Agricultural pesticide toxicity to aquatic organisms

- a literature review

by

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Introduction

Since the World War II, the number of pesticides used has dramatically increased. In the United Kingdom, the number of pesticide active ingredients approved for use increased from 11 to 105 between 1957 and 1995 (Sotherton and Holland, 2003). Similar increases in the use of pesticides occurred in North America. In the USA, herbicide usage increased by 180 % in the period 1971-1987 and in Canada herbicide usage increased threefold and insecticide usage fivefold during the same period (Sotherton and Holland, 2003). In Sweden there are today approximately 90 different active pesticide ingredients registered for use within the agricultural sector, and approximately 1700 tons of these are applied each year (KEMI, 2000). Although, more than 70% of the pesticides used in Sweden are associated with the industrial sector, also effluents from agriculture (>20 %), forestry (<1 %), golf courses, gardens (<1 %) and households (<4 %) constitute important sources of pesticides in the environment (KEMI, 2000; Naturvårdsverket and Statistiska Centralbyrån, 2000). Accidental spills and dumpsites also provide for a part of the environmental pesticide input. In contrast to many other man-made chemicals present in the environment, pesticides are deliberately spread into the environment. They are manufactured to be harmful to specific target organisms, or groups of organisms, and their toxic properties are essential to give the pesticides a satisfactory function. Due to pesticide's toxic properties there is an obvious risk that non-target organisms are affected, either at the application site, or due to unintentional spreading, at nearby, or even distant, areas.

Chemicals originating from agricultural activity enter the aquatic environment through atmospheric deposition, surface run-off or leaching (Kreuger, 1999) and frequently accumulate in soft-bottom sediments and aquatic organisms (Miles and Pfeuffer, 1997; Lehotay *et al.*, 1998; Kreuger *et al.*, 1999). In all parts of the world pesticides have been found in the aquatic ecosystem and often information of how these pesticides affect inhabiting organisms is missing. In canals in south Florida more than 700 pesticide detections were made between 1991 and 1995 (Miles and Pfeuffer, 1997). Atrazine, ametryn, and bromacil were most often detected in the water samples, whereas pesticides which bind strongly to soil, are highly persistent and/or used in large amounts, e.g. DDE, DDD and ametryn, were some of the more frequently found in sediments (Miles and Pfeuffer, 1997). In Chesapeak Bay, also USA, herbicides such as atrazine, simazine, cyanazine, and metolachlor were found in water samples, but not in oysters (Lehotay *et al.*, 1998). Another herbicide, trifluralin, was however detected in both water and oysters. In an oligotrophic coastal lagoon, the Mar Menor, located in the southeast of Spain, Pérez-Ruzafa *et al.* (2000) found mainly endosulfan, HCH, and endrin when analyzing water, sediment and organisms. The highest concentrations, up to 1.5 mg/kg (endrin), were recorded in organisms due to bioaccumulation of the hydrophobic pesticides. High concentrations were also found in the sediments, but in the water the levels were lower. In Sweden, a recent screening monitoring program of surface waters in nine small agricultural catchments revealed that the most frequently detected pesticides were those sold

in largest quantities (Ulén *et al.*, 2002). Bentazone and MCPA were detected in all streams and in most samples from the two rivers included in the study. Other herbicides commonly detected were glyphosate and mecoprop. Occasionally, also residues of herbicides that had been withdrawn from the Swedish market were detected, e.g. atrazine, 2,4-D, and simazine. In a few cases substances were found in concentrations that may cause adverse effects to aquatic life, with the herbicide cyanazine being the most frequently detected at concentrations exceeding the Dutch water quality guidelines (Crommentuijn *et al.*, 2000). In two earlier studies, Kreuger *et al.* (1999) and Kreuger (1998) determined commonly used pesticides in both water and sediment from a small catchment in southern Sweden. Fenpropimorph was the most dominant pesticide in sediments, but also fenvalerate, propiconazole, and ∑DDT were detected on several occasions. There was a more than 50% detection frequency for the herbicides atrazine, bentazone, dichlorprop, ethofumesate, MCPA, mecoprop, metazachlor, and terbuthyazine in the water samples. Agricultural pesticides are also found in areas not adjacent to agricultural land (Hoffman *et al.*, 2000). Eight urban streams from across the United States were monitored for high-use pesticides and pesticide transformation products. Among the 75 target pesticides and the 7 target transformation products, 13 insecticides, 28 herbicides, and one transformation product were found. The herbicides detected most frequently were prometon, simazine, atrazine, tebuthiuron, and metolachlor, and the insecticides detected most frequently were diazinon, carbaryl, chlorpyrifos, and malathion. In contrast to similar-sized agricultural streams, total insecticide concentrations commonly exceeded total herbicide concentrations in these urban streams. Consequently, the contributions of insecticides from urban and agricultural land to streams may be similar (Hoffman *et al.*, 2000). It is from all these studies clear that both commonly used pesticides and pesticides withdrawn from the market years ago do reach and/or accumulate in aquatic ecosystems and thereby constitute a threat to all aquatic organisms.

The toxicity of a chemical is totally dependent on the concentration of the chemical in the organism or even the concentration at the target receptor in the organism. Only the bioavailable fraction of the pesticide can be accumulated by aquatic organisms and thereby reach the specific target receptors (Hamelink and Spacie, 1977). The actual amount of the pesticide that enters the organism, is therefore of critical importance and determines the biological ramifications. In environmental toxicology, environmental concentration is often used as a substitute for knowing the actual amount of a chemical entering an organism. Care must be taken to realize that the entering dose may be only indirectly related to environmental concentration. The shape, characteristics of the organisms external covering, respiratory systems, and feeding selectivity can all dramatically affect the rate of a chemical's absorption from the environment (Bruner *et al.*, 1994a; Bruner *et al.*, 1994b; Gossiaux *et al.*, 1998; Landrum and Fisher, 1998). The entering dose and environmental concentration are therefore not always directly proportional or comparable from species to species (Landis and Yu, 1995).

Different aspects of pesticide toxicity have already been summarized in many review articles and books (Mulla and Mian, 1981; Matsumura, 1985; Ecobichon, 1991; Barron and Woodburn, 1995; DeLorenzo *et al.*, 2001). The aim of this literature review is therefore not to give a complete overview of pesticide toxicity, but rather summarize the main effects of typical groups of pesticides used today or found in the aquatic environment due to accumulation and persistence. I will focus on toxic effects on non-target organisms at different trophic levels, including microorganisms, invertebrates, and fish and discuss possible consequences on the food webs of aquatic ecosystems. All pesticides mentioned in this literature review are summarized in the appendix, including information about pesticide group and function.

Mechanisms of pesticide action

Pesticides can be classified according to their mechanisms of action. For example, insecticides like organochlorines, organophosphates, and carbamates act primarily by disrupting nervous system function, while herbicides often target photosynthesis pathways (Ecobichon, 1991; DeLorenzo *et al.*, 2001). In this section I will describe the mode of action of some common pesticides or pesticide groups present in the aquatic environment. For a more extensive description of pesticide action I refer to earlier published books and review articles (Matsumura, 1985; Ecobichon, 1991; DeLorenzo *et al.*, 2001).

Insecticides

Many insecticides affect the nervous system at different target sites (figure1). The insecticides interfere with the membrane transport of sodium, potassium, calcium, or chlorine ions, inhibit selective enzyme activities, and contribute to the release and/or the persistence of chemical transmitters at nerve endings (Ecobichon, 1991).

Organochlorines belong to one of the insecticide classes affecting the nerve system. Chemically they are relatively unreactive stable compounds and are characterized by their long-lasting effects. Among them is the most studied pesticide, DDT. Recently, Younis *et al.* (2002) identified the DDT target protein in insects. The target protein was shown to be a subunit of the adenosine triphosphatase synthase (ATPase), which is involved in the neuronal repolarization and thereby important for normal nerve function. DDT also acts through at least three more mechanisms (Matsumura, 1985). At the level of neuronal membrane, DDT affects the permeability to potassium ions, reducing potassium transport across the membrane. DDT also alters the channels, through which sodium ions pass, thereby interfering with the active transport of sodium out of the nerve axon during repolarization. Finally, DDT inhibits the ability of calmodulin, a calcium mediator in the nerve, to transport calcium ions essential for the release of neurotransmitters. Two other organochlorine insecticides, endrine and

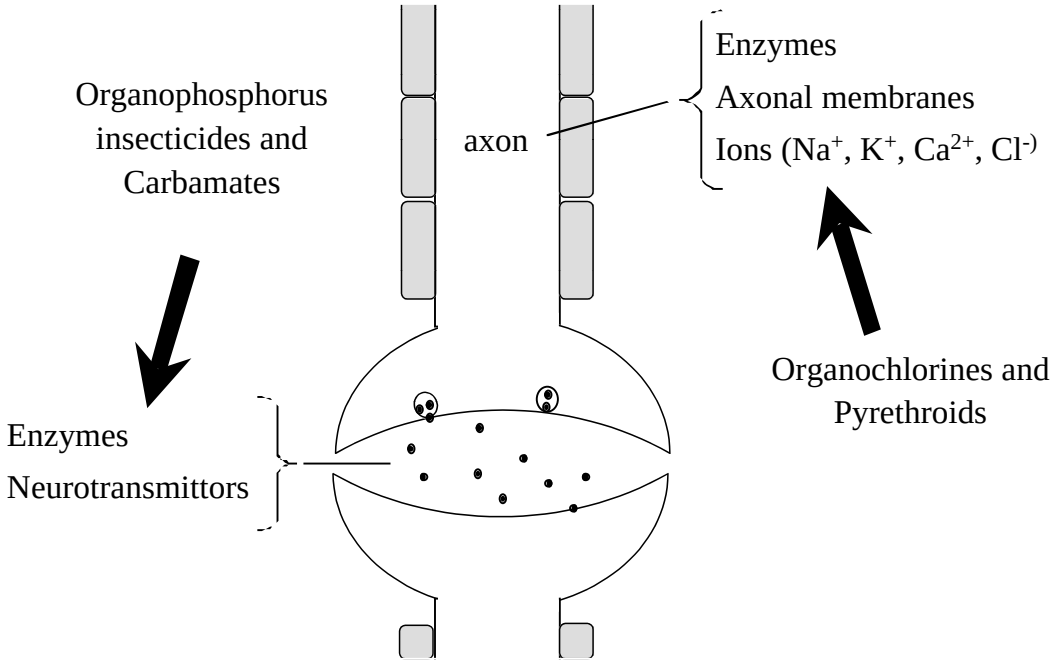

Figure 1. *Potential sites of action of classes of insecticides on the axon and the terminal portions of the nerve (modified from Ecobichon (1991)).*

lindane, are in addition to the DDT-resembling effects also inhibitor of the γ-aminobutyric acid (GABA) receptors in both insect and vertebrate central nervous system (Wafford *et al.*, 1989). The GABA receptor is an ion channel glycoprotein that traverses the cell membrane and functions as a receptor for the neurotransmitter GABA.

Organophosphorus insecticides, like malathion and parathion, replaced the organochlorines in e.g. malaria and other global programs for control of disease vectors when some the organochlorins, including DDT, were banned and withdrawn from the market in many countries (Mulla and Mian, 1981). The organophosphorus group of insecticides is also neurotoxic, although this group acts on the nervous system by inhibiting the enzyme acetylcholinesterase (AChE). The reaction between an organophosphorus insecticide and the active site in the AChE protein results in the formation of a transient, intermediate complex that partially hydrolyzes, leaving a stable, phosphorylated, and largely unreactive, inhibited enzyme that, under normal circumstances, can be reactivated only at very slow rate (figure 2; Ecobichon, 1991). With many organophosphorus insecticides, an irreversible inhibited enzyme is formed, and the signs and symptoms of intoxication are prolonged and persistent. However, some organophosphorus insecticides are thought to be toxic only after metabolism by the cytochrome P-450 monooxygenase enzyme systems. This bioactivation step creates a metabolite that is a much stronger inhibitor of the AChE than the parent compound (Belden and Lydy, 2000).

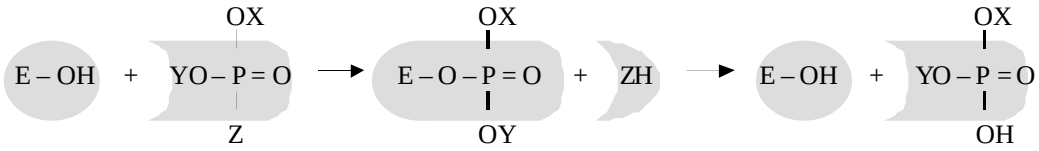

Figure 2. *The interaction between an organophosphorus insecticide with the hydroxyl group in the active site of the enzyme acetylcholinesterase (E-OH). The intermediate, unstable complex formed before the release of the "leaving" group (ZH) is not shown. The dephosphorylation of the inhibited enzyme is the rate-limiting step to forming free enzyme. (Modified from Ecobichon (1991)).*

Carbamates are also AChE inhibitors, attaching to the reactive site of this enzyme (Ecobichon, 1991). However, in contrast to organophosphorus insecicides, carbamates are poor substrates for the cholinesterase-type enzymes, resulting in a short and reversible inhibition of AChE. When carbamates attach to AChE they undergo hydrolysis in two stages. The first stage is the formation of a carbamylated enzyme by removal of an aryl (e.g. $–C_6H_5$) or alkyl group (e.g. $–CH_3$). The second stage is the decarbamylation of the inhibited enzyme, which results in the generation of a free and once again active enzyme. The process is very much the same as when organophosphorus pesticides bind to AChE, however the last step with regeneration of a free enzyme is much faster.

Synthetic pyrethroids belong to the newest of the major classes of insecticides. The pyrthroids show two different characteristic acidic portions, chrysanthemic or pyrethric acids resulting in type I and type II syndrome. Type II syndrome involves primarily an action in the central nervous system, whereas with the type I syndrome, peripheral nerves are also involved (Ecobichon, 1991). Both type I and II pyrthroid insecticides affect the sodium channels in the nerve membranes, causing repetitive neuronal discharge, with effects being quite similar to those produced by DDT. There appears to be a prolongation of sodium influx with a delay in the closing of the sodium activation gate, resulting in an increased and prolonged sodium tail current (Narahashi, 1986; Bradbury and Coats, 1989; Ecobichon, 1991). Type II pyrethroids prolongs the sodium channel open-time much more drastically than type I pyrethroids (Narahashi, 1986). Also other sites of action have been noted for the pyrethroid insecticides. Some of them inhibit Ca^{2+}, Mg^{2+}-ATPase, thereby interfering with calcium removal from the nerve endings, resulting in increased neurotransmitter release in the postsynaptic gap. In addition, the protein calmodulin, responsible for the intracellular binding of calcium ions to reduce spontaneous neurotransmitter release, can be inhibited. Type II pyrethroids have also been shown to bind to the GABA-receptor chloride channel complex, blocking chloride ion transport into the nerve cell. To summarize, there are many similarities between the mechanism of action of pyrethriod insecticides and organochlorines, consequently there is a risk for additive or even synergistic effects.

Herbicides

Herbicides may be classified in a number of ways. In this literature review I will classify the herbicides according to their chemical structure, despite the fact that there is a tremendous overlap of biological effects for a variety of chemical structures. Herbicides are produced to kill or injure plants and therefore affect various mechanisms associated with e.g. photosynthesis, respiration, growth, cell and nucleus division, or synthesis of proteins, carotenoids or lipids (Ecobichon, 1991).

The herbicide glyphosate, which is the active substance in the commonly used preparation Roundup™, controls weeds by inhibiting a single plant enzyme, EPSPS (5-enolpyruvoylshikimate 3-phosphate synthase; Sikorski and Gruys, 1997; Baylis, 2000). EPSPS is a key enzyme in the aromatic amino acid biosynthetic pathway and a blockage of this enzyme, results in severe effects on protein synthesis. Although EPSPS is the only known enzyme target of glyphosate, the herbicide affects many physicochemical and physiological processes (Cole, 1985). Among these are the reduction in photosynthesis and the degradation of chlorophyll, as well as inhibited transport of the plant growth hormone auxin and enhancement of auxin oxidation.

The commonly used chlorophenoxy herbicides, including 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), and MCPA (4-chloro-*o*-toloxyacetic acid) mimic the action of the growth hormone auxin in plants (Ecobichon, 1991; Grossmann, 2000). When the herbicides are present in low concentrations at the cellular site of action, growth by cell division and elongation is usually stimulated. However, with increasing concentrations, a variety of growth abnormalities are induced within 24 h of treatment (Grossmann, 2000). These include curvature of leaves and stem, growth inhibition of shoot and root, and intensified leaf pigmentation. A number of biochemical reactions are involved in the development of these abnormalities in the plants, e.g. overproduction of ethylene, leading to stomata closure and thereby carbon assimilation inhibition. Eventually, the exposure results in desiccation, necrosis and plant death.

Fungicides

The breakdown of organic molecules provides energy for the survival of living systems. In fungi, as well as in other eukaryotes, a part of this catabolic process take place in the mitochondria and lead to the synthesis of the high energy intermediate ATP. Several groups of fungicides disturb the energy supply in fungi and all such compounds are powerful inhibitors of spore germination (Leroux, 1996). Among them are the dithiocarbamates (e.g. maneb and thiram) and the R-S-CCl_3 compounds (e.g. captan and dichlofluanid). These fungicides have a multisite action by inhibiting several enzymes involved in the respiratory processes.

Another group of fungicides, the phenylpyrroles, including fenpiclonil and iprodionel are known to inhibit spore germentation and induce morphological alterations of germ tubes, i.e.

inhibit germ-tube elongation (Leroux *et al.*, 1992). In addition these fungicides are able to uncouple oxidative phosphorylation and to inhibit electron transport in respiration processes. However, more recent studies indicate that the main effect induced by fenpiclonil is an inhibition of wall glycan biosynthesis and an accumulation of natural sugars. These effects indicate that the mechanism of action may be related to glucose metabolism (Jespers and de Waard, 1995). The effect of phenylpyrroles in fungi are thereby very close to the effect of the herbicide dichlobenil in plants. According to Delmer *et al.* (1987), dichlobenil may interfere with a membrane-bound protein involved with the regulation of the *ß*-glucan synthesis. Consequently there is a risk for additive or even synergistic effects when the phenylpyrroles fungicides and the herbicide dichlobenil are present at the same time in the environment.

Pesticide toxicity to non-target aquatic microorganisms

Microorganisms are important inhabitants of aquatic ecosystems, where they fulfill critical roles in primary productivity, nutrient cycling, and decomposition. Microorganisms span three kingdoms, including > 50,000 different species of bacteria, algae, fungi, and protozoa. They cover a tremendous range of size classes and morphologies, exist in every habitat, and include multiple feeding types, mobile, and nonmobile forms, and a variety of reproductive strategies and growth rates. The majority of the available pesticide studies regarding aquatic microorganisms describe effects on algae. Studies of herbicide effects dominate, in particular effects caused by atrazine. Far fewer pesticide studies exist for aquatic bacteria, fungi, and protozoa. Recently, DeLorenzo *et al.* (2001) published an extensive review on pesticide toxicity to aquatic microorganisms. The mechanism of pesticide action in microbial species may not be the same as for the target-organisms. In microorganisms, pesticides have been shown to interfere with respiration, photosynthesis, and biosynthetic reactions as well as cell growth, division, and molecular composition (DeLorenzo *et al.*, 2001).

Insecticides

Few studies address direct toxic effects of insecticides to aquatic microorganisms. However, the toxicity of the two carbamate insecticides carbaryl and carbofuran to algae and cyanobacteria were tested in expected environmental concentrations (EEC) by Peterson *et al.* (1994). The EEC is a concentration estimate of a worst-case exposure scenario for an aquatic habitat in a lotic system, based on the input of the maximum proposed application rate. Peterson *et al.* (1994) showed that growth inhibition, following carbaryl exposure (3.7 mg/L), ranged from 35% to 86% with the cyanobacteria *Pseudoanabaena* and *Anabaena inaequalis* being the most sensitive species tested. Actually, >50% inhibition was found in nine of the ten species included in the study. In contrast, carbofuran had relatively low toxicity to most species tested when applied at the EEC of 0.67 mg/L. Also the organophosphorus insecticides fenitrothion and pyridaphenthion cause growth inhibition in algae and cyanobacteria (Sabater and Carrasco, 2001a; Sabater and Carrasco, 2001b). The EC_{50} (96 h) for fenitrothion and

pyridaphenthion ranged from 0.84 to 5.5 mg/L for the green algae *Scenedesmus acutus*, *Scenedesmus subspicatus*, and the cyanobacteria *Pseudanabaena galeata* and from 5.7 to 30.9 mg/L for the green algae *Chlorella vulgaris* and *Chlorella saccharophila*. *Scenedesmus acutus* was the most sensitive species for both insecticides and its EC_{10} (96 h) range from 0.14 to 0.32 mg/L, which is lower than the concentrations found in natural waters (Sabater and Carrasco, 2001a; Sabater and Carrasco, 2001b).

In contrast to single species tests, DeLorenzo *et al.* (1999a), tested the effects of the agricultural insecticides endosulfan and chlorpyrifos on an estuarine microbial food web. Endosulfan was primary found to target the phototropic portion of the bacterial community, i.e. the cyanobacteria. Total bacterial abundance, but not heterotrophic bacterial productivity, was reduced with endosulfan treatments of 1 and 10 µg/L. Cyanobacterial taxa like *Melosira* and *Oscillatoria* were among those that were eliminated from the high-endosulfan treatment. In the same study, chlorpyrifos affected many of the phototrophic endpoints tested, e.g. both chlorophyll *a*, and the phototrophic biovolume were reduced in 1 and 10 µg/L. Several diatom genera (*Asterionella, Bacillaria, Gomphonema,* and *Tabellaria*) present in the controls were absent in the 10 µg/L treatment and heterotrophic ciliates and flagellates were reduced. In contrast, the bacterial abundance increased significantly compared to the control when chlorpyrifos was added. However, the changes in the chlorpyrifos treatments occurred mainly in the high dose (10 µg/L). At typical environmental concentrations of chlorpyrifos (<0.02 µg/L; Miles and Pfeuffer, 1997), the microbial populations would be likely to recover before any adverse effects are transferred to higher trophic levels (DeLorenzo *et al.*, 2001). However, Widenfalk *et al.* (2004) recently showed that microbial communities of natural sediments can be affected by low concentrations of pesticides, including the insecticides deltamethrin and pirimicarb. Widenfalk *et al.* (2004) used microcosm experiments to show that deltamethrin and pirimicarb inhibited the bacterial activity, whereas deltamethrin also affected the microbial biomass negatively.

Herbicides

Peterson *et al.* (1994) tested the growth inhibition of green algae (*Scenedesmus quadricauda and Selenastrum capricornutum*), diatoms (*Nitzschia* sp. and *Cyclotella meneghiana*), and cyanobacteria (*Microcystis aeruginosa, Oscillatoria* sp., *Pseudoanabaena* sp., *Anabaena inaequalis* and *Aphanizomenon flos-aquae*) in response to exposure of 23 different pesticides in EECs. Each of the five triazine herbicides tested (atrazine, cyanazine, hexazinone, metribuzin, and simazine; 2.7-2.9 mg/L) and diquat (0.7 mg/L) caused > 50% inhibition of growth in all test species. Later studies by Peterson *et al.* (1997) showed that already 5% of the EEC of hexazinone (0.14 mg/L) inhibited growth more than 80% in both diatoms and green algae. In addition, 0.7-14% of the EEC of diquat (0.005-0.1 mg/L) caused a 50% inhibition of diatoms and cyanobacteria (Peterson *et al.*, 1997). The earlier study by Peterson *et al.* (1994) also revealed that glyphosate (2.8 mg/L) was highly toxic to diatoms and the

nitrogen-fixing cyanobacterium *Anabaena flos-aquae* at EECs, but relatively non-toxic to the remaining algal species. In contrast, Peterson *et al.* (1994) found that four sulfonylurea herbicides (e.g. chlorsulfuron) caused minor or no inhibition of any of the algal species at EECs (0.003-0.020 mg/L). In fact, each of the sulfonylurea herbicides caused stimulation of growth in either a diatom or a cyanobacteria. The phenoxyalkane herbicide 2,4-D (2.9 mg/L) did not either cause any growth inhibition, whereas MCPA (1.4 mg/L), also a phenoxyalkane, inhibited growth in one species of the green algae, diatoms, and cyanobacteria, respectively (Peterson *et al.*, 1994).

In contrast to Peterson *et al.* (1994), Nyström *et al.* (1999) found that two sulfonylurea herbicides, chlorsulfuron and metsulfuronmethyl, caused severe growth inhibition in the cyanobacteria *Synechococcus leopoliensis* at concentrations lower than the EEC. In addition, the cyanobacteria *Anabaena flos-aquae* and two marine algae, *Amphidinium cararae* and *Nodularia harveyana*, had EC_{50}-values below or equal to the EEC-value for metsulfuronmethyl (Nyström *et al.*, 1999). However, the species-dependent variation in sensitivity to sulfonylurea herbicides among algal taxa appears to be very high. Among the 20 freshwater and the 20 marine species investigated by Nyström and coworkers (1999), the lowest and the highest EC_{50}-value differed six orders of magnitude. Cyanobacteria and dinoflagellates seem to be especially sensitive to the sulfonylurea herbicides, according to this study.

Although a number of studies have addressed herbicide effects on single species of algae or bacteria (DeLorenzo *et al.*, 2001), few studies have focused on the interaction between pesticides and natural sediment microbial communities, including benthic algae, bacteria, fungi, and protozoans. However, DeLorenzo *et al.* (1999b) showed that the herbicide atrazine induced functional and structural changes of estuarine microbial communities. Atrazine, in the concentrations 40 and 160 µg/L, was found to reduce chlorophyll *a*, phototrophic carbon assimilation, and phototrophic biovolume in test chambers, simulating tidal creek ecosystems. Further more, Widenfalk *et al.* (2004) recently exposed microbial communities of a natural sediment to concentrations, that are consider to be environmentally safe, of the phenylurea herbicide isoproturon. It was shown that isoproturon inhibited the bacterial activity and negatively affected the microbial biomass. However, the observations did not show a consistent, inverse relationship with pesticide concentration, indicating the difficulty with proposing the effects of low pesticide doses to microorganisms.

Fungicides

According to the review by DeLorenzo *et al.* (2001) there is very little information available about agricultural fungicide toxicity to aquatic microorganisms. Knowledge about fungicide toxicity in low concentrations is even scarcer. However, in a single species test Peterson *et al.* (1994) compared the sensitivity of ten algal species to the triazole derivative fungicide

propiconazole. At the EEC of 0.08 mg/L, inhibition of ^{14}C uptake was <20% to all species of algae. Propicanazole, actually stimulated growth to some extent in the cyanobacteria. The commercial propiconazole formulation stimulated growth in the diatoms.

In whole microbial community tests, low concentrations (10 µg/kg) of the commonly used fungicide fenpropimorph, decreased denitrification in a lake sediment (Svensson and Leonardson, 1992). Since fenpropimorph has lipophilic properties it will probably adsorb to organic sediment particles and constitute a persistent threat to microorganisms. Prolonged exposure also resulted in an enhanced effect on the denitrification bacteria (Svensson and Leonardson, 1992). In addition, Widenfalk *et al.* (2004) showed that environmentally safe concentrations of the phtalimide fungicide captan affected bacterial activity in a natural sediment. In contrast, microbial biomass, respiration, and denitrification were not affected by captan. Surprisingly, the negative effect on bacterial activity, observed at environmental safe concentrations, disappeared at a higher test concentration, indicating a non-linear relationship of the toxicity.

Pesticide toxicity to aquatic invertebrates

Benthic communities are functionally important in transferring environmental contaminants to higher trophic levels (fish, waterfowl and humans), and play a key role in sediment energy, nutrient, and contaminant fluxes (Burton *et al.*, 1992; Horne and Goldman, 1994). Accumulation of toxic pesticides in the sediments may hit key organisms/processes and thereby cause serious damage to the ecosystem. Routes of pesticide exposure in aquatic invertebrates include uptake from pore water and overlying water across body walls and across respiratory surfaces, and ingestion of contaminated sediment particles (Power and Chapman, 1992). The bioavailability, and hence the toxicity, is highly dependent on the sorptive behavior of the pesticide, i.e. if the pesticide is predominantly sediment-sorbed or dissolved in the water. The sediment is a highly complex medium and its sorption characteristics are for contaminants largely dependent on the quality and quantity of organic matter (e.g. chemical stability and cation exchange capacity) and pH (Landrum *et al.*, 1996; Davies *et al.*, 1999; Gunnarsson *et al.*, 1999; Lacey *et al.*, 1999). For example, strong associations of contaminants with humic acid coatings may render these contaminants biologically unavailable (Decho and Luoma, 1994), while contaminants associated with other food sources (e.g. artificial food, phytoplankton, and biofilms) may be highly bioavailable (Lotufo *et al.*, 2000; Schlekat *et al.*, 2000). In all cases, if the pesticide is bioavailable, there will be uptake in the organism and interaction with a receptor site, which may elicit measurable biological effects.

Whole-sediment toxicity tests with benthic macroinvertebrates are important tools in risk assessments of pesticides (Streloke and Köpp, 1995; U.S. Environmental Protection Agency,

2000; Organisation for Economic Co-operation and Development, 2001a; Organisation for Economic Co-operation and Development, 2001b). It is, however, important to realize that there are numerous sources of variation associated with toxicity tests performed in laboratory environment. In a recent study by Goedkoop and Peterson (2003) the effects of bioturbation and sediment organic matter on the toxicity of lindane to *Chironomus riparius* larvae were tested. It was shown that both larval burrowing activity and sediment organic matter strongly modified test conditions, and consequently the toxicity of lindane. Earlier, confounding effects of food additions on the toxicity test results have been addressed in various studies (Ankley *et al.*, 1993; Ristola, 1995; Harkey *et al.*, 1997; Ristola *et al.*, 1999). In addition, a recent study by Åkerblom and Goedkoop (2003) clearly showed that *Chironomus riparius* in standardized toxicity test to a high degree selectively feeds on added uncontaminated food particles. This preferential feeding behavior affects exposure pathways and ultimately toxicity test results. With all these confounding factors in mind, it is very difficult to compare toxicity tests with different experimental designs, e.g. different test sediments, number of individuals per area, and feeding regimes.

However, several studies have shown the toxicity of currently used pesticides to aquatic invertebrates (e.g. Moore *et al.*, 1998; Schroll *et al.*, 1998; Woin, 1998; Conrad *et al.*, 1999). Moore *et al.* (1998), for example, showed that the invertebrates *Daphnia magna*, *Hyalella azteca*, and *Chironomus tentans* were ≥200 times more sensitive than the fish fathead minnow (*Pimephales promels*) to the AChE inhibiting organophosphorus insecticide chlorpyrifos. In the 48 h acute toxicity tests they also showed that *C. tentans* was, not surprisingly, the most sensitive species to the organochlorine chlordane and the carbamate aldicarb with LC_{50}-values of 5.8 and 20 µg/L, respectively. The most sensitive species to chlorpyrifos was, however, *H. azteca* with a LC_{50}-value of 0.1 µg/L. Effects of chronic application of chlorpyrifos on invertebrates were studied in outdoor artificial streams by Ward *et al.* (1995). Ninety-eight different taxa, including 24 chironomid taxa, were recorded during the study. Both the number of taxa and total invertebrate abundance were reduced in response to low (0.1 µg/L) and high (5 µg/L) chlorpyrifos concentrations. The Shannon-Weaver diversity decreased in both concentrations, whereas the evenness increased in the high dose stream. The subsequent change in overall taxonomic composition, including decreased invertebrate grazing pressure, could possibly lead to increased periphyton growth and perhaps even stimulate algal blooms (Ward *et al.*, 1995).

Lindane has been extensively used and is frequently found in aquatic environments (Hoffman *et al.*, 2000; Pérez-Ruzafa *et al.*, 2000; Ulén *et al.*, 2002). Schultz and Liess (1995) summarized available toxicity data of lindane and parathion for several aquatic macroinvertebrates. Lindane was acutely toxic (EC_{50} and LC_{50} 24-96 h) in the range 9.6-7300 µg/L, which indicate the variability in sensitivity among species. Hirthe *et al.* (2001) showed that short-term exposure (48h) to 500 µg/L lindane to 4th instar larvae of *Chironomus riparius* affected organisms during the exposure, but also after the exposure as adults. The normal larval behavior, e.g. burrowing, was affected, the emergence was delayed for both sexes, and

as adults the midges got shorter wings and lower fecundity. A similar result was obtained when *C. riparius* larvae were exposed to much lower concentrations of lindane as 2nd instars over 10 days (Maund *et al.*, 1992). The emergence time increased by 20 % and 45 %, respectively, in larvae exposed to 0.8 and 2.0 µg/L in comparison with emergence in the control. In addition, the larval growth was reduced at 1 µg/L lindane. The caddisfly *Limnephilus lunatus* is among the most sensitive species to lindane exposure (Schulz and Liess, 1995). The emergence, and hence survival rate, of the caddisfly was reduced by lindane exposure (90d) at 0.1 ng/L, a value nearly five orders of magnitude lower than the 96 h LC_{50}.

Parathion, with acute and chronic toxicity similar to that of lindane, did surprisingly not, alter the emergence rate of *Limnephilus lunatus* (Schulz and Liess, 1995). In contrast, this substance reduced the emergence rate of the closely related species *Limnephilus bipunctatus* Curtis at 1 ng/L, even though this species was less susceptible than *L. lunatus* to parathion at higher concentrations. Mulla and Mian (1981) reviewed the biological and environmental impacts of parathion and malathion on the aquatic ecosystem. Immature non-target insects, e.g. caddisfly, stonefly, and mayfly, showed high sensitivity to these pesticides, with LC_{50}-values (24-96 h) in the range 1-6 µg/L.

Some of the modern insecticides are extremely toxic to non-target aquatic invertebrates (Bradbury and Coats, 1989; Woin, 1998; Conrad *et al.*, 1999; Schulz and Liess, 2001a; Schulz and Liess, 2001b; Hedlund, 2002; Friberg-Jensen *et al.*, 2003; Wendt-Rasch, 2003; Wendt-Rasch *et al.*, 2003b). The pyrethriod deltamethrin, for example, affects *Chironomus riparius* at concentrations much lower than the detection limit (30 ng/L; Hedlund, 2002). In whole-sediment toxicity tests with artificial sediment the LC_{50} (28d) were 15 pg/L in tests with spiked water and 26 µg/kg dw in spiked sediment tests, indicating the impossibility to detect this pesticide at highly toxic concentrations, which is a serious and devastating problem. Schultz and Liess (2001a) showed that another pyrethroid insecticide, fenvalerate, at short-term exposures at environmentally relevant concentrations causes long-term effects to the trichoptera *Limnephilus lunatus*. Significant-effect concentrations were 0.001 µg/L in water and 0.2 µg/kg in suspended sediment. The results of this study indicate that the effects of exposure to fenvalerate are weaker for all endpoints studied when the insecticide is associated with suspended particles (5 g dw/L) than when fenvalerate is in the aqueous phase. The effect of particle-associated fenvalerate, on stream macroinvertebrates, was further studied by Schultz and Liess (2001b) by simulating runoff events in outdoor microcosms. Six of the eight exposed species exhibited an increased downstream drift as an acute concentration-dependent response to fenvalerate runoff simulation, with the amphipod *Gammarus pulex* and the trichoptera *Hydropsyche angustipennis* as the most sensitive species. Particle-associated fenvalerate caused sublethal responses at 1 µg/kg and lethal effects at 10 µg/kg, indicating that both sublethal acute effects and lethal chronic effects may be expected following short-term exposure during runoff events (Schulz and Liess, 2001b). Earlier, Woin (1998) showed that fenvalerate had both direct and secondary effects on the structure of pond ecosystems when studying freshwater mesocosms, simulating natural fish-free eutrophic ponds. The

direct effect of pesticide exposure was death of many invertebrate taxa, especially insects, while the secondary effects were altered interspecies relationships and life history cycles, leading to ecosystem breakdown.

Even though it may be difficult to compare toxicity data due to a variety of confounding factors (see above), I think it is possible to draw the conclusion that single pesticide exposure can be hazardous to aquatic invertebrates even at low exposure concentrations. It is important to remember that pesticide exposure in the field seldom is a single compound exposure, but rather an exposure of a mix of substances with the possibility to additive or even synergistic effects.

Joint toxicity of pesticides

Additive effects can be expected for compounds with a similar mode of action, while synergistic effects trigger a more than additive effect in the exposed organism due to e.g. changes in chemical biotransformation (Belden and Lydy, 2000). There are a few studies indicating joint toxicity of pesticides in aquatic invertebrates (Bailey *et al.*, 1997; Pape-Lindström and Lydy, 1997; Belden and Lydy, 2000). For example, Bailey and coworkers (1997) showed that two organophosphorus insecticides, diazinon and chlorpyriphos, exhibit additive toxicity to *Ceriodaphnia dubia*. Additivity between diazinon and chlorpyriphos is reasonable given that both are metabolically activated organophosphorus insecticides and act similarly with respect to binding with AChE (Ecobichon, 1991).

The herbicide atrazine is not acutely toxic (96 h), even at high concentrations (20,000 µg/L), to larvae of the midge *Chironomus tentans* (Pape-Lindström and Lydy, 1997). However, atrazine in binary combination with the organophosphorus insecticides chlorpyrifos, malathion, trichlorfon, and methyl parathion exhibit greater than additive (synergistic) toxicity to the fourth instar of the midge. In contrast, exposure of the organophosphorus mevinophos or the organochlorine methoxychlor together with atrazine results in less than additive toxicity to the larvae (Pape-Lindström and Lydy, 1997). Pape-Lindström and Lydy, suggest two possible explanations for the synergistic toxicity of atrazine and several organophosphorus insecticides. First, atrazine may increase the penetration of the insecticides through the midge cuticula or increase the cellular permeability. Second, atrazine may increase the biotransformation of the organophosphorus insecticides, converting them into more toxic metabolites. In a later study, Belden and Lydy (2000), reported synergistic toxicity to *Chironomus tentans* larvae at much lower concentrations of atrazine (10-200 µg/L) in combination with the organophosphorus chlorpyrifos and methyl parathion. In addition, exposure to the organophosphorus diazinon showed similar results. Already at 40 µg/L atrazine, the EC_{50}-value (representing unnormal larval swimming motion when pinched with a pair of forceps in 50% of the population) of chlorpyrifos had decreased to approximately half the value when no atrazine was added (from 0.44 µg/L to 0.24 µg/L). The mechanisms for the synergistic toxicity were investigated and found to be similar to those earlier suggested by Pape-Lindström and Lydy (1997). Atrazine caused an increased uptake rate of the

organophosphorus insecticides, however, the following increase in toxicity would be minimal as compared to the nearly 50 % decrease in the EC_{50}-value. Instead, body residue analyses of exposed larvae indicated that larger amounts of metabolites were generated in atrazine-treated larvae as compared to the controls. Additionally, *in vitro* assays showed that atrazine-treated larvae generated an increase in a specific toxic chlorpyrifos metabolite. Therefore, the increase in toxicity is thought to result from an increase in biotransformation rates of the organophosphorus insecticides resulting in metabolites more toxic than the parent compounds (Belden and Lydy, 2000). In contrast to the earlier study by Pape-Lindström and Lydy (1997), Belden and Lydy (2000) could not reveal any synergistic toxicity between atrazine and malathion. This discrepancy was explained by the difference of malathion's chemical structure compared to the other organophosphorus insecticides (an aliphatic side chain instead of an aromatic side chain). The difference in malathion's structure may result in slightly different biotransformation pathways that reduce the influence of atrazine, unless atrazine is present at very high levels. These finding indicate that two commonly used pesticides, in environmentally relevant concentrations, may constitute synergistic toxicity towards aquatic invertebrates.

Pesticide toxicity to fish

Pesticide toxicity to fish has been investigated in several studies (e.g. Hamelink and Spacie, 1977; Kumaraguru and Beamish, 1981; Mulla and Mian, 1981; Barry *et al.*, 1995; Steinberg *et al.*, 1995; Moore and Waring, 1996; Waring and Moore, 1997; Moore *et al.*, 1998; Csillik *et al.*, 2000; Moore and Waring, 2001). However, fish are not usually target organisms for pesticides, and knowledge about effects of pesticides in the field is still sparse. Suprisingly, only a few studies have shown that fish, inhabiting natural freshwater ecosystems, may be affected by unintentional spreading of pesticides (Bálint *et al.*, 1997; Csillik *et al.*, 2000).

Herbicides are often regarded as relatively harmless to fish. Direct effects caused by, for example, the herbicide atrazine are scarce. However, studies have demonstrated that atrazine concentrations as low as 5 µg/L affect the swimming behavior of zebrafish (*Brachydanio rerio*; Steinberg *et al.*, 1995). The fish was exposed for atrazine in low concentrations for four weeks, during which its preference of light or dark habitats was investigated. Already in the lowest concentration tested (5 µg/L; more than three orders of magnitude below acute toxicity data) the zebrafish altered its swimming behavior and preferred the dark habitats. As a consequence, the feeding behavior of individuals or schools and the schooling behavior may change, possibly increasing predatory susceptibility.

Reproduction disorders in response to low pesticide concentrations

During periods of reproductive activity, fish are frequently physiologically stressed and their susceptibility to naturally occurring hazards such as diseases and predation may increase. Additionally, reproducing fish may be exposed to a range of anthropogenic compounds,

including pesticides. This may increase the risks associated with reproduction and also increase the susceptibility of spawning fish to toxicants compared with non-spawning individuals. The pyrethroid insecticides, for example, have been shown to affect mechanisms involved in fish reproduction (Barry *et al.*, 1995; Tanner and Knuth, 1996; Moore and Waring, 2001). Moore and Waring (2001) revealed that exposure of mature male salmon parr (*Salmo salar*) to low concentrations (≤0.028 µg/L) of cypermethrin inhibited the ability for salmon males to detect and respond to the female salmon priming pheromone prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). The increase in expressive milt and the levels of plasma sex hormones were reduced in the presence of the pyrethroid as a result of impaired olfactory detection of the priming pheromone. The authors suggest that the exposure to the pyrethroid acted directly on the sodium channels and thereby inhibited the nervous transmissions within the olfactory system. Exposure of salmon eggs and milt to cypermethrin also reduced the level of fertilization, suggesting a further toxic impact of the insecticide on salmon reproduction (Moore and Waring, 2001). Another pyrethroid, esfenvalerate, has earlier been shown to cause a reduction in the fecundity and failure of eggs to hatch among the Australian crimson-spotted rainbowfish (*Melanotaenia fluviatilis*; Barry *et al.*, 1995) and further, reduced fry growth, delayed spawning and reduced hatching in bluegills (*Lepomis macrochirus*; Tanner and Knuth, 1996).

Also other pesticides, originating from very diverse pesticide groups, inhibit the ability of male salmon to detect and respond to female priming pheromones, and may therefore affect salmon reproduction success (Moore and Waring, 1996; Waring and Moore, 1997; Moore and Waring, 1998). The two insecticides diazinon (organophosphorus) and carbofuran (carbamate), showed similar effects as cypermethrin at concentrations as low as 1.0 µg/L (Moore and Waring, 1996; Waring and Moore, 1997). Also the herbicide atrazine, which seems to be a relatively harmless toxicant for fish, with calculated 96 h LC_{50}-values of >18 and 6.3 mg/L for rainbow trout and brook trout, respectively, affect mechanisms involved in salmon reproduction. The male salmon response to female pheromones was affected already at 0.04 µg atrazine/L, with reduced priming effect on milt and reduced plasma sex steroid levels as a result (Moore and Waring, 1998).

Are pesticides a threat to the aquatic environment?

Few studies provide evidence for ecological disturbance in natural aquatic environments due to unintentional pesticide spreading. However, the major threat to the aquatic ecosystems may be the lack of information available about pesticides. Even though we know that the mode of action for several groups of pesticides is similar (Narahashi, 1986; Bradbury and Coats, 1989; Ecobichon, 1991; Belden and Lydy, 2000), there is little information available about joint toxicity. Agricultural pesticides are often used in combination with each other to protect crops, so the risk for additive or even synergistic effects is obvious. In a field-study, Liess and Schulz (1999) and Schulz and Liess (1999) investigated runoff-related insecticide input

(parathion-ethyl, fenvalerate, and deltamethrin) on stream macroinvertebrate dynamics. Transient increase in discharge and insecticide contamination (maximum 6µg/L parathion-ethyl in water and 302 µg/kg fenvalerate in suspended particles) was observed in the stream subsequent to surface runoff from arable land. In the macroinvertebrate community, eight of eleven abundant species disappeared, and the remaining three were reduced significantly in abundance following the three most highly contaminated runoff events (Liess and Schulz, 1999; Schulz and Liess, 1999). In microcosms, operated in the bypass to the stream, the hydraulic stress during runoff events was eliminated (Liess and Schulz, 1999). By using the in-parallel bypass microcosms, the authors were able to distinguish the effects of the insecticides from the hydraulic stress also associated with surface runoff. They concluded that hydraulic stress only plays a minor role for the population reduction, whereas the agricultural insecticide input altered the dynamics of the total macroinvertebrate community and thereby constitute a serious threat to the stream ecosystem. Interestingly, the reaction of *Gammarus pulex* and *Limnephilus lunatus* were stronger in this field and microcosm study than the response predicted by laboratory data (Liess and Schulz, 1999). Also the insecticide endosulfan caused negative effects on the macroinvertebrate community following runoff events from cotton fields in the catchment of Namoi River in Australia (Leonard *et al.*, 1999). In this field study, five of the six dominant taxa were affected by elevated endosulfan concentrations. However, other factors were collinear with endosulfan and could be important in explaining the variation in the densities of the studied taxa. For example, water temperature, turbidity, and elevated concentrations of the herbicides prometryn and trifluralin are mentioned, although these factors are not relevant according to the authors. The presence of profenofos, on the other hand, cannot be discounted, despite the weaker correlation to the densities of the studied taxa than endosulfan. Profenofos is an organophosphorus insecticide that is extremely toxic to fish and macroinvertebrates. A similar organophosphorus insecticide is known to have toxicological synergistic effects with endosulfan (Arnold *et al.*, 1995).

Also devastating fish deaths have been recorded in response to unintentional spreading of pesticides. In 1991 and 1995 huge amounts of eels (*Anguilla anguilla*) died in the largest freshwater lake in Europe, Lake Balaton in Hungary (Bálint *et al.*, 1997). Several research institutes were involved to determine the factors that could have contributed to the massive fish death. Water temperature and eutrophication, the parasite *Anguillicola crassus*, bacterial infections, and pesticides were among those factors investigated. Even though there are several contradictory opinions about the reason for the eel death in Lake Balaton, the main reason seems to be pyrethriod intoxication. Both deltamethrin and permethrin were used in the area against mosquitoes. The insecticides were found in fish tissues, with the highest residues in gills. In addition, both eels and other fish species had a strong inhibition of AChE, which is a common symptom of pesticide exposure. The fact that mainly eels were affected was explained as a micro-layer theory, i.e. the insecticides formed a very thin layer on the top of the water. Eels often poke their head out of the water, and thereby got exposed to the insecticides. Even though the effects are clear and devastating as in this case, extensive

research is needed to be able to exclude other factors than pesticides. There are even more complicated cases when the effects are not so clear and easily measured. In the UK, for example, another pyrethroid, cypermethrin is mainly used in sheep dips to prevent and treat ticks, lice, and scab on sheep and as a treatment against infestation by the parasitic sea louse (*Lepeophtheirus salmonis*) in intensive salmonid aquaculture. The sheep are usually treated late in the year (October) and cypermethrin has been found in many salmon spawning rivers and tributaries during Novermber and December (Moore and Waring, 2001). Pyrethroids are more toxic at low than at high temperatures in mammals, fish, and insects (Kumaraguru and Beamish, 1981; Song and Narahashi, 1996). The risk for toxic effects by pyrethroids in the aquatic ecosystem is therefore higher during the cold season, during which e.g. salmon spawning occurs. In addition to the direct possible negative effects on salmon reproduction, the pyrethroids may effect olfactory imprinting to the natal river and thereby seriously decrease the ability of the mature salmons to return to the native river for spawning (Moore and Waring, 2001). Such effects are difficult to quantify, explaining that there are yet no studies made on pyrethroid effetcs on salmon spawning areas. Further, pyrethroid insecticides are toxic in very low concentrations, sometimes in concentrations much lower than the detection limit (Hedlund, 2002), further complicating the detection of toxic levels in natural water.

Direct effects of pesticide exposure are sometimes difficult to detect. However, indirect effects are even more complicated to describe. Woin (1998) showed that the pyrethroid fenvalerate resulted in indirect structural changes in a macroinvertebrate community when studying mesocosms. More than 2 years after the fenvalerate treatment, the most exposed system (1.3 µg/L) was still different compared with non-exposed ones, suggesting that non-persistent pesticides may produce detrimental effects resulting in long-term changes at the ecosystem level of organization (Woin, 1998). Also Wendt-Rasch (2003) studied ecological indirect effects of pesticides in freshwater model systems exposed to concentrations within the range that may contaminate natural aquatic ecosystems following normal agricultural practices. A proliferation of algae was observed in the microcosms following exposure to both the pyrethroid insecticide cypermethrin (Friberg-Jensen *et al.*, 2003; Wendt-Rasch *et al.*, 2003b) and the sulfonylurea herbicide metsulfuron methyl (Wendt-Rasch *et al.*, 2003a). This suggests that exposure to pesticides may shift an aquatic ecosystem over to an algal dominated turbid state, i.e. cause alterations similar to changes induced by eutrophication. It is thus not unlikely that pesticide contamination contribute to the effects caused by increased nutrient loading in agricultural dominating areas. Since exposure to both herbicides and insecticides may induce a competitive advantage for algae in relation to macrophytes, it is possible that the indirect effects of these two groups of pesticides can act synergistically at the ecosystem level, even though their direct toxic effects are exerted through very different modes of action.

In addition to the parent compounds, we also need to consider that most pesticides applied in agriculture are transformed by physical, chemical, and biological processes into one or more transformation products. Both parent pesticides and transformation products may exert a toxic action in aquatic organisms, whenever the concentration is sufficient to trigger such an effect. In fact, the transformation product is sometimes more toxic than the parent compound (Belden and Lydy, 2000). In spite of this, the environmental fate and toxic effects of pesticide transformation products are seldom published in the scientific literature. Belfroid *et al.* (1998) tried to summarize and evaluate available physico-chemical and ecotoxicological information on transformation products of 20 widely used pesticides in the Netherlands. For the 78 transformation products included in the study, the available information was scarce and often not reliable (figure 3 and 4). In many cases there is a need for more information, especially on the persistency and no-observed effect concentrations of the pesticide's transformation products. For approximately one third of the transformation products, the amount of physico-chemical information is sufficient, but seldom reliable. For over 50 %, however, the physico-chemical information is considered not sufficient (figure 3). The ecotoxicological information for the transformation products is also scarce, with no information available for more than 45 % of the compounds (figure 4). Consequently, this lack of information constitutes a huge problem and there are reasons to believe that pesticide transformation products are potential threats to the aquatic environment.

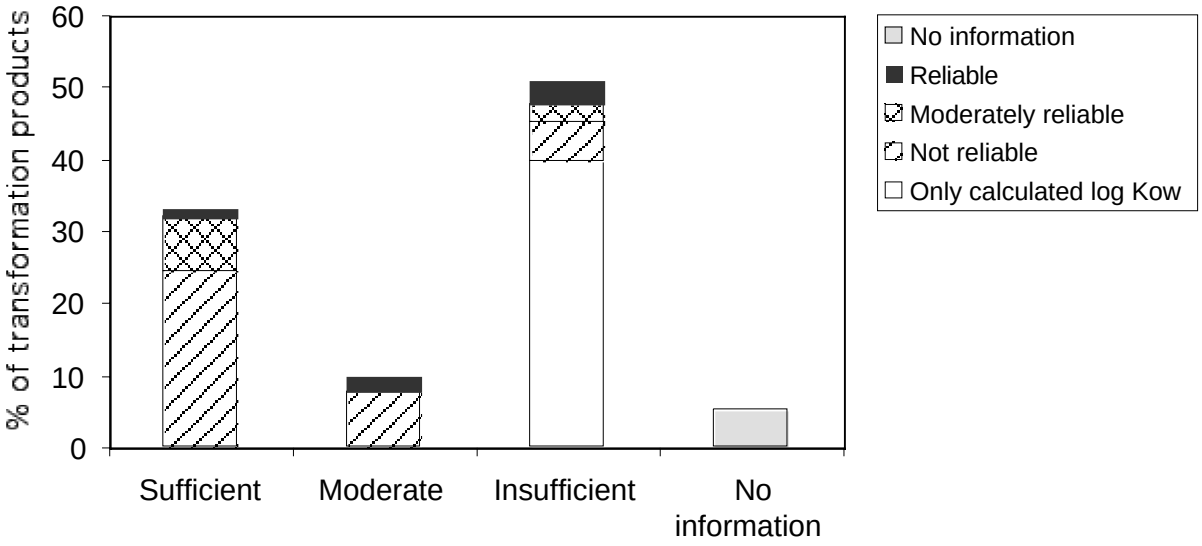

Figure 3. *Availability and reliability of physico-chemical data for 78 transformation products of pesticides (modified from Belfroid et al. (1998)).*

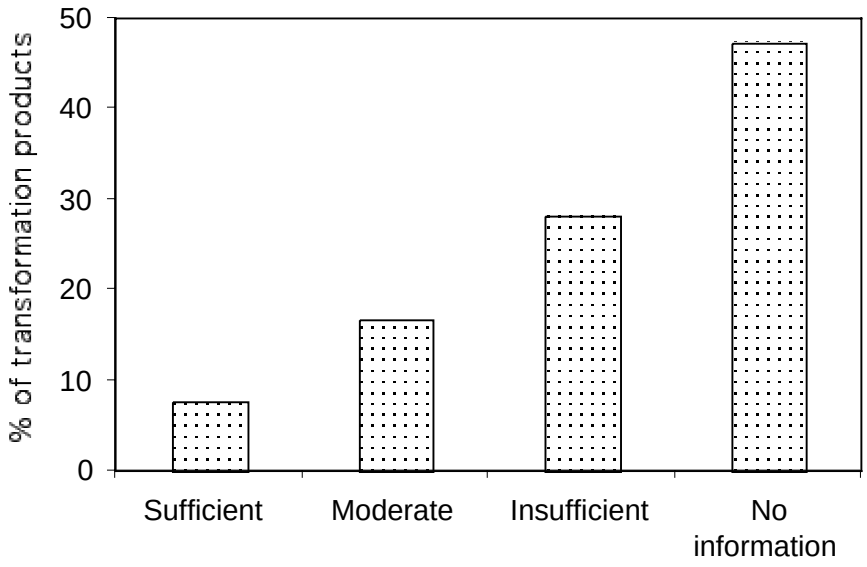

Figure 4. *Availability of ecotoxicological data for 78 transformation products of pesticides. Compounds are divided in four groups for which sufficient (> 10 data), moderate (6-10 data), few (1-5 data) or no information is available. The reliability of the data set is considered to be sufficient. (Modified from Belfroid et al. (1998)).*

In conclusion, few studies have clearly accounted for the cause and effect regarding suspected pesticide contamination of the aquatic environment. However, a huge amount of pesticides are used and they are frequently detected in aquatic ecosystems. In future research we need to consider, not only direct effects of single parent compounds, but also indirect effects caused by mixtures of pesticides, including possible pesticide transformation products.

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Appendix

Pesticide	Pesticide group	Pesticide function
2,4-D	chlorophenoxy	herbicide
2,4,5-T	chlorophenoxy	herbicide
aldicarb	carmamate	insecticide
ametryn		herbicide
atrazine	triazine	herbicide
bentazone		herbicide
bromacil		
captan	phtalimide (R-S-CCl_3)	fungicide
carbaryl	carbamate	insecticide
carbofuran	carbamate	insecticide
chlorpyrifos	organophosphorus	insecticide
chlorsulfuron	sulfonylurea	herbicide
cyanazine	triazine	herbicide
cypermethrin	pyrethroid	insecticide
DDT	organochlorine	insecticide
deltamethrin	pyrethroid	insecticide
diazinon	organophosphorus	insecticide
dichlobenil		herbicide
dichlofluanid	R-S-CCl_3	fungicide
dichlorprop		herbicide
diquat		herbicide
endosulfan	pyrethroid	insecticide
endrine	organochlorine	insecticide
esfenvalerate	pyrethroid	insecticide
etofumesate		herbicide
fenitrothion	organophosphorus	insecticide
fenpiclonil	phenylpyrroles	fungicide
fenpropimorph		fungicide
fenvalerate	pyrethroid	insecticide
glyphosate		herbicide
hexazinone	triazine	herbicide
iprodionel	phenylpyrroles	fungicide
isoproturon	phenylurea	herbicide
lindane	organochlorine	insecticide
malathion	organophosphorus	insecticide
maneb	dithiocarbamate	fungicide
MCPA	chlorophenoxy	herbicide
mecoprop		herbicide
metazachlor		herbicide
methoxychlor	organochlorine	insecticide

Appendix continuation

Pesticide	Pesticide group	Pesticide function
methyl parathion	organophosphorus	insecticide
metolachlor		herbicide
metribuzine	triazine	herbicide
metsulfuron methyl	sulfonylurea	herbicide
mevinophos	organophosphorus	insecticide
parathion-methyl		insecticide
parathion	organophosphorus	insecticide
permethrin	pyrethroid	insecticide
pirimicarb	carbamate	insecticide
profenofos	organophosphorus	insecticide
prometon		herbicide
propiconazole	triazole	fungicide
pyridaphenthion	organophosphorus	insecticide
simazine	triazine	herbicide
tebuthivron		herbicide
terbuthyazine	triazine	herbicide
thiram	dithiocarbamate	fungicide
trichlorfon	organophosphorus	insecticide