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Review

Invertebrate biomarkers: links to toxicosis that predict population decline

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Abstract

The application of biochemical measurements that can be used as individual biomarkers of impaired biological function in invertebrates is reviewed to evaluate whether biochemical biomarkers of aquatic invertebrates can predict changes in natural populations. Biomarkers that measure toxic effects at the molecular level (e.g., the inhibition of brain acetylcholinesterase activity by organophosphorus pesticides) have been shown to provide rapid quantitative predictions of a toxic effect upon individuals in laboratory studies. Such biomarkers should not be used as a replacement for conventional aquatic monitoring techniques, but should be applied as supplementary approaches for demonstrating links between sublethal biochemical and adverse effects in natural populations in field studies. The research challenge for using biomarker measurements in aquatic invertebrates is to predict effects at the population level from effects at the individual level measured upon individuals collected in the field.

Keywords: Biomarkers; Invertebrates; Population changes; Toxicity; Pesticide resistance; Detoxication enzymes

Contents

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1. Introduction

In Australia, through monitoring initiatives such as the National River Health Program, emphasis has been placed on measuring changes in riverine macroinvertebrate communities or populations (Simpson and Norris, 2000). The problem with measuring changes only at the population level is that only indirect inference can be made with regard to the cause of the population decline, such as the decreases in macroinvertebrate densities that have been measured in cotton-growing regions (Leonard et al., 1999, 2000). Contaminants can cause changes at all levels of biological organization (Table 1). It is now recognized that if the focus of contaminant studies in streams or rivers is only on community change, subtle or chronic biological effects that may result in irreversible long-term changes could be occurring in apparently healthy ecosystems but would not be initially detected (Maher et al., 1999).

Although the population, community, and ecosystem are the important levels at which to monitor toxic effects because of environmental management regulatory purposes, toxic effects are also manifested at the molecular–subcellular level by impaired biological function (Table 1).

This paper reviews the general use of biochemical measurements that can be used as individual biomarkers of impaired biological function in invertebrates. The emphasis is on biomarkers that can be potentially used to assess changes in aquatic macroinvertebrates at population and/or community levels. The lack of knowledge on the linkages between biochemical biomarker variations and macroinvertebrate population response is highlighted. The primary focus of the present review is the measurement of biochemical biomarkers in macroinvertebrates under field conditions, where effects of exposure to environmental chemicals at different levels of biological organization can be examined.

2. Concept of biomarkers

Biomarkers were originally defined as any biochemical, histological, or physiological alterations or manifestations of environmental stress (NRC, 1987). They have been classified as biomarkers of exposure to a toxicant, biomarkers of effects of exposure, or biomarkers of susceptibility to the effects of exposure (Peakall and Shugart, 1993). More recently, this definition has been challenged by several authors (Adams, 1990; McCarty and Munkittrick, 1996; Engel and Vaughan, 1996) and the term biomarker is now more commonly used in a more restrictive sense, namely biochemical sublethal changes resulting from individual exposure to xenobiotics. A summary of the major biochemical biomarkers that have been used to assess impairment of biological function in organisms is given in Table 2. Some of these biomarkers are not applicable to invertebrates because sufficient biological material cannot be obtained for analysis.

For the most part, studies on biochemical biomarkers are carried out in organisms exposed to selected toxicants under controlled laboratory conditions. These laboratory studies can help establish cause and effect relationships that can be applied for predicting effects of specific contaminants on natural populations in the field (Clements, 2000). However, a number of biotic and abiotic factors can influence the extrapolation of individual biomarkers to the field monitoring of contaminant effects at population and community levels (Adams, 1990; Lagadic et al., 1994). The relationships between biomarker responses and population or community structures and functions are complicated because of the compensatory mechanisms which regulate population dynamics in natural systems.

3. Linking the responses of individuals to populations

The use of biochemical biomarkers to measure the exposure of organisms to chemicals has been extensively

Table 1
Levels of biological organization and measurements

Level	Measurement	Function affected
Community	Structure/function	Diversity
Population	Mortality/genetic pool/disease/abundance/recovery rates	Population changes
Organism	Metabolism/fitness/fecundity/behavior/lifespan/susceptibility	Reproduction
Cellular	Metabolism/immunosuppression/fertilization/tumors	Homeostasis and individual mortality
Subcellular	Neurone function/stability of lysosomes/organelles/necrosis/genotoxicity	Homeostasis and individual mortality
Molecular	Enzyme activity/adenylate energy change/RNA:DNA ratio	Metabolic dysfunction and individual mortality

Table 2
Summary of major biomarkers of exposure presently used to assess impaired biological function

Biomarker	Tissue	Use
Mixed-function oxidases	Liver	Indicator of exposure to organic chemicals such as PAHs and PCBs
Glutathione *S*-transferases	Liver	Indicator of exposure to pesticides and metalloids
Cellulase/carbohydrase	Stomach	Indicator of exposure to pesticides
Acetylcholinesterase	Brain	Indicator of exposure to organophosphorus or carbamate pesticides
Carboxylesterase	Various	Indicator of exposure to pyrethroid and carbamate pesticides
DNA strand breakage, adduct formation, chromatid exchange	Various	Indicator of exposure to alkylating or arylating agents
Aminolevulinic acid dehydratase	Blood	Indicator of exposure to lead
Metallothionein	Various	Indicator of exposure to metals
Retinoids	Liver	Indicator of exposure to dioxin and furans
Porphyrins	Liver	Indicator of exposure to chlorinated aromatic hydrocarbons
Adenylate energy change and ATP/ADP ratio	Various	Indicator of exposure to stress
Stress proteins	Various	Indicator of cells experiencing stress
Glutathione	Liver	Indicator of oxidative stress

Note: Adapted from Peakall (1992), McCarthy and Shugart (1990), Benson and DiGiulio (1992).

reviewed (McCarthy and Shugart, 1990; Benson and DiGiulio, 1992; Mayer et al., 1992; Lagadic et al., 1994). Some biochemical biomarkers (e.g., induction of metallothioneins from exposure to trace metals) do not appear to have a direct relationship to a mechanism of toxicity. In this case, the use of the biomarker will not give a reliable prediction of toxic effects upon organisms and is, therefore, only ever likely to indicate exposure to chemicals. In using such biomarkers of exposure, it is not possible to predict effects at the population level from biomarker changes measured in a sample of individuals. To relate effects on individuals to higher levels of biological organization, the biomarker response should be related to a degree of impairment of growth, reproductive output, or metabolic function which directly affects the survival of the organism and which can be attributed to exposure to a known amount of the specific contaminant (Depledge and Fossi, 1994).

The effects of neuroactive compounds, which have been used as insecticides for the last 50 years, and the exposure to neurotoxicants can be assessed through the measurement of biomarkers related to their target activity or detoxification processes (Casida and Quistad, 1998). Resistance to pesticides in invertebrates by detoxification is the only population-level response that has been directly correlated with enzyme biomarkers such as mixed-function oxidase (MFO) activity, glutathione *S*-transferase (GST) activity, acetylcholinesterase (AChE) activity, or other esterase activity (Lagadic et al., 1994; Gunning et al., 1997). The development of resistance depends on genetic variability already present in a population or arising during the period of the selection.

4. Field use of biomarkers

Biochemical techniques offer the possibility of rapidly detecting the initial stages of resistance in a population and the mechanism(s) of resistance involved (Table 3). Among the various mechanisms so far identified, enhanced detoxication by pesticide-metabolizing enzymes and decreased target site sensitivity are the most efficient and the best documented (Terriere, 1984; Brown and Brogdon, 1987; Soderlund and Bloomquist, 1989; Gunning et al., 1997, 1998).

Table 3
Resistance mechanisms to insecticides

Effect/enzyme	Chemical class
Insensitive target	
Voltage-dependent Na^+ channel	DDT, pyrethroids
GABA-gated chloride channel	Cyclodienes
Acetycholinesterase	Organophosphorus, carbamates
Enhanced detoxification	
Cytochrome P450	Chlorinated hydrocarbons, pyrethroids, organophosphorus, carbamates
Carboxyesterases and other esterases	Pyrethroids, organophosphorus, carbamates
Glutathione *S*-transferase	Chlorinated hydrocarbons, organophosphorus

Note: Adapted from Casida and Quistad (1998).

The cotton bollworm, *Helicoverpa armigera* (Hubner), is a serious pest for cotton and other summer crops in Australia and insecticides are widely used for its control on cotton. Studies have shown that resistance of cotton bollworm to exposure to pyrethroid, carbamate, and organophosphorus pesticides is directly related to specific biochemical mechanisms of detoxication existing in natural populations (Gunning et al., 1996, 1998). Pyrethroid resistance in Australian *H. armigera* is largely attributable to an increased production of esterase enzymes that are not present in susceptible *H. armigera,* which apparently detoxify pyrethroids by sequestration and hydrolysis (Gunning et al., 1996).

Table 4
Insecticide mode of action

Target	Chemical class	Pesticide example
Nerve target		
Voltage-dependent Na^+ channel	DDT and pyrethroids	Deltamethrin
γ-Aminobutyric acid (GABA)	Cyclodienes	Dieldrin, endosulfan
Nicotinic acetylcholine receptor	Nicotinoids	Imidacloprid, spinosad
Acetylcholinesterase	Organophosphorus compounds	Chloropyrifos
Other targets		
Mitochondria	Respiratory inhibitors/uncouplers	Chlorfenapyr
Insect growth hormone receptors	Juvenile hormone and ecdysone agonists	Methoprene, tebufenozide

Note: Adapted from Casida and Quistad (1998).

The presence of an insensitive AChE has been identified as the mechanism causing resistance to methyl parathion and profenofos in *H. armigera* (Gunning et al., 1998). Fortunately, for the Australian cotton industry, *H. armigera* remain susceptible to chlorpyrifos.

Nontarget macroinvertebrate species should also be studied to determine whether reduced acetylcholinesterase activity or an insensitive acetylcholinesterase or increased esterase activity is developing in riverine macroinvertebrates exposed to pesticides in agricultural regions. Choice of the appropriate biomarker for monitoring chemical exposure in the field requires knowledge of a variety of factors (Mayer et al., 1992; Peakall and Shugart, 1993). Gender, reproductive status, age, and diet (composition and amount) are among the factors that can influence the biomarker response of many invertebrates. There is evidence that multiple forms of some enzyme biomarkers exist in a variety of insects and aquatic invertebrates (Terriere, 1984; Clark, 1989; Habig and DiGiulio, 1991). Each form of the enzyme may differ in its sensitivity to the contaminant under study. These enzyme biomarkers can also vary in form, location, and activity within a species.

The concentration or activity of biochemical biomarkers in a given tissue can also vary in response to characteristics of the organism such as development stage and age within a particular development stage. Variation in biomarker response with organism development is of particular importance when studying biomarkers in invertebrate species of streams or rivers where the immature nymphs and larvae are typically at various stages of development. It is critical to use well-defined biological material, where the variation in biochemical activity with development, age, and tissue in an organism is known, for prediction of toxicity from changes in biochemical biomarker response as a result of exposure to a chemical.

Many biomarker responses have a transient temporal feature. Exposure to a contaminant may elicit a response that persists for only a few hours. However, some biomarker responses persist for weeks or months with continued exposure of the organisms. Species-specific differences in the rate of recovery to exposure from a particular contaminant have been demonstrated (Benke and Murphy, 1974). Hence, it is vital to establish in advance the duration of response that can be expected. Determining the temporal response of biochemical biomarkers in invertebrates is essential if they are used to measure the environmental impact of chemicals.

At present the selection of biomarkers of exposure that may be applied in many species is limited by lack of knowledge of their basic mechanisms of action. The reliability of use of biomarkers depends on knowledge of the mechanisms involved in the particular response. For insecticides there is a growing understanding of their mechanistic basis which can assist in the identification of suitable biochemical biomarkers that can be linked to population effects (Table 4). Once suitable biomarkers are selected, it is important to conduct field studies to establish how environmental and biotic factors will modify the biomarker responses to toxicants relative to those seen in laboratory conditions where these factors are controlled. The prediction of higher-level effects in natural populations from biochemical biomarker measurements of individuals can be achieved by using statistically correlated studies of population densities as discussed by McCarty and Munkittrick (1996) using a biomarker/bioindicator approach. The potential use of biochemical biomarkers in aquatic macroinvertebrates to predict population effects will now be discussed.

5. Biomarkers of organic chemical exposure

5.1. Mixed-function oxidases

Cytochrome P450 (or simply P450) refers to a family of enzymes that transform the structure of organic chemicals (for review, see Nebert et al., 1981). The reactions catalyzed by these proteins are of a type generally referred to as mixed-function oxidase or monooxygenase reactions which function through the hemoprotein cytochrome P450. The toxicity of organic

chemicals such as pesticides can be drastically altered by structural transformation. By affecting chemical structures, cytochrome P450 enzymes may render a given compound nontoxic or, by contrast, drastically increase its toxicity. Many organophosphorus pesticides require bioactivation of sulfur analogues to more potent oxygen analogues prior to exerting their anticholinesterase effects. This desulfuration reaction is mediated by microsomal MFO enzyme systems.

The quantities of some types of P450 that can be induced (increased) vary in response to an organism's exposure to chemicals. As a result, the rate of chemical transformation catalyzed by these enzymes is altered. P450 induction can also serve as a highly sensitive indicator of an organism's exposure to chemical inducers in the environment.

The properties of MFO systems in aquatic invertebrates have been detailed in recent reviews (Livingstone, 1993; Snyder, 2000). Invertebrate microsomal enzymes catalyze transformation of a diverse suite of xenobiotic substrates including aromatic hydrocarbons, but the rates of these processes in invertebrates are substantially lower than those in most fish. Thus, at present, there seems to be little potential for using monooxygenase activity or P450 levels in invertebrates to assess their exposure to contaminants such as aromatic and chlorinated hydrocarbons.

5.2. Glutathione S-transferases

The glutathione *S*-transferases represent an important family of enzymes because of their role as catalysts for the conjugation of various electrophilic compounds (e.g., epoxides of PAH) with the tripeptide glutathione (Clark, 1989). These enzymes exist in multiple forms and are known to be involved in insect resistance to organophosphorus insecticides (Terriere, 1984). The glutathione *S*-transferases of larval insects are induced up to 18-fold by chemicals in plants (Terriere, 1984). Few studies have been carried out on chemically induced levels of GST in freshwater invertebrates, but its responsiveness to organic xenobiotics is very low (Blat et al., 1988; Boryslawskyj et al., 1988).

Therefore, because of the lack of specificity and qualitative response of some of the detoxication enzymes to contaminants, research is needed before they can be considered for use as biomarkers.

6. Biomarkers of pesticide exposure

6.1. Acetylcholinesterase

Many insecticides are toxic because they inhibit the animal nervous system enzyme AChE. Since this reaction is substantially irreversible for many pesticides, percentage inhibition of acetylcholinesterase activity can be used as an indicator of exposure of an organism to organophosphorus pesticides for a considerable period after the contaminant itself is metabolized or eliminated from the organism's body. The monitoring of acetylcholinesterase activity in the brains of fish and birds in the field has become a technique commonly used for diagnosing their exposure to cholinergic poisons (Greig-Smith, 1991; Zinkl et al., 1991).

Although aquatic invertebrates have been used routinely as biological indicators, the use of acetylcholinesterase inhibition as a biochemical biomarker in these species has been largely neglected. Aquatic invertebrates are more sensitive to organophosphorus insecticides than vertebrates (Giesy et al., 1999) and inhibition of acetylcholinesterase activity has been measured in a number of aquatic invertebrates species (Day and Scott, 1990; Kozlovskaya et al., 1993; Diamantino et al., 2000). Percentage inhibition of acetylcholinesterase activity in nymph heads of stoneflies was shown to be linearly correlated with concentration in nymphs treated with fenitrothion (Flannagan et al., 1978). Day and Scott (1990) and Ibrahim et al. (1998) demonstrated similar effects for stoneflies and chironomids exposed to organophosphorus compounds. Repeated exposures of the freshwater shrimp, *Paratya australiensis*, to chlorpyrifos and profenofos at sublethal concentrations caused increased suppression of acetylcholinesterase activity (Abdullah et al., 1993, 1994). Sibley et al. (2000) demonstrated in a microcosm study that acetylcholinesterase activity could be used as a reliable biomarker of exposure and mortality at the individual organism level and had potential for use in predicting responses at the population level for zooplankton. Similarly, acetylcholinesterase activity also provided a rapid indicator of organophosphate exposure to the amphipod *Gammarus pulex*, but the authors questioned the sensitivity of this biomarker in predicting sublethal, higher-order effects (McLoughlin et al., 2000). These results indicate that the choice of the aquatic invertebrate species, and a knowledge of its acetylcholinesterase activity, would be important in any hazard assessment program that attempts to link changes in acetylcholinesterase activities to decreases in field population densities.

6.1.1. Multiple and insensitive acetylcholinesterase

Although vertebrate acetylcholinesterases are primarily membrane-bound enzymes, there is evidence to indicate that invertebrate acetylcholinesterase exists in hydrophilic (buffer-soluble) and amphiphilic (membrane-bound) states. This has been reported for Coleoptera, Lepidoptera, Diptera, Hymenoptera, Plecoptera, Ephemeroptera, and other aquatic taxa (Krysan and Kruckberg, 1970; Belzunces et al., 1988; Day and Scott, 1990), with the proportions of both states

varying from species to species. A major concern is that multiple forms of acetylcholinesterase might obscure the correlation between symptoms and inhibition of acetylcholinesterase activity (Edwards and Fisher, 1991). For the housefly, there is evidence to indicate that the inhibition of thoracic acetylcholinesterase is more important in producing symptoms of intoxication than acetylcholinesterase derived from other sites (Edwards and Fisher, 1991). The honey bee, *Apis mellifera*, is the only terrestrial insect in which inhibition of head acetylcholinesterase activity by organophosphorus pesticides has been correlated with a neurotoxic effect and used for diagnostic purposes (Bendahou et al., 1999).

To be used as a biomarker, candidate species should be screened carefully for the anatomical location of multiple forms of acetylcholinesterase activity and the relative sensitivity of each molecular form to cholinergic inhibitors determined. The predictive value of the diagnosis will very much depend on how carefully these distinctions are made.

6.1.2. Variations in endogenous acetylcholinesterase activity

In addition to acetylcholinesterase activity varying in its form and location, as discussed in the previous section, the amount of acetylcholinesterase activity in a given tissue can also vary in response to characteristics of the organism such as the developmental stage and age at a particular instar. Casida (1955) measured different cholinesterase activity in the eggs, larvae, pupae, and adult heads of the housefly and other insects, with the highest specific activity in the heads of adults. Quantifiable levels of acetylcholinesterase activity first appeared at stage V of grass shrimp embryonic development and increased as development progressed (Lund et al., 2000).

It is also important to determine whether the enzyme activity being studied is exclusively acetycholinesterase. Cholinesterases are typically subdivided into two major classes, the true or acetycholinesterases and butytylcholinesterases (ChEs), sometimes referred to as pseudocholinesterases. These cholinesterase enzymes can be distinguished by differences in their catalytic properties toward various substrates and by determining the effect of iso-OMPA and BW284C51, specific inhibitors of butytylcholinesterase and acetylcholinesterase activity, respectively (Belzunces et al., 1988).

6.1.3. Characteristics of chemicals which affect acetylcholinesterase

The two major classes of cholinergic insecticides, organophosphorus and carbamate compounds, differ fundamentally in their acetylcholinesterase inhibitory properties. Organophosphorus compounds are considered to be functionally irreversible inhibitors of acetylcholinesterase since the time necessary to liberate the enzyme from inhibition may be in excess of the time required for new enzyme synthesis. Carbamates can have a fairly rapid decarbamylation step so that substantial recovery of the enzyme can occur within 24–48 h (Habig and DiGiulio, 1991). Recovery from the effects of organophosphorus compounds is both chemical and species specific (Zinkl et al., 1991). Abdullah et al. (1994) reported that acetylcholinesterase activity of the Australian freshwater shrimp, *Paratya australiensis*, required 7 days for recovery from inhibition by sublethal concentrations of profenofos. This difference between the effects of these two types of pesticides must be taken into account when making diagnoses of field samples, with field samples from a suspected carbamate contamination needing to be analyzed with 24 h of the initial impact.

6.1.4. Chemical reactivation of inhibited acetylcholinesterase

Chemical reactivation is a means of confirming whether low acetylcholinesterase activity is due to pesticide inhibition. Reactivation of acetylcholinesterase activity is produced by addition of aqueous pyridine-2-aldoxime methiodide (2-PAM), which will produce a substantial recovery of activity in cases where there has been inhibition by organophosphorus pesticides (Martin et al., 1981). Measurement of spontaneous reactivation can also help to identify the action of a carbamate compound (Martin et al., 1981). Results obtained by the application of this technique might be biased by the pesticides involved, for it is known that dimethyl-substituted organophosphates are less prone to reactivation by 2-PAM than are diethyl-substituted compounds (Martin et al., 1981).

6.2. Cellulase/carbohydrase

The physiology of digestion in aquatic macroinvertebrates has received little attention, but the few published studies suggest that the digestive processes of various functional groups are adapted to the locally available food supply, primarily terrestrial leaves (Martin et al., 1980; Kesler, 1982). Aquatic insects, including shredders, show a positive selection for "conditioned" leaves, that is, leaves in the early stages of decay due to the presence of colonized aquatic hyphomycete fungi and bacteria (Barlocker and Kendrick, 1975). The fungi species on leaves have been shown to secrete three enzymes, collectively known as cellulase, which degrade cellulose to glucose (Martin, 1983). The digestive systems of most macroinvertebrate shredders are designed to decompose dietary polysaccharides and oligosaccharides. Thus their digestive systems contain either endogenous or ingested cellulose and cellobiase (β-glucosidase) activity (Kesler, 1982; Martin, 1983; Barlocher and Porter, 1986) and α-glucosidases such as amylase or α-amylose. Amylase catalyzes the digestion

of starch, the nutrient reservoir in plants (Bernard and Lagadic, 1993), whereas α-amylose catalyzes the digestion of α-1,4-glucans in the fungal tissue associated with plant debris (Martin et al., 1980). Caddisflies and mayflies feed very little as adults, thus essentially all the nutrients required for pupation and reproduction must be accumulated by the larva. Some species preferentially ingest lipid-coated detritus during the last larval instar (Cargill et al., 1985). Pyrethroids at sublethal concentrations have been shown to decrease gut amylase activity in larvae of the beetle *Tribolium castaneum* (Saleem and Shakoori, 1987). The organophosphorus insecticide fenitrothion has also been reported to inhibit the gut carbohydrase activity of earthworms (Patnaik and Dash, 1993). Decreased feeding will result in decreased growth and reproduction, which can severely affect the survival of some populations. Therefore, the measurement of decreased cellulase, α-glucosidase, or β-glucosidase activity in shredders such as the mayfly nymphs of *Jappa kutera* and *Atalophlebia* sp., which are dominant macroinvertebrate species in the Namoi River, New South Wales, Australia, has potential as a biomarker of pesticide exposure. However, De Coen et al. (2001) have found that relationships between enzymatic endpoints in carbohydrate metabolism and population level effects observed in *Daphnia magna* were toxicant specific, and no single enzyme in carbohydrate metabolism could predict quantitative changes in population characteristics.

6.3. Carboxyesterase

The primary target for pyrethroids and dichlorodiphenyltrichloroethane (DDT) analogues in insects is the sodium channel of nerve-cell membranes (Narashashi, 1992) where the insecticides prolong the opening time of the channels (Bloomquist and Miller, 1986). An important mechanism that confers resistance to pyrethroids and DDT, known as knockdown resistance or *kdr*, due to single mutations, has been reported in many insect species (Soderlund and Bloomquist, 1989; Gunning et al., 1991). Induction of detoxifying enzymes in insects is another important mechanism for resistance to these insecticides (Terriere, 1984). After the introduction of a pest management strategy that placed restrictions on the agricultural use of pyrethroids against the Australian cotton bollworm, *H. armigera*, resistance due to *kdr* nerve insensitivity rapidly declined to virtually undetectable levels in field populations (Gunning et al., 1996). However, pyrethroid resistance in *H. armigera* persists and is largely attributable to a massive overproduction of esterase enzymes, which detoxify pyrethroids by sequestration and hydrolysis (Gunning et al., 1996). The increased esterase activity of up to 50-fold is likely to be the result of gene amplification (Devonshire and Field, 1991). Quantitative changes in esterase enzyme activity were correlated to pyrethroid resistance determined by laboratory bioassay from eggs collected in the field (Gunning et al., 1996).

Carboxyesterase activity is present in a variety of tissues in vertebrate and invertebrate species (Leinweber, 1987). Other studies have demonstrated that carboxyesterases also protect against the toxicity of organophosphorus pesticides (Maxwell, 1992). Whether carboxyesterases also protect against the toxicity of organophosphorus pesticides in field populations of aquatic invertebrates in agricultural catchments needs to be investigated.

7. Conclusions

Aquatic macroinvertebrates are commonly used in biological monitoring programs, but their use in biomarker studies has been limited. To link a biomarker measurement in individuals to population changes, it is necessary to understand the mechanisms of ecotoxicological damage by the causative agent. Quantitative dose–response measurements of the biomarker will then link the molecular effect of the toxicant to the toxic response of the individual organism. Linkage of whole organism responses to changes in field populations can then be obtained by statistical correlations.

The slow induction of monoxygenase activity and P450 in response to contaminants in invertebrates precludes the use of mixed-function oxidases as biomarkers of exposure to environmental contaminants. Two biochemical mechanisms that confer high resistance to organophosphorus and pyrethroid insecticides are detoxification by the overproduction of esterases and insensitive acetylcholinesterases. Measurement of the activities of these enzymes in aquatic macroinvertebrates could be used as a biomarker of susceptibility to toxicity (decreased acetylcholinesterase activity) or as a biomarker of resistance (insensitive acetylcholinesterase or increased carboxyesterase activity) that could be linked to changes in population densities. Other enzymes such as α-glucosidase or β-glucosidase, which are involved in the digestion of carbohydrates in plant material in the gut, have potential as biomarkers of exposure to pesticides that could be linked to the survival of aquatic macroinvertebrate populations. The ability of these macroinvertebrate biomarkers to predict effects on field populations needs to be validated by studying a known contaminated catchment.

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