exposures of $\geq$ 1.5 ppb reduced reproduction at 14d but not 24d ($p<0.01$, ANOVA)	Decreased reproductive output	Duquesne, et al, 2006
Aquatic	Carbamate	Carbaryl	*Calineuria californica Cinygma* sp.	Nymph	Single pulse exposures of 15-60 min	96hr LC_{50} values were similar for the two species; however, *Cinygma* sp were 1000x more sensitive than *C. californica* when exposed for 1hr or less (SPSS probit analysis).	Physiology governing early exposure uptake and elimination may explain inter-species differences in sensitivity.	Peterson, et al, 2001

Environ-mental Compartment	Pesticide class	Pesticide name	Species tested	Life stage tested	Number and frequency of pulses	Detected effects	Potential long-term effects	Reference
Aquatic	Carbamate	Carbaryl	*Daphnia ambigua*	Egg, embryo first, second and third instars	Single 10hr pulse	Significant delayed mortality in egg, 1st, and 2nd instars exposed to 0.05ppb; growth suppression observed at all life stages at 0.5 ppb ($p<0.05$, ANOVA)	Recovery of weight; unclear long-term effects	Hanazato, 1991
Aquatic	Carbamate	Carbaryl	*Daphnia pulex, Daphnia galeata, Daphnia mendotae, Daphnia retrocurva* and *Daphnia lumholtzi*	Gravid females	Single 8hr to 14hr pulse exposure	Longer neckteeth in *D. pulex* exposed to ≥ 15 ppb; high helmet production in *D. galeata* exposed to 5-10 ppb; reduced growth and high helmets in *D. retrocurva* exposed to ≥ 20 ppb; long tailspines in *D. lumholtzi* exposed to ≥10 ppb ($p<0.05$, ANOVA)	Unnecessary defensive growth may negatively affect energy budget	Hanazato and Dodson, 1993
Aquatic	Carbamate	Carbaryl	*Gammarus pulex*	Adult	Four 48h pulses on day 0, 5, 10, 15; Three 48hr pulses on day 0, 10 15; Two 48hr pulses on day 0 and 15	As measured at day 20, pulses of 0.5 ppb significantly increased latent mortality (versus control and chronic 0.05 ppb exposure). Increasing number of pulses caused a steeper cumulative mortality curve. ($p<0.05$)	Unclear, but greater effects observed following pulsed vs. chronic exposure	Ashauer, et al, 2007a

Environ-mental Compartment	Pesticide class	Pesticide name	Species tested	Life stage tested	Number and frequency of pulses	Detected effects	Potential long-term effects	Reference
Aquatic	Organophosphate, and carbamate	Aldicarb, carbaryl, carbofuran, malathion, parathion andpropoxur	*C. riparius*	4th instar	Either single 2hr exposure, or two 1hr exposures with intervening recovery time	Malathion and parathion exhibited no significant reversal of effect with 24 hr recovery time; carbamate recovery occurred with >2hr recovery time ($p<0.05$, Tukey's Student test)	Unclear	Kallander, et al, 1997
Aquatic	Organophosphate and carbamate	Chlorpyrifos and carbaryl	*G. pulex*	Adult	1) Single 24hr pulse of carbaryl, 14d depuration, single 24hr pulse of chlorpyrifos 2)Single 24hr pulse of chlorpyrifos, 14d depuration, single 24hr pulse of carbaryl	Carbaryl was more toxic to *G. pulex* when preceded by exposure to chlorpyrifos. Mortality in treatment 1 = 45%, mortality in treatment 2 = 60% (binomial test for two proportions).	*G.pulex* populations that suvive exposures to organophosphates may be more sensitive to future AChE exposures	Ashauer et al, 2007b
Aquatic	Organophosphate and carbamate	Dimethoate and pirimicarb	*Daphnia magna*	24hr and 3-day old	Single pulses or double-pulses separated by 48h	Exposure to 30 ppm dimethoate or 100ppm pirimicarb resulted in reduced growth, delayed time to first reproduction, and decreased reproductive output ($p<0.05$, t-test)	Limited reproduction window, fewer offspring	Andersen, et al, 2006

Environ-mental Compartment	Pesticide class	Pesticide name	Species tested	Life stage tested	Number and frequency of pulses	Detected effects	Potential long-term effects	Reference
Aquatic	Synthetic pyrethroid	Cypermethrin	*Acartia tonsa*	Adult	Single 1hr or 24hr pulse exposures	1hr exposure to 0.7-2.2 ppb and 24hr exposure to 0.2-0.8ppb significantly decreased survival 144h following exposure ($p<0.05$, ANOVA)	Significant delayed toxicity and the particular susceptibility of males suggest that populations structure may be significantly altered	Medina, et al, 2004
Aquatic	Synthetic pyrethroid	Cypermethrin	*Trichodactylus borellianus*	Juvenile and Adult	Single 3hr pulse exposure	Exposure to ≥0.001 ppb cypermethrin cause significant depression in oxygen consumption; ammonia excretion significantly increased	Alteration in metabolic efficiency; long-term effects unclear	Veronica and Collins, 2003
Aquatic	Synthetic pyrethroid	Esfenvalerate	*Cloeon dipterum*	3^{rd}-4^{th} instar	Single 1hr pulse	29d survival reduced following exposure to 0.1 ppb; low food availability increased morality ($p<0.05$, ANOVA)	Decreased reproductive output; resource competition altered sensitivity to pesticide	Beketov and Liess, 2005
Aquatic	Synthetic pyrethroid	Esfenvalerate	*D. magna*, *plus* various mesocosm invertebrate species	N/A	15min pulse flow-through exposure every two weeks for 3 months; laboratory determination of 48hr *D. magna* LC_{50}	Reduced mesocosm macroinvertebrate abundance following exposures ≥ 0.25 ppb ($p<0.05$ NPANOVA, LSD); *D. magna* 48hr LC50 = 0.27 ppb	May significantly alter aquatic community structure; *D. magna* sensitivity may not be predictive of aquatic communities' sensitivities	Fairchild,et al, 1992

Environ-mental Compartment	Pesticide class	Pesticide name	Species tested	Life stage tested	Number and frequency of pulses	Detected effects	Potential long-term effects	Reference
Aquatic	Synthetic pyrethroid	Esfenvalerate	*G. pulex*	Juvenile Adult	Single 1hr pulse	Exposure to ≥ 0.1 ppb disrupted mating pairs and increased mortality. Repairing required 6x longer when exposed, and resulted in fewer offspring ($p<0.05$, Kaplan-Meier or Kruskal-Wallis analysis)	Decreased reproductive output	Cold and Forbes, 2004
Aquatic	Synthetic pyrethroid	Esfenvalerate	Mesocosm invertebrates	Stable population structure	Two pulse applications (one per month), followed by dissipation	Multiple species abundances were reduced by exposures as low as 0.08 ppb ($p<0.05$, ANOVA); *H. azteca* did not recover	Only Chironomid species showed signs of recovery 70 d after second exposure; significant long-term impacts on community structure	Lozano, et al, 1992
Aquatic	Synthetic pyrethroid	Fenvalerate	*D. magna*	Stable population structure	Single 48hr pulse	Significantly reduced abundances at 1ppb and greater ($p<0.05$, ANOVA); older age classes required significantly more recovery time	Recovery from a single pulse exposure required 60 days (as measured by population structure recovery)	Liess, et al, 2006
Aquatic	Synthetic pyrethroid	Fenvalerate	*D. magna*	Modeled population	Data from single 24hr pulse exposures	Model results suggest that sublethal fenvalerate exposure affects food acquisition and assimilation leading to reduced growth	Decreased growth could result in higher disease susceptibility, delayed reproduction, and lowered reproductive output	Pieters, et al, 2006

Environ-mental Compartment	Pesticide class	Pesticide name	Species tested	Life stage tested	Number and frequency of pulses	Detected effects	Potential long-term effects	Reference
Aquatic	Synthetic pyrethroid	Fenvalerate	*D. magna*	<24hr old neonates from either high or low maternal food stock	Single 24hr pulse exposures	Female *D. magna* provided with high food stock produced smaller offspring more sensitive to fenvalerate (significant mortality at 0.6 ppb and greater); low food stock females produced larger, more resilient offspring (significant mortality at 3.2 ppb and greater); $p<0.05$, Gehan-Wilcoxon survival analysis	Environmental conditions preceding pesticide exposure can affect individuals' sensitivities.	Pieters and Liess, 2006
Aquatic	Synthetic pyrethroid	Fenvalerate	*D. magna*	<24hr old neonates	Single 24hr pulse exposure with low or high food availability	Low food *D. magna* LOEC = 0.6 ppb; high food *D. magna* LOEC = 3.2 ppb ($p<0.01$, Gehan-Wilcoxon survival analysis). Low food + 0.3 ppb exposure and greater caused significantly reduced growth ($p<0.001$, ANOVA)	Differences in *D. magna* size recovered after two weeks; however, fenvalerate alone was demonstrated to reduce reproduction.	Pieters, et al 2005
Aquatic	Synthetic pyrethroid	Fenvalerate	*D. magna*	<24hr old neonates	Single 24hr pulse exposure	Filtering rate, 15N assimilation and growth were all reduced following exposures of 0.3 ppb and greater ($p<0.05$, ANOVA with Dunnett's test); only filtering rates recovered 2d post-exposure	Reduced growth led to significantly greater time to first reproduction, and lowered reproductive output.	Reynaldi, et al, 2006

Environ-mental Compartment	Pesticide class	Pesticide name	Species tested	Life stage tested	Number and frequency of pulses	Detected effects	Potential long-term effects	Reference
Aquatic	Synthetic pyrethroid	Fenvalerate	*L. lunatus*	2^{nd}-3^{rd} instar	Single 1hr pulse exposure to solubilized fenvalerate or particulate-bound fenvalerate	Sediment-bound fenvalerate is less toxic to *L. lunatus* (possibly due to decreased bioavailability). Exposures of 0.1 µg/L and greater reduced emergence success, and exposures of ≥0.001 µg/L or ≥ 2 µg/kg altered emergence phenology ($p< 0.05$, ANOVA, Fisher's PLSD)	Long-term impacts on caddisfly fitness are likely to occur after fenvalerate exposure. However, the length of time required for manifestation of effects is so long that linking exposure to effects would be near-impossible in the field.	Schultz and Liess, 2001b
Aquatic	Synthetic pyrethroid	Fenvalerate	*Limnephilus lunatus*	2^{nd}-3^{rd} instar	Single 1hr or 10hr pulse exposures (concentrations converted to µg/hr to determine importance of pulse duration)	Exposure to 0.1 µg/hr caused decreased emergence ($p<0.05$, ANOVA, Fisher's PLSD); timing of emergence and adult weight was altered at 0.001 µg/hr for 1hr exposures, but not for 10hr exposures	Effects occurred 81-231 days post-exposure, signifying significant long-term effects. Shorter pulses affected greater impact than longer pulses when equivalent doses were compared.	Schultz and Liess, 2000

Environ-mental Compartment	Pesticide class	Pesticide name	Species tested	Life stage tested	Number and frequency of pulses	Detected effects	Potential long-term effects	Reference
Aquatic	Synthetic pyrethroid	Fenvalerate	Mesocosm invertebrates	Stable population structure	Single 1hr pulse exposure to sediment-bound fenvalerate	Exposure to ≥ 13.6 µg/kg increased *G. pulex* and *H. angustipennis* drift; *Anabolia nervosa*, *Plectrocnemia conspersa*, *L. lunatus*, and *Tipula maxima* drift significantly increased following exposures of ≥ 136.5 µg/kg. *A. nervosa* emergence was delayed at 1365 µg/kg, and was *G. pulex* and *T. maxima* abundances ($p<0.05$, ANOVA)	Sediment-bound fenvalerate adversely impacted emergence up to 3 months after cessation of exposure.	Schulz and Liess, 2001a
Aquatic	Synthetic pyrethroid	Lambda-cyhalothrin	*Baetis rhodani* *Leuctra fusca* *Leuctra digitata* *G. pulex*	Unclear; similar sizes	Single flow-through pulse exposure of 1hr	≥ 0.01ppb significantly increased three species drift ($p<0.05$, Mann-Whitney test)	Authors surmise that annual pyrethroid pulses could shift community structure to more resilient species	Lauridsen and Friberg, 2005
Aquatic	Synthetic pyrethroid	Lambda-cyhalothrin	*G. pulex*	Adult	Single flow-through mesocosm pulse	Precopulatory behavior reduced at concentrations >0.35 ppb; significant mortality at >0.05 ppb ($p<0.05$, ANOVA)	Possible reduction in reproductive output	Heckmann, et al, 2005

Environ-mental Compartment	Pesticide class	Pesticide name	Species tested	Life stage tested	Number and frequency of pulses	Detected effects	Potential long-term effects	Reference
Aquatic	Organophosphate, carbamate and synthetic pyrethroid	Fenitrothion, carbofuran, carbaryl, and permethrin	*Aedes aegypti*	3rd instar	Two 1hr pulses separated by 6h recovery time or single 2h pulse	The inclusion of recovery time increased microencapsulated permethrin toxicity as measured as survival to adult stage ($p<0.05$, t-test); recovery time had no effect on the effect of other compounds	6hr recovery time insufficient to reverse effects; multiple pulses in short time period may reduce survival to adult.	Parsons and Surgeoner, 1991
Aquatic	Metal	Arsenic	*D. magna*	<24hr old neonates	Single pulses ranging from 3 to 120 hrs in duration; double pulses with variable intervening recovery times	Recovery times had no effect, and may have exacerbated effects. Time to first reproduction was significantly lengthened by 6-9hr exposures of 6000 ppb, 24hr exposures of 4000-5000 ppb, and 120hr exposures of 3000ppb ($p<0.05$)	Potentially fewer offspring due to restriction of reproductive period.	Hoang, et al, 2007
Aquatic	Metal	Selenium	*D. magna*	<24hr old neonates	Single exposures of 4-24hr in duration; double exposures of 3-12hr in duration with intervening recovery times of 72hr to 288hr	Increased exposure concentration and duration increased cumulative 21-day mortality. Significant cumulative mortality occurred at 12hr of 800 ppb, 9hrs of 1200 ppb, 6hr of 1600 or 2000 ppb ($p<0.05$, F-test)	Potentially altered population structure or decreased abundance	Hoang and Klaine, 2008

Environ-mental Compartment	Pesticide class	Pesticide name	Species tested	Life stage tested	Number and frequency of pulses	Detected effects	Potential long-term effects	Reference
Aquatic	Biorational	Azadirachtin (neem)	Macroinvertebrate assemblages	Stable population structures	Single flow-through pulse exposure of 5h	0.84 mg/L caused significantly increased *Isogenoides* sp. drift and *Isonychia* sp. and *Hydropysche* sp. mortality ($p<0.05$, G-test)	Authors conclude no long-term effects on mesocosm community	Kreutz-weiser, et al, 1999
Aquatic	Biorational	Fenoxycarb	*D. magna*	<24hr old neonate	Single exposures reduced 50% every 10hrs (to mimic natural degradation)	50 ppb exposure (initial concentration) resulted in significantly reduced reproductive output ($p<0.05$, ANOVA)	Possible reduction in number of viable offspring	Hosmer, et al, 1998
Aquatic	Biorational	Imidacloprid	*C. tentans* *H. azteca*	~8-9d old *C. tentans* ~2-9d old *H. azteca*	Single 96h pulse exposures	Pulse exposures were less toxic than chronic exposures to *C. tentans.* Reduction in *H. azteca* growth rate (LOEC = 3.53 ppb) was a more sensitive endpoint than mortality (NOEC = 11.93 ppb)	Unclear	Stoughton, et al, 2008
Aquatic	Biorational	Margosan-O (Neem product)	*D. magna*	<48hr old	Repeated pulse exposures of 4h or 12h with 4d or 7d recovery time for a total of 28d	4d recovery time was insufficient to reverse effects at either pulse duration, whereas recovery time of 7d reduced mortality at both pulse durations.	Unclear	Scott and Kaushik 1998
Aquatic	Biorational	Methoprene	*H. americanus*	Larval	Single 4hr pulse exposure	Exposure to 50ppb resulted in significant accumulation in eyestalk, epithelial, and gonadal tissues	Unclear; may result in future tissue-specific damage.	Walker, et al, 2005

Environ-mental Compartment	Pesticide class	Pesticide name	Species tested	Life stage tested	Number and frequency of pulses	Detected effects	Potential long-term effects	Reference
Aquatic	Herbicide	Hexazinone	Mesocosm invertebrates	Stable population structure	Single 24hr flow-through pulse	No significant effects on abundance, drift, community composition, or size of individual invertebrates.	No evidence of long-term effects.	Schneider, et al, 1995
Aquatic	Herbicide	Linuron	Mesocosm invertebrates	Stable population structures	Static 7d pulse (with degradation), followed by flowing conditions	*Keratella quadrata* abundances significantly but briefly reduced ($p<0.05$, Monte Carlo permutation); macroinvertebrate taxa richness was slightly reduced at 50 ppb.	No long-term impact observed by authors.	Van Geest, et al, 1999
Aquatic	Algaecide	BULAB 6002	*Dreissena polymorpha*	Adult	Single pulses of 48hr-96hr in duration at 12°C or 22°C	Survival at 240hr after exposure was significantly decreased only at 22°C at exposures ≥ 4 ppm	Warm temperatures increased *D. polymorpha* sensitivity to BULAB 6002 ($p<0.05$, ANOVA)	Martin, et al, 1993
Aquatic	Multiple (review article)	Multiple (review article)	Aquatic invertebrates	Various	Time varied pulse exposures	Doubling exposure time decreased survival by a factor of 1.4 to 8.4 (*C. riparius* and *Hydropsyche angustipennis*); Effects of insect growth regulator (IGR) may be delayed until molt/emergence (weeks)	Variable	Reinert, et al 2002

Environ-mental Compartment	Pesticide class	Pesticide name	Species tested	Life stage tested	Number and frequency of pulses	Detected effects	Potential long-term effects	Reference
Terrestrial	Organophosphate	Chlorfenvinphos	*Trybliographa rapae*	Adult	Single topical application	Number of eggs reduced 20d after exposure to 0.5 µl/insect. Reduced adult longevity, reduced pairing also observed ($p< 0.05$, t-test)	Decreased reproductive output	Alix, et al, 2001
Terrestrial	Synthetic pyrethroid	Lambda-cyhalothrin	*Phyllonorycter blancardella Pholetesor omigis* Chalcididae	Eggs and larvae of *P. blancardella*, and adult *P.omigis* and Chalcididae	Three single spray applications, one/month during summer	*P. blancardella* eggs and larvae were significantly reduced by 2.5 g a.s./ha and greater ($p<0.05$, Scheffe's F test). *P.omigis* and Chalcididae abundances were also significantly reduced	Unclear whether parasitoids decreased because of direct toxicity or insecticide-mediated community alterations.	Li, et al, 1991
Terrestrial	Synthetic pyrethroid	Permethrin	*Apis mellifera*	Adult	Single topical application	Exposure to 0.001 µg caused significantly increased rotating, trembling, and cleaning behaviors, and reduced foraging. Exposure to 0.009 µg caused disorientations and prevented all exposed bees from returning to hive ($p<0.05$, t-test)	High worker bee mortality possibly resulting in hive debilitation	Cox and Wilson, 1984

Environ-mental Compartment	Pesticide class	Pesticide name	Species tested	Life stage tested	Number and frequency of pulses	Detected effects	Potential long-term effects	Reference
Terrestrial	Organophosphate, and synthetic pyrethroid	Dimethoate, deltamethrin, and lambda-cyhalothrin	*Aphidius uzbekistanicus*	Early and late instar larvae (in aphids); adults	Single spray applications of 6.25 g a.s./ha lambda-cyhalothrin, 7.5 g a.s./ha deltamethrin, or 400 g a.s./ha dimethoate	100% percent host aphid and early instar mortality after all exposuresDeltamethrin and dimethoate significantly reduced larval emergence and male longevity; female longevity significantly reduced at all exposures ($p<0.05$, t-test)	Possible reduction in reproductive output resulting from reduced adult lifespan	Krespi, et al, 1991
Terrestrial	Synthetic pyrethroid, organophosphate, carbamate,and AChE inhibitor	Lambda-cyhalothrin, chlorpyrifos, pirimicarb and triazamate	*Aphidius ervi*	Adults	Single 48hr pulse exposure to contaminated surface	Highest triazamate exposure (18.75 ng/cm) significantly disrupted aphid odour detection ($p<0.001$, Kolmogorov–Smirnov test)	May lower reproductive output through decreased host detection	Desneux, et al, 2004
Terrestrial	Organophosphate and synthetic pyrethroid	Propoxur and deltamethrin	*Blattella germanica*	Adult	Single topical application (LD_{10}-LD_{50}, only survivors studied)	LC_{30} and greater significantly reduced adult longevity, ootheca production and viability ($p<0.05$ ANOVA)	Both maternal and paternal exposure led to reduced reproductive output	Lee, et al, 1998
Terrestrial	Biorational	BT endotoxin	*Leptinotarsa decemlineata*	Larvae	Single 24hr dietary exposure	Single feeding with diet contaminated with 62 ppm BT caused significant latent mortality in later larval and pupal stages. Weight reduced at dietary exposure to 31 ppm. Acute exposure more toxic than chronic ($p<0.05$, ANOVA, or Scheffe's test)	Altered population dynamics (decreased population growth rate and reduced net reproductive output)	Costa, et al, 2000

Environ-mental Compartment	Pesticide class	Pesticide name	Species tested	Life stage tested	Number and frequency of pulses	Detected effects	Potential long-term effects	Reference
Terrestrial	Biorational	Neem	*C. septempunctata and Acyrthosiphon pisum*	Adult	Single spray exposure	100 ppm Neem exposure significantly reduced *A. pisum* population growth rate; also reduced *C. septempunctata* ovipositioning, larval feeding, pupation ($p<0.05$, t-test LSD)	Altered population dynamics	Banken and Stark, 1998
Terrestrial	Biorational	Neem compounds: neem oil (NO), neem kernel, NeemAzal (NA)	*Coccinella septempunctata, Chrysoperla carnea, Episyrphus balteatus*	Egg and larval life stages	Single one-day dietary pulse (aphids); single spray on eggs	*C. septempunctata:* significantly altered larval development time, increased pupal time, adult deformities (NO=1.2 ppm a.s.; NA= 40 ppm) *C. carnea:* same concentrations increased larval/pupal development time, reduced adult longevity and increased adult deformities. *E. balteatus:* same concentrations as soil and foliar applications increased mortality ($p<0.05$, ANOVA)	Delayed larval and pupal mortality; pupal deformities	Ahmad, et al, 2003

Environ-mental Compartment	Pesticide class	Pesticide name	Species tested	Life stage tested	Number and frequency of pulses	Detected effects	Potential long-term effects	Reference
Terrestrial	Organophosphate, synthetic pyrethroid and biorational	Methyl-parathion, azinphos-methyl, cypermethrin, fenoxycarb, diflubenzuron and tebufenozide	*Micromus tasmaniae*	Larvae	Reared on surfaces sprayed with emergence LC_{50}s (e.g. concentration that results in 50% reduction in emergence)	Diflubenzuron: reduced male:female ratio, female longevity, reproductive output. Fenoxycarb: reduced female longevity and reproductive output. Tebufenozide exposure altered offspring reproductive output ($p<0.05$, ANOVA)	Effects of insect growth regulators can occur weeks following exposure, and can alter offspring as well as parental reproductive fitness	Rumpf, et al, 1998
Terrestrial	Fungicide	Sulphur compounds	*Typhlodromus pyri*	Adults	6 to 10 spray applications during two summers, separated by 7-10d	Six spray applications of 800 ppm fungicide required for significant reduction; 10 spray pulses of 400 ppm showed significant reductions at all concentrations ($p<0.05$, ANOVA)	Altered population dynamics	Blumel, et al, 1997
Terrestrial	Organophosphate, carbamate and fungicide	Chlorpyrifos, carbaryl, mancozeb and thiophanate-methyl	*T. pyri*	Adult	Repeated field sprays every three weeks May-August comprised of single or mixture of compounds.	3600 g a.s./ha mancozeb significantly reduced *T. pyri* numbers, and mancozeb+chlorpyrifos (960g a.s./ha) and thiophanate-methyl (1100 g a.s./ha) +chlorpyrifos treatments exhibit synergistic action. Host populations increased as *T. pyri* abundances decreased. ($p<0.05$, ANOVA)	Disruption of parasitoid-host population balance	Cross and Berrie, 1996

Environ-mental Compartment	Pesticide class	Pesticide name	Species tested	Life stage tested	Number and frequency of pulses	Detected effects	Potential long-term effects	Reference
Terrestrial	Synthetic pyrethroid and fungicide	Deltamethrin, prochloraz and difenoconazole	*A. mellifera*	Adult	Single topical exposure	Both deltamethrin and fungicide concentrations ~12-15x lower than application rate induced hypothermic reactions in bees (2.5 ng/bee and 1250 ng/bee). $p<0.05$, Pearson correlation	Disruption of behavioural homeostasis may leave worker bees disoriented, causing reduced numbers of foragers	Vandame and Belzunces, 1998
Terrestrial	Organophosphate and fungicide	Diazinon and benomyl	*Porcellionides pruinosus*	Adult	Single substrate application	At diazinon substrate concentrations ≥ 0.5 µg/g, glycogen content fell to zero; at ≥ 1.1 µg/g protein content was significantly lower; at $s \geq 0.24$ µg/g, lipid content was significantly lowered 6 weeks following exposure. ($p < 0.05$ Spearman correlation)	Alteration in metabolic efficiency; long-term effects unclear	Vink, et al, 1995
Terrestrial	Multiple (review article)	Multiple (review article)	Beneficial arthropod species	Multiple	Generally topical application	Biochemical, developmental, longevity and immunology	Unclear	Desneux, et al, 2007

3.2 Version 2 (in order of environmental compartment, followed by frequency of pulses)

Environ-mental Compartment	Number and frequency of pulses	Pesticide class	Pesticide name	Species tested	Life stage tested	Detected effects	Potential long-term effects	Reference
Aquatic	2-20 min single pulse exposure	Organochlorine	Lindane	*G. pulex*	Pre-copulatory	10 and 20min pulses of 1 and 2 ppm significantly inhibited pre-copula behavior ($p<0.05$, Mann-Whitney test)	Interruption of pre-copula caused increased male cannibalism on females (altered sex abundances and potentially decreased reproductive output)	Malbouisson, et al, 1994
Aquatic	Single pulse exposures of 15-60 min	Carbamate	Carbaryl	*Calineuria californica* *Cinygma* sp.	Nymph	96hr LC_{50} values were similar for the two species; however, *Cinygma* sp were 1000x more sensitive than *C. californica* when exposed for 1hr or less (SPSS probit analysis).	Physiology governing early exposure uptake and elimination may explain inter-species differences in sensitivity.	Peterson, et al, 2001
Aquatic	Single 1hr pulse	Synthetic pyrethroid	Esfenvalerate	*Cloeon dipterum*	3^{rd}-4^{th} instar	29d survival reduced following exposure to 0.1 ppb; low food availability increased morality ($p<0.05$, ANOVA)	Decreased reproductive output; resource competition altered sensitivity to pesticide	Beketov and Liess, 2005
Aquatic	Single 1hr pulse	Synthetic pyrethroid	Esfenvalerate	*G. pulex*	Juvenile Adult	Exposure to ≥ 0.1 ppb disrupted mating pairs and increased mortality. Repairing required 6x longer when exposed, and resulted in fewer offspring ($p<0.05$, Kaplan-Meier or Kruskal-Wallis analysis)	Decreased reproductive output	Cold and Forbes, 2004

Environ-mental Compartment	Number and frequency of pulses	Pesticide class	Pesticide name	Species tested	Life stage tested	Detected effects	Potential long-term effects	Reference
Aquatic	Single 1hr pulse exposure to solubilized fenvalerate or particulate-bound fenvalerate	Synthetic pyrethroid	Fenvalerate	*L. lunatus*	2^{nd}-3^{rd} instar	Sediment-bound fenvalerate is less toxic to *L. lunatus* (possibly due to decreased bioavailability). Exposures of 0.1 µg/L and greater reduced emergence success, and exposures of ≥0.001 µg/L or ≥ 2 µg/kg altered emergence phenology ($p < 0.05$, ANOVA, Fisher's PLSD)	Long-term impacts on caddisfly fitness are likely to occur after fenvalerate exposure. However, the length of time required for manifestation of effects is so long that linking exposure to effects would be near-impossible in the field.	Schultz and Liess, 2001b
Aquatic	Single 1hr pulse exposure to sediment-bound fenvalerate	Synthetic pyrethroid	Fenvalerate	Mesocosm invertebrates	Stable population structure	Exposure to ≥ 13.6 µg/kg increased *G. pulex* and *H. angustipennis* drift; *Anabolia nervosa*, *Plectrocnemia conspersa*, *L. lunatus*, and *Tipula maxima* drift significantly increased following exposures of ≥ 136.5 µg/kg. *A. nervosa* emergence was delayed at 1365 µg/kg, and was *G. pulex* and *T. maxima* abundances ($p<0.05$, ANOVA)	Sediment-bound fenvalerate adversely impacted emergence up to 3 months after cessation of exposure.	Schulz and Liess, 2001a

Environ-mental Compartment	Number and frequency of pulses	Pesticide class	Pesticide name	Species tested	Life stage tested	Detected effects	Potential long-term effects	Reference
Aquatic	Single flow-through pulse exposure of 1hr	Synthetic pyrethroid	Lambda-cyhalothrin	*Baetis rhodani* *Leuctra fusca* *Leuctra digitata* *G. pulex*	Unclear; similar sizes	$\geq$ 0.01ppb significantly increased three species drift ($p<0.05$, Mann-Whitney test)	Authors surmise that annual pyrethroid pulses could shift community structure to more resilient species	Lauridsen and Friberg, 2005
Aquatic	Single 3hr pulse exposure	Synthetic pyrethroid	Cypermethrin	*Trichodactylus borellianus*	Juvenile and Adult	Exposure to $\geq$0.001 ppb cypermethrin cause significant depression in oxygen consumption; ammonia excretion significantly increased	Alteration in metabolic efficiency; long-term effects unclear	Veronica and Collins, 2003
Aquatic	Single 4h flow though pulse exposure	Organochlorine	Methoxychlor	Stream macro-invertebrates	Stable population structures	Exposure to 10ppm caused massive drift, biomass loss, and significant reduction in standing macroinvertebrate abundances	Significant alteration in invertebrate community structure and function (losses of certain functional feeding groups)	Wallace, et al, 1989
Aquatic	Single 4hr pulse exposure	Biorational	Methoprene	*H. americanus*	Larval	Exposure to 50ppb resulted in significant accumulation in eyestalk, epithelial, and gonadal tissues	Unclear; may result in future tissue-specific damage.	Walker, et al, 2005
Aquatic	Single flow-through pulse exposure of 5h	Biorational	Azadirachtin (neem)	Macroinvertebrate assemblages	Stable population structures	0.84 mg/L caused significantly increased *Isogenoides* sp. drift and *Isonychia* sp. and *Hydropysche* sp. mortality ($p<0.05$, G-test)	Authors conclude no long-term effects on mesocosm community	Kreutz-weiser, et al, 1999

Environ-mental Compartment	Number and frequency of pulses	Pesticide class	Pesticide name	Species tested	Life stage tested	Detected effects	Potential long-term effects	Reference
Aquatic	Single 6hr flow-through pulse exposure	Organophosphate	Chlorpyrifos	Mesocosm invertebrates	Stable population structure	6hr exposure to 5ppb reduced abundances of *Ferrissia* sp., *Nanocladius* sp., *Cladotanytarsus bilinearis*, and an unidentified nematode; *Procladius paludicola* abundance increased ($p<0.05$, ANOVA)	Recovery was evident by day 21, but resilience of invertebrate community would depend on richness and timing of exposure relative to reproductive period.	Pusey, et al 1994
Aquatic	Single 10hr pulse	Carbamate	Carbaryl	*Daphnia ambigua*	Egg, embryo first, second and third instars	Significant delayed mortality in egg, 1st, and 2nd instars exposed to 0.05ppb; growth suppression observed at all life stages at 0.5 ppb ($p<0.05$, ANOVA)	Recovery of weight; unclear long-term effects	Hanazato, 1991
Aquatic	Single 1hr or 10hr pulse exposures (concentrations converted to µg/hr to determine importance of pulse duration)	Synthetic pyrethroid	Fenvalerate	*Limnephilus lunatus*	2nd-3rd instar	Exposure to 0.1 µg/hr caused decreased emergence ($p<0.05$, ANOVA, Fisher's PLSD); timing of emergence and adult weight was altered at 0.001 µg/hr for 1hr exposures, but not for 10hr exposures	Effects occurred 81-231 days post-exposure, signifying significant long-term effects. Shorter pulses affected greater impact than longer pulses when equivalent doses were compared.	Schultz and Liess, 2000

Environ-mental Compartment	Number and frequency of pulses	Pesticide class	Pesticide name	Species tested	Life stage tested	Detected effects	Potential long-term effects	Reference
Aquatic	Single 8hr to 14hr pulse exposure	Carbamate	Carbaryl	*Daphnia pulex, Daphnia galeata, Daphnia mendotae, Daphnia retrocurva* and *Daphnia lumholtzi*	Gravid females	Longer neckteeth in *D. pulex* exposed to ≥ 15 ppb; high helmet production in *D. galeata* exposed to 5-10 ppb; reduced growth and high helmets in *D. retrocurva* exposed to ≥ 20 ppb; long tailspines in *D. lumholtzi* exposed to ≥10 ppb ($p<0.05$, ANOVA)	Unnecessary defensive growth may negatively affect energy budget	Hanazato and Dodson, 1993
Aquatic	Single 1hr or 24hr pulse	Organophosphate	Azamethiphos	*Mytilus edulis*	Adult	Exposure to 100 ppb for 24hr significantly reduced phagocytic activity ($p<0.05$, ANOVA)	Unclear, possible increased disease susceptibility	Canty, et al, 2007
Aquatic	Single static exposures of 12hr or 24hr, followed by initiation of flow	Organochlorine	Endosulfan	Macro-invertebrate assemblages	Stable population structures	Certain caddisfly and mayfly species abundances were reduced at 12 hr of 50 ppb or 48hr of 5-25 ppb ($p<0.05$, CANOCO). Recovery was noted, preceded by algal blooms	Possible temporary alteration of community function	Hose, et al 2003
Aquatic	Single 2hr, 6hr or 24hr pulse exposure	Organochlorine	Lindane	*D. magna*	<24hr old neonates	Reduced clearance rate at all exposures to 241 ppb ($p<0.05$, ANOVA)	Fast recovery, no foreseeable long-term impacts	Hartgers, et al, 1999

Environ-mental Compartment	Number and frequency of pulses	Pesticide class	Pesticide name	Species tested	Life stage tested	Detected effects	Potential long-term effects	Reference
Aquatic	Single 1hr or 24hr exposure	Organophosphate	Paraoxon-methyl	*D. magna 0*	<24hr old neonates	Single 1hr exposure to 100 or 1000 ppb significantly reduced 21d reproduction; 24hr exposures of ≥ 1.5 ppb reduced reproduction at 14d but not 24d ($p<0.01$, ANOVA)	Decreased reproductive output	Duquesne, et al, 2006
Aquatic	Single 1hr or 24hr pulse exposures	Synthetic pyrethroid	Cypermethrin	*Acartia tonsa*	Adult	1hr exposure to 0.7-2.2 ppb and 24hr exposure to 0.2-0.8ppb significantly decreased survival 144h following exposure ($p<0.05$, ANOVA)	Significant delayed toxicity and the particular susceptibility of males suggest that populations structure may be significantly altered	Medina, et al, 2004
Aquatic	Data from single 24hr pulse exposures	Synthetic pyrethroid	Fenvalerate	*D. magna*	Modeled population	Model results suggest that sublethal fenvalerate exposure affects food acquisition and assimilation leading to reduced growth	Decreased growth could result in higher disease susceptibility, delayed reproduction, and lowered reproductive output	Pieters, et al, 2006

Environ-mental Compartment	Number and frequency of pulses	Pesticide class	Pesticide name	Species tested	Life stage tested	Detected effects	Potential long-term effects	Reference
Aquatic	Single 24hr pulse exposures	Synthetic pyrethroid	Fenvalerate	*D. magna*	<24hr old neonates from either high or low maternal food stock	Female *D. magna* provided with high food stock produced smaller offspring more sensitive to fenvalerate (significant mortality at 0.6 ppb and greater); low food stock females produced larger, more resilient offspring (significant mortality at 3.2 ppb and greater); $p<0.05$, Gehan-Wilcoxon survival analysis	Environmental conditions preceding pesticide exposure can affect individuals' sensitivities.	Pieters and Liess, 2006
Aquatic	Single 24hr pulse exposure with low or high food availability	Synthetic pyrethroid	Fenvalerate	*D. magna*	<24hr old neonates	Low food *D. magna* LOEC = 0.6 ppb; high food *D. magna* LOEC = 3.2 ppb ($p<0.01$, Gehan-Wilcoxon survival analysis). Low food + 0.3 ppb exposure and greater caused significantly reduced growth ($p<0.001$, ANOVA)	Differences in *D. magna* size recovered after two weeks; however, fenvalerate alone was demonstrated to reduce reproduction.	Pieters, et al 2005
Aquatic	Single 24hr pulse exposure	Synthetic pyrethroid	Fenvalerate	*D. magna*	<24hr old neonates	Filtering rate, 15N assimilation and growth were all reduced following exposures of 0.3 ppb and greater ($p<0.05$, ANOVA with Dunnett's test); only filtering rates recovered 2d post-exposure	Reduced growth led to significantly greater time to first reproduction, and lowered reproductive output.	Reynaldi, et al, 2006

Environ-mental Compartment	Number and frequency of pulses	Pesticide class	Pesticide name	Species tested	Life stage tested	Detected effects	Potential long-term effects	Reference
Aquatic	Single 24hr flow-through pulse	Herbicide	Hexazinone	Mesocosm invertebrates	Stable population structure	No significant effects on abundance, drift, community composition, or size of individual invertebrates.	No evidence of long-term effects.	Schneider, et al, 1995
Aquatic	48hr pulsed sediment exposure	Organophosphate	Chlorpyrifos	*Chironomus riparius*	Larvae	Exposure to 0.1 ppm caused reduced burrowing behavior, decreased male emergence, and reduced adult female weight. AChE was significantly inhibited by exposure to 0.01 ppm ($p<0.05$, ANOVA, or $\chi 2$ analysis)	Potentially reduced reproductive output.	Callaghan, et al, 2001
Aquatic	Single 48hr exposure to contaminated sediment	Organochlorine	Lindane	*C. riparius*	Larvae	Male wing length and adult emergence reduced at >0.5 ppm lindane; reduced ovipositioning >0.75 ppm ($p<0.05$, ANOVA)	Possible reduction in reproductive output	Hirthe, et al , 2000
Aquatic	Single 48hr pulse	Synthetic pyrethroid	Fenvalerate	*D. magna*	Stable population structure	Significantly reduced abundances at 1ppb and greater ($p<0.05$, ANOVA); older age classes required significantly more recovery time	Recovery from a single pulse exposure required 60 days (as measured by population structure recovery)	Liess, et al, 2006
Aquatic	Single flow-through mesocosm pulse	Synthetic pyrethroid	Lambda-cyhalothrin	*G. pulex*	Adult	Precopulatory behavior reduced at concentrations >0.35 ppb; significant mortality at >0.05 ppb ($p<0.05$, ANOVA)	Possible reduction in reproductive output	Heckmann, et al, 2005

Environ-mental Compartment	Number and frequency of pulses	Pesticide class	Pesticide name	Species tested	Life stage tested	Detected effects	Potential long-term effects	Reference
Aquatic	Single exposures reduced 50% every 10hrs (to mimic natural degradation)	Biorational	Fenoxycarb	*D. magna*	<24hr old neonate	50 ppb exposure (initial concentration) resulted in significantly reduced reproductive output ($p<0.05$, ANOVA)	Possible reduction in number of viable offspring	Hosmer, et al, 1998
Aquatic	Single 96h pulse exposures	Biorational	Imidacloprid	*C. tentans* *H. azteca*	~8-9d old *C. tentans* ~2-9d old *H. azteca*	Pulse exposures were less toxic than chronic exposures to *C. tentans.* Reduction in *H. azteca* growth rate (LOEC = 3.53 ppb) was a more sensitive endpoint than mortality (NOEC = 11.93 ppb)	Unclear	Stoughton, et al, 2008
Aquatic	Single pulses of 48hr-96hr in duration at 12°C or 22°C	Algaecide	BULAB 6002	*Dreissena polymorpha*	Adult	Survival at 240hr after exposure was significantly decreased only at 22°C at exposures ≥ 4 ppm	Warm temperatures increased *D. polymorpha* sensitivity to BULAB 6002 ($p<0.05$, ANOVA)	Martin, et al, 1993
Aquatic	Static 7d pulse (with degradation), followed by flowing conditions	Herbicide	Linuron	Mesocosm invertebrates	Stable population structures	*Keratella quadrata* abundances significantly but briefly reduced ($p<0.05$, Monte Carlo permutation); macroinvertebrate taxa richness was slightly reduced at 50 ppb.	No long-term impact observed by authors.	Van Geest, et al, 1999

Environ-mental Compartment	Number and frequency of pulses	Pesticide class	Pesticide name	Species tested	Life stage tested	Detected effects	Potential long-term effects	Reference
Aquatic	Single pulse exposure; double pulse exposures with intervening recovery time	Organophosphate	Chlorpyrifos	*D. magna*	<24hr old neonate	Survival was the most sensitive endpoint with significant decreases occurring following pulses of 0.5ppb of 12 hrs or less ($p< 0.05$, ANOVA)	Increased recovery time mediated effects of chlorpyrifos	Naddy, et al, 2000
Aquatic	Either single 2hr exposure, or two 1hr exposures with intervening recovery time	Organophosphate, and carbamate	Aldicarb, carbaryl, carbofuran, malathion, parathion andpropoxur	*C. riparius*	4th instar	Malathion and parathion exhibited no significant reversal of effect with 24 hr recovery time; carbamate recovery occurred with >2hr recovery time ($p<0.05$, Tukey's Student test)	Unclear	Kallander, et al, 1997
Aquatic	Two 1hr pulses separated by 6h recovery time or single 2h pulse	Organophosphate, carbamate and synthetic pyrethroid	Fenitrothion, carbofuran, carbaryl, and permethrin	*Aedes aegypti*	3rd instar	The inclusion of recovery time increased microencapsulated permethrin toxicity as measured as survival to adult stage ($p<0.05$, t-test); recovery time had no effect on the effect of other compounds	6hr recovery time insufficient to reverse effects; multiple pulses in short time period may reduce survival to adult.	Parsons and Surgeoner, 1991

Environ-mental Compartment	Number and frequency of pulses	Pesticide class	Pesticide name	Species tested	Life stage tested	Detected effects	Potential long-term effects	Reference
Aquatic	Single exposures of 4-24hr in duration; double exposures of 3-12hr in duration with intervening recovery times of 72hr to 288hr	Metal	Selenium	*D. magna*	<24hr old neonates	Increased exposure concentration and duration increased cumulative 21-day mortality. Significant cumulative mortality occurred at 12hr of 800 ppb, 9hrs of 1200 ppb, 6hr of 1600 or 2000 ppb ($p<0.05$, F-test)	Potentially altered population structure or decreased abundance	Hoang and Klaine, 2008
Aquatic	1)Single 24hr pulse of carbaryl, 14d depuration, single 24hr pulse of chlorpyrifos 2)Single 24hr pulse of chlorpyrifos, 14d depuration, single 24hr pulse of carbaryl	Organophosphate and carbamate	Chlorpyrifos and carbaryl	*G. pulex*	Adult	Carbaryl was more toxic to *G. pulex* when preceded by exposure to chlorpyrifos. Mortality in treatment 1 = 45%, mortality in treatment 2 = 60% (binomial test for two proportions).	*G.pulex* populations that suvive exposures to organophosphates may be more sensitive to future AChE exposures	Ashauer et al, 2007b
Aquatic	Single pulses or double-pulses separated by 48h	Organophosphate and carbamate	Dimethoate and pirimicarb	*Daphnia magna*	24hr and 3-day old	Exposure to 30 ppm dimethoate or 100ppm pirimicarb resulted in reduced growth, delayed time to first reproduction, and decreased reproductive output ($p<0.05$, t-test)	Limited reproduction window, fewer offspring	Andersen, et al, 2006

Environ-mental Compartment	Number and frequency of pulses	Pesticide class	Pesticide name	Species tested	Life stage tested	Detected effects	Potential long-term effects	Reference
Aquatic	Two pulse applications (one per month), followed by dissipation	Synthetic pyrethroid	Esfenvalerate	Mesocosm invertebrates	Stable population structure	Multiple species abundances were reduced by exposures as low as 0.08 ppb ($p<0.05$, ANOVA); *H. azteca* did not recover	Only Chironomid species showed signs of recovery 70 d after second exposure; significant long-term impacts on community structure	Lozano, et al, 1992
Aquatic	Single pulses ranging from 3 to 120 hrs in duration; double pulses with variable intervening recovery times	Metal	Arsenic	*D. magna*	<24hr old neonates	Recovery times had no effect, and may have exacerbated effects. Time to first reproduction was significantly lengthened by 6-9hr exposures of 6000 ppb, 24hr exposures of 4000-5000 ppb, and 120hr exposures of 3000ppb ($p<0.05$)	Potentially fewer offspring due to restriction of reproductive period.	Hoang, et al, 2007
Aquatic	3-4 1hr pulses	Organophosphate	Azamethiphos	*Homarus americanus*	Female adults	Spring time exposure to pulses of 10 ppb caused significant disorientation, paralysis, mortality and decreased spawning ($p<0.05$, z-test or $\chi 2$ analysis)	Reduced reproductive output	Burridge, et al, 2008

Environ-mental Compartment	Number and frequency of pulses	Pesticide class	Pesticide name	Species tested	Life stage tested	Detected effects	Potential long-term effects	Reference
Aquatic	Four 48h pulses on day 0, 5, 10, 15; Three 48hr pulses on day 0, 10 15; Two 48hr pulses on day 0 and 15	Carbamate	Carbaryl	*Gammarus pulex*	Adult	As measured at day 20, pulses of 0.5 ppb significantly increased latent mortality (versus control and chronic 0.05 ppb exposure). Increasing number of pulses caused a steeper cumulative mortality curve. ($p<0.05$)	Unclear, but greater effects observed following pulsed vs. chronic exposure	Ashauer, et al, 2007a
Aquatic	15min pulse flow-through exposure every two weeks for 3 months; laboratory determination of 48hr *D. magna* LC_{50}	Synthetic pyrethroid	Esfenvalerate	*D. magna, plus* various mesocosm invertebrate species	N/A	Reduced mesocosm macroinvertebrate abundance following exposures $\geq$ 0.25 ppb ($p<0.05$ NPANOVA, LSD); *D. magna* 48hr LC50 = 0.27 ppb	May significantly alter aquatic community structure; *D. magna* sensitivity may not be predictive of aquatic communities' sensitivities	Fairchild,et al, 1992
Aquatic	Repeated pulse exposures of 4h or 12h with 4d or 7d recovery time for a total of 28d	Biorational	Margosan-O (Neem product)	*D. magna*	<48hr old	4d recovery time was insufficient to reverse effects at either pulse duration, whereas recovery time of 7d reduced mortality at both pulse durations.	Unclear	Scott and Kaushik 1998

Environ-mental Compartment	Number and frequency of pulses	Pesticide class	Pesticide name	Species tested	Life stage tested	Detected effects	Potential long-term effects	Reference
Aquatic	Time varied pulse exposures	Multiple (review article)	Multiple (review article)	Aquatic invertebrates	Various	Doubling exposure time decreased survival by a factor of 1.4 to 8.4 (*C. riparius* and *Hydropsyche angustipennis*); Effects of insect growth regulator (IGR) may be delayed until molt/emergence (weeks)	Variable	Reinert, et al 2002
Terrestrial	Single topical application	Synthetic pyrethroid	Permethrin	*Apis mellifera*	Adult	Exposure to 0.001 µg caused significantly increased rotating, trembling, and cleaning behaviors, and reduced foraging. Exposure to 0.009 µg caused disorientations and prevented all exposed bees from returning to hive ($p<0.05$, t-test)	High worker bee mortality possibly resulting in hive debilitation	Cox and Wilson, 1984
Terrestrial	Single topical application	Organophosphate	Chlorfenvinphos	*Trybliographa rapae*	Adult	Number of eggs reduced 20d after exposure to 0.5 µl/insect. Reduced adult longevity, reduced pairing also observed ($p< 0.05$, t-test)	Decreased reproductive output	Alix, et al, 2001

Environ-mental Compartment	Number and frequency of pulses	Pesticide class	Pesticide name	Species tested	Life stage tested	Detected effects	Potential long-term effects	Reference
Terrestrial	Single spray applications of 6.25 g a.s./ha lambda-cyhalothrin, 7.5 g a.s./ha deltamethrin, or 400 g a.s./ha dimethoate	Organophosphate, and synthetic pyrethroid	Dimethoate, deltamethrin, and lambda-cyhalothrin	*Aphidius uzbekistanicus*	Early and late instar larvae (in aphids); adults	100% percent host aphid and early instar mortality after all exposuresDeltamethrin and dimethoate significantly reduced larval emergence and male longevity; female longevity significantly reduced at all exposures ($p<0.05$, t-test)	Possible reduction in reproductive output resulting from reduced adult lifespan	Krespi, et al, 1991
Terrestrial	Single 48hr pulse exposure to contaminated surface	Synthetic pyrethroid, organophosphate, carbamate,and AChE inhibitor	Lambda-cyhalothrin, chlorpyrifos, pirimicarb and triazamate	*Aphidius ervi*	Adults	Highest triazamate exposure (18.75 ng/cm) significantly disrupted aphid odour detection ($p<0.001$, Kolmogorov–Smirnov test)	May lower reproductive output through decreased host detection	Desneux, et al, 2004
Terrestrial	Single topical application (LD_{10}-LD_{50}, only survivors studied)	Organophosphate and synthetic pyrethroid	Propoxur and deltamethrin	*Blattella germanica*	Adult	LC_{30} and greater significantly reduced adult longevity, ootheca production and viability ($p<0.05$ ANOVA)	Both maternal and paternal exposure led to reduced reproductive output	Lee, et al, 1998
Terrestrial	Single 24hr dietary exposure	Biorational	BT endotoxin	*Leptinotarsa decemlineata*	Larvae	Single feeding with diet contaminated with 62 ppm BT caused significant latent mortality in later larval and pupal stages. Weight reduced at dietary exposure to 31 ppm. Acute exposure more toxic than chronic ($p<0.05$, ANOVA, or Scheffe's test)	Altered population dynamics (decreased population growth rate and reduced net reproductive output)	Costa, et al, 2000

Environ-mental Compartment	Number and frequency of pulses	Pesticide class	Pesticide name	Species tested	Life stage tested	Detected effects	Potential long-term effects	Reference
Terrestrial	Single spray exposure	Biorational	Neem	*C. septempunctata and Acyrthosiphon pisum*	Adult	100 ppm Neem exposure significantly reduced *A. pisum* population growth rate; also reduced *C. septempunctata* ovipositioning, larval feeding, pupation ($p<0.05$, t-test LSD)	Altered population dynamics	Banken and Stark, 1998
Terrestrial	Single one-day dietary pulse (aphids); single spray on eggs	Biorational	Neem compounds: neem oil (NO), neem kernel, NeemAzal (NA)	*Coccinella septempunctata, Chrysoperla carnea, Episyrphus balteatus*	Egg and larval life stages	*C. septempunctata:* significantly altered larval development time, increased pupal time, adult deformities (NO=1.2 ppm a.s.; NA= 40 ppm) *C. carnea:* same concentrations increased larval/pupal development time, reduced adult longevity and increased adult deformities. *E. balteatus:* same concentrations as soil and foliar applications increased mortality ($p<0.05$, ANOVA)	Delayed larval and pupal mortality; pupal deformities	Ahmad, et al, 2003

Environ-mental Compartment	Number and frequency of pulses	Pesticide class	Pesticide name	Species tested	Life stage tested	Detected effects	Potential long-term effects	Reference
Terrestrial	Reared on surfaces sprayed with emergence LC_{50}s (e.g. concentration that results in 50% reduction in emergence)	Organophosphate, synthetic pyrethroid and biorational	Methyl-parathion, azinphos-methyl, cypermethrin, fenoxycarb, diflubenzuron and tebufenozide	*Micromus tasmaniae*	Larvae	Diflubenzuron: reduced male:female ratio, female longevity, reproductive output. Fenoxycarb: reduced female longevity and reproductive output. Tebufenozide exposure altered offspring reproductive output ($p<0.05$, ANOVA)	Effects of insect growth regulators can occur weeks following exposure, and can alter offspring as well as parental reproductive fitness	Rumpf, et al, 1998
Terrestrial	Single topical exposure	Synthetic pyrethroid and fungicide	Deltamethrin, prochloraz and difenoconazole	*A. mellifera*	Adult	Both deltamethrin and fungicide concentrations ~12-15x lower than application rate induced hypothermic reactions in bees (2.5 ng/bee and 1250 ng/bee). $p<0.05$, Pearson correlation	Disruption of behavioural homeostasis may leave worker bees disoriented, causing reduced numbers of foragers	Vandame and Belzunces, 1998
Terrestrial	Single substrate application	Organophosphate and fungicide	Diazinon and benomyl	*Porcellionides pruinosus*	Adult	At diazinon substrate concentrations ≥ 0.5 µg/g, glycogen content fell to zero; at ≥ 1.1 µg/g protein content was significantly lower; at $s \geq 0.24$ µg/g, lipid content was significantly lowered 6 weeks following exposure. ($p < 0.05$ Spearman correlation)	Alteration in metabolic efficiency; long-term effects unclear	Vink, et al, 1995

Environ-mental Compartment	Number and frequency of pulses	Pesticide class	Pesticide name	Species tested	Life stage tested	Detected effects	Potential long-term effects	Reference
Terrestrial	Three single spray applications, one/month during summer	Synthetic pyrethroid	Lambda-cyhalothrin	*Phyllonorycter blancardella Pholetesor omigis* Chalcididae	Eggs and larvae of *P. blancardella*, and adult *P.omigis* and Chalcididae	*P. blancardella* eggs and larvae were significantly reduced by 2.5 g a.s./ha and greater ($p<0.05$, Scheffe's F test). *P.omigis* and Chalcididae abundances were also significantly reduced	Unclear whether parasitoids decreased because of direct toxicity or insecticide-mediated community alterations.	Li, et al, 1991
Terrestrial	6 to 10 spray applications during two summers, separated by 7-10d	Fungicide	Sulphur compounds	*Typhlodromus pyri*	Adults	Six spray applications of 800 ppm fungicide required for significant reduction; 10 spray pulses of 400 ppm showed significant reductions at all concentrations ($p<0.05$, ANOVA)	Altered population dynamics	Blumel, et al, 1997
Terrestrial	Repeated field sprays every three weeks May-August comprised of single or mixture of compounds.	Organophosphate, carbamate and fungicide	Chlorpyrifos, carbaryl, mancozeb and thiophanate-methyl	*T. pyri*	Adult	3600 g a.s./ha mancozeb significantly reduced *T. pyri* numbers, and mancozeb+chlorpyrifos (960g a.s./ha) and thiophanate-methyl (1100 g a.s./ha) +chlorpyrifos treatments exhibit synergistic action. Host populations increased as *T. pyri* abundances decreased. ($p<0.05$, ANOVA)	Disruption of parasitoid-host population balance	Cross and Berrie, 1996

Environ-mental Compartment	Number and frequency of pulses	Pesticide class	Pesticide name	Species tested	Life stage tested	Detected effects	Potential long-term effects	Reference
Terrestrial	Generally topical application	Multiple (review article)	Multiple (review article)	Beneficial arthropod species	Multiple	Biochemical, developmental, longevity and immunology	Unclear	Desneux, et al, 2007

Chapter 4: Literature Search

4.1 Database search protocols

To ensure a thorough search of available literature, a number of useful descriptors were identified. These were organised into two separate combinations for use in literature searches, and are listed below.

Search 1:

short term short-duration brief acute pulse* multiple repeated transient	and	exposure bio-availability dos*	and	long-term sub-lethal chronic repro* recovery population communities microcosm mesocosm	and	*invertebrate *worm aquatic organism soil organism arthropod* crustacea* cladoceran insect* arachnid* acari mollusc* annelid* cirriped* copepod radiat* coelenterat* infusaria amphipod* nematod*

Search 2:

pulsed exposure multiple applications	and	mesocosm microcosm model ecosystem field stud* semi-field stud*	and	ecotox* environ*

Note: * indicates any combination before or after the *, e.g. stud* would catch the words "study" and "studies". Hyphenated terms should be run with and without the hyphen, e.g. semi-field and semi field.

These keywords (in English) were searched for in the titles, abstracts and indexing terms of the available literature. The above combinations of terms were represented in the search protocols utilised in the course of this project. Senior research specialist Jane Ibl of Exponent International at Menlo Park conducted the searches (during January 2009) that returned the following results:

Set	**Items**	**Description**
S1	492784	(SHORT-TERM OR SHORT()TERM OR BRIEF OR ACUTE OR PULSE? ? OR PULSING OR MULTIPLE? ? OR REPEATED OR TRANSIENT)/TI,DE,ID,AB AND (EXPOSURE? ? OR EXPOSED OR BIOAVAIL? OR BIO-AVAIL? OR DOSE? ? OR DOSAGE? ?)/TI,DE,ID,AB
S2	158499	S1 AND (LONG-TERM OR LONG()TERM OR SUBLETHAL? OR SUB-LETHAL? OR CHRONIC? OR REPRO? OR RECOVERY OR POPULATION? ? OR COMMUNIT? OR MICRO-COSM? OR MICROCOSM? OR MESO-COSM? OR MESOCOSM?)/TI,DE,ID,AB
S3	29944	S2 AND (INVERTEBRAT? OR WORM? ? OR AQUATIC? OR SOIL? ? OR ARTHROPOD? OR CRUSTACEA? OR CLADOCER? OR INSECT? OR ARACHN? OR ACARI? OR MOLLUS? OR ANNELID? OR CIRRIPED? OR COPEPOD? OR RADIAT? OR COELENTERAT? OR INFUSAR? OR AMPHIPOD? OR NEMATOD?)/TI,DE,ID,AB
S4	73	S3 AND (PLANT? ?)(4N)(PROTECT?)/TI,DE,ID
S5	6187	S3 AND (PESTICID? OR HERBICID? OR ACARICID? OR INSECTICID? OR FUNGICID? OR PLANT? ?(4N)GROW??? OR MITICID?)/TI,DE,ID
S6	5229	S5 AND (TOXICIT? OR SHORT-TERM OR SHORT()TERM OR DURATION OR BRIEF OR ACUTE OR PULSE? ? OR PULSING OR MULTIPLE OR REPEATED OR TRANSIENT? OR EXPOSURE? ? OR EXPOSED OR BIOAVAIL? OR BIO-AVAIL? OR DOSE? ? OR DOSAGE? ? OR LONG-TERM OR LONG()TERM OR SUBLETHAL? OR SUB-LETHAL? OR CHRONIC? OR REPRO? OR RECOVERY OR POPULATION? ? OR COMMUNIT? OR MICRO-COSM? OR MICROCOSM? OR MESOCOSM? OR MESO-COSM?)/TI,DE,ID
S7	60	RD S4 (unique items)
S8	39	S7/ENG
S9	**39**	**Sort S8/ALL/PY,D [Search 1 – Lot 5]**
S10	755	S6 AND (SHORT-TERM OR SHORT()TERM OR DURATION? ? OR BRIEF OR ACUTE OR PULSE? ? OR PULSING OR MULTIPLE OR REPEATED OR

Set	Items	Description
		TRANSIENT?)(6N)(EXPOSURE? ? OR EXPOSED OR BIOAVAIL? OR BIO-AVAIL? OR DOSE? ? OR DOSAGE? ?)/TI,DE,ID
S11	754	S10 NOT S4
S12	730	S11/ENG
S13	365	RD (unique items)
S14	**365**	**Sort S13/ALL/PY,D [Search 1 – Lot 5]**
S15	7978	(PULSE? ? OR PULSING OR MULTIPLE)(4N)(EXPOSURE? ? OR EXPOSED OR APPLICATION? OR APPLIED)/TI,DE,ID,AB AND (MESO-COSM? OR MESOCOSM? OR MICRO-COSM? OR MICROCOSM? OR ECOSYSTEM? OR MODEL? ?OR FIELD(4N)(STUDY OR STUDIES OR STUDIED) OR (SEMI-FIELD OR SEMIFIELD?)(4N)(STUDY OR STUDIES OR STUDIED))/TI,DE,ID,AB
S16	1060	S15 AND (ECOTOX? OR ECO-TOX? OR ENVIRON?)/TI,DE,ID
S17	691	S15 AND (TOXICIT?)/TI,DE,ID
S18	1554	S16 OR S17
S19	1500	S18 NOT (S4 OR S10)
S20	1479	S19/ENG
S21	896	RD (unique items)
S22	**896**	**Sort S21/ALL/PY,D [Search 2 – Lot 5]**

4.2 Databases searched

As defined in the technical offer, the following databases were searched:

DIALOG DATABASES:

File 50:CAB Abstracts 1972-2009/Jan W3
(c) 2009 CAB International
File 10:AGRICOLA 70-2009/Jan
(c) format only 2009 Dialog
File 203:AGRIS 1974-2009/Dec
Dist by NAL, Intl Copr. All rights reserved
File 6:NTIS 1964-2009/Feb W1
(c) 2009 NTIS, Intl Cpyrght All Rights Res
File 66:GPO Mon. Cat. 1978-2008/Dec
(c) format only 2008 Dialog
File 156:ToxFile 1965-2008/Nov W2
(c) format only 2008 Dialog
File 65:Inside Conferences 1993-2009/Jan 26
(c) 2009 BLDSC all rts. reserv.
File 144:Pascal 1973-2009/Jan W2
(c) 2009 INIST/CNRS
File 143:Biol. & Agric. Index 1983-2009/Dec
(c) 2009 The HW Wilson Co

File 24:CSA Life Sciences Abstracts 1966-2009/Jan
(c) 2009 CSA.
File 40:Enviroline(R) 1975-2008/May
(c) 2008 Congressional Information Service
File 76:Environmental Sciences 1966-2009/Mar
(c) 2009 CSA.
File 44:Aquatic Science & Fisheries Abstracts 1966-2009/Feb
(c) 2009 CSA.

STN DATABASES:

CAPLUS [Chemical Abstracts; 1907-present]
CROPU [Derwent Crop Protection File]

4.3 References to be reviewed

The search hits retrieved have been subject to the following considerations as a preliminary reference filter, and those considered to be not relevant have been discarded:

- If a title of a reference reads to be relevant to this literature review then it has been included in the search results.

- Where the relevance of a reference title was unclear if an abstract was provided then this was assessed for relevance (e.g. exposure of invertebrate species to pulses of plant protection products) and included in the search results where appropriate.

- If the relevance of a reference title was unclear but no abstract has been provided initially then this has also been included in the search results.

From the large number of hits retrieved in a 1st step, 97 references have been found to be relevant according to the screening explained above.

Once all abstracts of the following 97 references have been requested (where not already retrieved) they will be reviewed and their relevance to the project confirmed. All relevant references will be retrieved in full, reviewed under this project and collated in an 'EndNote' file. The relevance of retrieved referenced were judged based on the following criteria:

1) Use of invertebrates as test species: A few abstracts were unclear as to the specific test organisms utilized. As a result, some human health studies and fish toxicological experiments were erroneously included in the original group of 97 references identified.

2) Investigation of the effects of short-term exposures: Those invertebrate studies that reported the effect of low-concentration chronic exposures were excluded as inappropriate for the stated purposes.

3) Explanation of effects occurring after the exposure period: References detailing potential long-term effects (defined as effects observed following cessation of chemical exposure) were identified for use in this report. Special preference was given to longer-term effects (e.g., those occurring in subsequent life stages), and reproductive endpoints (e.g., reduced reproductive output) that could affect population viability.

4) Klimisch score[1]: References were scored based on a) use of standardised testing prodecures as recognised/developed by a competent regulatory agency, and b) chemical analysis and validation of pesticide concentrations. References satisfying both requirements were scored (1); references satisfying one requirement (usually requirement b) were scored (2). Those references meeting neither requirement were scored (3). However, because a number of identified

[1] Klimish, H.J., Andreae, M. and Tillmann, U. (1997). A Systematic Approach for Evaluating the Quality of Experimental Toxicological and Ecotoxicological Data. Regulatory Toxicology and Pharmacology **25**, 1-5.

terrestrial studies applied pesticides as commercially formulated sprays (e.g., not analytically verified), these were included to provide additional valuable information concerning effects in terrestrial ecosystems, despite low Klimisch scores.

5) Use of non-standard organisms, especially beneficial terrestrial insects: References detailing the long-term effects of short-term pesticide exposures on diverse invertebrate taxa is likely to provide a more complete evaluation of total effects. Since different invertebrate taxa exhibit different sensitivities, life histories, ecosystem niches, and population dynamics, inclusion of effects observed in multiple taxa will be a more thorough account of likely effects of pesticides introduced into a natural system.

6) Comparison of effects between different exposure regimes: References that provide information on the effects of pulse duration (e.g. short-term, acute versus chronic), frequency or exposures, or mixtures of plant protection products were also singled out. Since pesticides are likely to occur in mixtures or a series of short-term pulses, these references were considered especially useful in eludiating potential effect in natural systems.

Following the selection of appropriate references using the above criteria, additional references from the in-house Exponent library were selected to complete review (e.g., Cox and Wilson 1984, which described the effects of extremely low-level pyrethroid exposure on honeybees). This small number of additional references were chosen to supplement and augment information provided by referenced identified during the search.