Lydy, M., J. Belden, C. Wheelock, B. Hammock, and D. Denton. 2004. Challenges in regulating pesticide mixtures. Ecology and Society **9**(6): 1. [online] URL: http://www.ecologyandsociety.org/vol9/iss6/art1

Synthesis

Challenges in Regulating Pesticide Mixtures

Michael Lydy[1], *Jason Belden*[1], *Craig Wheelock*[2], *Bruce Hammock*[2], and *Debra Denton*[3]

ABSTRACT. This paper introduces the field of mixture toxicity and the challenges in regulating pesticide mixtures. Even though pesticides are unique chemical stressors designed to have biological activity that can affect a number of nontarget species, they are intentionally placed into the environment in large quantities. Currently, methods and terminology for evaluating mixture toxicity are poorly established. The most common approach used is the assumption of additive concentration, with the concentrations adjusted for potency to a reference toxicant. Using this approach, the joint action of pesticides that have similar chemical structures and modes of toxic action can be predicted. However, this approach and other modeling techniques often provide little insight into the observed toxicity produced by mixtures of pesticides from different classes. Particularly difficult to model are mixtures that involve a secondary toxicant that changes the toxicokinetics of a primary toxicant. This may result in increased activation or a change in the persistence of the primary toxicant within the organism and may be responsible for a several-fold increase or decrease in toxicity. At present, the ecological effects caused by mixtures of pesticides are given little consideration in the regulatory process. However, mixtures are being considered in relation to human health in the pesticide registration process, setting a precedent that could be followed for ecological protection. Additionally, pesticide mixtures may be regulated through toxicity testing of surface water under the *Clean Water Act.* The limits of our basic knowledge of how mixtures interact are compromising both these avenues for regulating mixtures. We face many challenges to adequately protecting the environment from mixture toxicity; these challenges include understanding the interactions of toxicants within an organism, identifying the mixtures that most commonly occur and cause adverse effects, and developing a regulatory structure capable of minimizing environmental impacts.

INTRODUCTION

Ecological risk assessments and regulatory standards typically apply to the effects of single stressors on ecosystem components. However, organisms in the environment often experience many stressors simultaneously, including those of a physical, biological, and chemical nature. There are many challenges in dealing with the interactions of multiple stressors. For example, how do we compare the interactions among a biological stressor such as an exotic species, a physical stressor such as sedimentation, and a chemical stressor such as a pesticide? This workshop was organized to address the issues associated with multiple stressors to wildlife species. Most of the papers in this session discussed issues associated with large-scale stressors such as acid rain coupled with habitat fragmentation or global climate change. This paper will take a more narrow view of environmental stressors by focusing on a single group of chemical stressors, namely pesticides.

The objectives of this paper are to introduce some basic terminology used in toxicology, including the mode of toxic action and the models generally used in these types of mixture assessments; to review the pesticide literature on mixture studies; to discuss the status of current environmental regulations governing mixture effects; and to provide a list of what we consider to be the major challenges in working with pesticide mixtures. To simplify the review, we have chosen to restrict our discussion primarily to aquatic systems.

Pesticides are unique chemical stressors in that they are designed to have biological activity but are intentionally placed into the environment in large quantities. In the United States alone, 4.14×10^8 kg of conventional pesticides, e.g., herbicides, insecticides, fungicides, and nematicides, containing approximately 1290 registered active ingredients were applied in

[1]Southern Illinois University at Carbondale; [2]University of California at Davis; [3]U.S. Environmental Protection Agency

1999 (Donaldson et al. 2002). In addition, the co-application of active ingredients is common. For example, 89% of the field corn grown in the United States in 2002 was treated with herbicides and 24% with insecticides. In cotton production, 85–100% of acreage was treated with herbicides, 53–100% with insecticides, and up to 20% with fungicides. Many fruit and vegetable crops receive even higher pesticide concentrations, and multiple active ingredients from a pesticide group are often used on a single crop. For example, in 2002, 59% of lettuce crops received herbicide treatment, 89% were treated with insecticides, and fungicides were applied to 70% of the acreage. Four different herbicides were applied to more than 10% of corn acreage, and five different insecticides were applied to more than 35% of total lettuce acreage (U.S. Department of Agriculture 2003).

Potential pesticide mixing may also occur when crops are intermingled. Even in regions that primarily grow row crops, two to four crops in a rotation system are common. In regions where fruit and vegetables are the primary agricultural products, numerous crops may be planted in a watershed, each with a unique mixture of pesticides that may potentially contaminate surface water and groundwater. In addition to agricultural contributions, pesticides may be present in the watershed from urban sources such as lawn and garden care, domestic pest control, and golf courses.

Chemical analysis of surface water conducted by the U.S. Geological Survey under the National Water Quality Assessment Program indicates that pesticide mixtures are contaminating surface waters. Streams throughout the continental United States were tested for 83 pesticides, 77 of which were detected in at least one sample (Gilliom et al. 1999). Ninety-five percent of surface water samples contained at least one pesticide, and several high-usage herbicides were frequently detected. For example, atrazine was detected in 78% of surface water samples, and metolachlor was detected 68% of the time. More than 50% of all stream samples contained five or more pesticides (U.S. Geological Survey 1998). It is therefore evident that we must consider mixtures to be the most common exposure scenario when evaluating the ecological effects of pesticides.

TOXICOLOGICAL INTERACTIONS OF PESTICIDES

Pesticides can be classified in a variety of ways. In the most general sense, they are grouped based on target pests, e.g., insecticide vs. herbicide. In a more detailed framework, pesticides are grouped into classes of compounds that have similar chemical structures and modes of toxic action. The term “mode of toxic action” is defined in this paper as a series of key processes that begins with the interaction of a pesticide with a receptor site and proceeds through operational and anatomical changes in an organism that result in sublethal or lethal effects (EPA 2000*a*). An example of a pesticide class is the organophosphate insecticides (OPs) such as malathion, chlorpyrifos, and diazinon. OPs contain phosphorus and are derivatives of phosphoric and similar acids (Matsumura 1975). These compounds inhibit the enzyme acetylcholinesterase (AChE), a key enzyme that hydrolyzes the neurotransmitter acetylcholine (Carlock et al. 1999). Inhibition of AChE results in the accumulation of acetylcholine and the overstimulation of cholinergic receptors, which in turn overstimulates neurological activity in the organism (Gallo and Lawryk 1991). Many other insecticide families also exhibit neurological activity. Carbamates, e.g., carbaryl and aldicarb, are another class of insecticides that inhibit AChE, the same enzyme targeted by the OPs (Baron 1991). Pyrethroids, e.g., permethrin and esfenvalerate, are another widely used insecticide class that also causes neurological damage, but at a different target site (Leahey 1985). These insecticides are potent sodium and potassium channel blockers that produce subtle changes in the channel's function, causing repetitive neuronal discharge (Soderlund et al. 2002). Other classes of insecticides such as stomach poisons and a number of different insect growth regulators have completely different modes of toxic action. The designed mode of toxic action between pesticide groups, e.g., herbicides and insecticides, is almost always different. Often, the toxicity caused by insecticides to plants or by herbicides to animals is through secondary modes of toxic action that are not clearly understood.

An important concept in examining pesticide mixtures is deciphering the language of chemical interactions. This can be a daunting task, because many terms are used interchangeably in the literature. In this paper, the term “additivity” is used when the effect of the combination of chemicals can be estimated directly from the sum of the concentrations, (concentration addition) or the sum of the responses (response addition). Sometimes the toxicity measured when performing a study does not match the model used to

calculate additivity because of chemical-chemical interactions; toxicokinetic interactions such as uptake, biotransformation, distribution, and elimination; or toxicodynamic, e.g., receptor site, interactions. This shift is best referred to either as less than additive toxicity, e.g., antagonism, or greater than additive toxicity, e.g., synergism.

Knowledge of a chemical's mode of toxic action is essential in understanding how mixtures may act jointly. For example, if two organophosphate insecticides (OPs) are applied together, it is expected that they will both inhibit AChE, thus working jointly at the same receptor site, and that their effects would be additive. To calculate their combined effect, the applied concentrations must first be normalized (Bailey et al. 1996). This is generally accomplished by using the concentration addition method. In this ideal case, the pesticides are assumed to behave similarly in terms of their primary toxicokinetic and toxicodynamic processes.

One of the most common methods of assessing concentration addition is to use toxic units (TU), and this approach was chosen for several reasons. First, it is a straightforward method that requires little training. Second, the only information that is necessary about the dose-response relationship is the concentration that causes the effect of interest, for example, lethal concentration 50 (LC50), which is defined as the external media concentration that causes 50% lethality in a test population. Other models would require a knowledge of the entire dose-response curve, which has not been historically reported in the literature.

As described by Faust et al. (1993), toxic units are calculated for a two-component mixture using the formula:

$$x_1/\mathrm{LC}(X_1) + x_2/\mathrm{LC}(X_2) = \mathrm{TU} \quad (1)$$

In this equation, x_1 and x_2 are the concentrations of mixture components X_1 and X_2, and LC(X_1) and LC(X_2) are the effect concentrations of the individual compounds that produce the same effect measured in the mixture test. This formula has been extended in previous studies to include any number of components (Berenbaum 1985). Using the TU approach, a shift from additivity has been described quantitatively by testing multiple levels of the mixture and assigning each test concentration a TU value. The TU values are regressed against the effect observed using standard log-probit procedures. The effect level originally used to calculate the TU is entered into the regression, for example, 50% if an LC50 was used. The value corresponding to this effect is the TU determined for the mixture. If this value is equal to 1.0, the joint action is additive; if the value is less than 1, the joint action is greater than additive; and if the value is greater than 1, the joint action is less than additive. The TU value calculated by this approach can be multiplied against the effective concentration to obtain a “corrected” value for the mixture combination. For example, if the TU equals 0.5 and the LC50 value for compound A is 2.0 μg/L, the corrected LC50 is estimated to be 1.0 μg/L. This calculation is useful in evaluating the risk of the mixture as compared to the variability found in intra- and interspecies toxicity testing.

If compounds exhibit completely different modes of toxic action, they may exhibit no interaction at all. For example, if a metal or herbicide co-occurs with an insecticide, the modes of toxic action may differ. In this case, the joint toxicity may occur as independent action (response addition). Independent action is different from concentration addition in that it does not assume similar toxicokinetics or similar modes of toxic action or that the concentration-response curves have similar shapes. Under independent action, the pesticides in the mixture are assumed to behave independently of one another, so that the organism's response to the first pesticide is the same whether or not the second pesticide is present. Independent action indicates that the toxicity of the compounds is predicted to occur based on simple probability statistics. If a concentration of compound A generally kills 25% of the organisms and a concentration of compound B kills 25% of the organisms, then the two concentrations of compounds A and B combined would result in their individual effects added together, minus that portion of the population in which sensitivities overlap. The following equation shows this relationship for a binary mixture; the effects are entered as proportions:

$$\textit{Mixture Effect} = \textit{Effect of Compound A} + \textit{Effect of Compound B} - (\textit{Effect of Compound A} * \textit{Effect of Compound B}) \quad (2)$$

Therefore, in our example, the combination would result in less than 50% effect (43.8%). In some combinations, the sensitivities to the compound may

completely overlap or not overlap at all because of the genetic makeup of the population. Although Bliss introduced independent action into pharmacological literature as early as 1939, few ecological studies have investigated the concept (Faust et al. 2003).

One of the complications in using either of these models to estimate toxicity is that few pesticide combinations have exactly the same mode of toxic action. On the other hand, few combinations act completely independently. It has been suggested that the joint action of chemicals is a spectrum of interactions with these two perfect cases as the extremes (Broderius and Kahl 1985). The following hypothetical cases of OPs occurring with other pesticides are discussed to clarify this point. If two OPs are jointly applied, they have very similar modes of toxic action. However, although OPs and carbamates share the same receptor site, they may have very different affinities for the receptor. By the same token, although OPs and pyrethroids are both neurotoxins that can increase neuronal depolarization, they have different enzyme targets. In contrast, OPs and herbicides have completely different modes of toxic action, yet they may overlap at an organ or system level or through a baseline narcotic effect.

A second complication to using the concentration addition (TU) and independent action methods to estimate toxicity is that these models account only for interactions at the target sites, e.g., toxicodynamics. In many cases, a jointly acting chemical can influence the toxicokinetics of the other compound. Many pesticide formulations take advantage of this property. For instance, piperonyl butoxide (PBO) is used as a synergist for pyrethroid insecticides (Casida 1970). Although PBO itself has little insecticidal activity, it inhibits cytochrome P450-dependent mixed-function oxidases (P450s). Inhibition of P450s can prevent the biotransformation and elimination of pyrethroid insecticides, thereby enhancing and/or prolonging the toxic response of the targeted host and increasing toxicity > 300-fold (Ando et al. 1983). In this case, the effect of PBO is referred to as “potentiation,” an effect that occurs when a component of the mixture, although not toxic by itself, increases the toxicity of one or more of the other compounds in the mixture. Conversely, PBO decreases the toxicity of many OP insecticides that require oxidative transformation to become toxic. For OPs that require bioactivation, PBO actually decreases toxicity (Bailey et al. 1997). Pharmacologically, the effects of PBO may not be entirely via P450, but may alter the action of other enzymes or even the penetration of the active compound into the organism.

We have attempted to provide a brief overview of the basic terminology and concepts necessary to discuss pesticide toxicology and the interactions of chemical mixtures. As previously noted, two main methods are used to interpret chemical interactions: concentration addition, e.g., TU, and independent action, e.g., response addition. These models both have advantages and disadvantages associated with their use and application (George et al. 2003). However, an underlying problem with the application of these models is a general lack of understanding of the mode of toxic action of chemical interactions. As a result, our ability to predict and understand observed toxicity from chemical mixtures, especially across compound classes, is greatly impaired. As our understanding of these complex interactions increases, so will our ability to select and apply the appropriate method for study design and data interpretation.

TOXICITY OF PESTICIDE MIXTURES

Toxicity studies involving pesticide mixtures have resulted in a full spectrum of responses in which the complexity of the interactions depends on differences in the chemical properties and modes of toxic action of the pesticides. Studies that examine the effects of pesticides from the same class are usually the easiest to interpret, because the observed effects are often additive in nature. For example, Bailey et al. (2000) observed that the organophosphate insecticides (OPs) chlorpyrifos and diazinon were strictly additive in their toxicity toward the cladoceran *Ceriodaphnia dubia* in toxicity studies performed in natural, storm-, and laboratory waters (Bailey et al. 1996,1997). Additive effects were also noted in the aquatic midge (*Chironomus tentans*) when it was exposed to binary mixtures of several OPs, including chlorpyrifos, azinphos methyl, methidathion, and diazinon (Lydy and Austin 2004). Faust et al. (1993) found concentration additivity for binary mixtures of the s-triazine herbicides atrazine and cyanazine in reproductive tests with the green alga *Chlorella fusca.* The mode of toxic action for these herbicides is to interrupt the electron transport chain in photosytem II. Finally, the additivity of acetylcholinesterase (AChE) inhibitors has been shown with *C. dubia* dosed with the carbamate insecticide carbofuran and the OPs methyl parathion and malathion (Norberg-King et al. 1991).

Understanding toxicity across chemical classes with slightly different modes of toxic action is more challenging. However, we still have some understanding of what processes cause the observed toxic responses. For example, Pape-Lindstrom and Lydy (1997) found an additive toxic response in midges exposed to two neurotoxicants, the organochlorine insecticide methoxychlor and the OP methyl parathion. Faust et al. (1994) found that herbicides with different modes of toxic action were generally additive in nature in binary combinations in algae. Twenty-four of the combinations were additive; two combinations exhibited greater than additive toxicity, i.e., observed EC50 (the molar concentration that produces 50% of the maximum possible response) values were 25–30% lower than expected; and two combinations exhibited less than additive toxicity, i.e., observed EC50 values were 138–200% of the expected value.

The effects of simultaneous pyrethroid insecticide and OP exposure have also been studied by a number of researchers (Tripathi and Agarwal 1998, Moreby et al. 2001, Denton et al. 2003). Given that P450-activated OPs will inhibit esterases, thus decreasing an organism's ability to detoxify pyrethroids, greater than additive toxicity is often observed. Denton et al. (2003) demonstrated that exposure to esfenvalerate and diazinon resulted in greater than additive toxicity in fathead minnows. Similar toxic effects have been observed in exposures to pyrethroids and carbamates. Permethrin and the carbamate propoxur elicited greater than additive toxicity in the mosquito *Culex quinquefasciatus* (Corbel et al. 2003). These greater than additive effects were attributed to the complementary modes of toxic action of these two insecticide classes, which act on different components of nerve impulse transmission.

Our predictive ability begins to break down when we examine the toxic effects of chemicals from different classes with completely different modes of toxic action, e.g., insecticides with herbicides, or pesticides with other stressors. Research on the impacts of these mixtures on organisms has yielded mixed results. For example, no toxic interaction was noted when *C. tentans* were exposed to a binary mixture of the triazine herbicide atrazine and the carbamate insecticide carbofuran (Douglas et al. 1993). The joint toxicity of the OP diazinon and ammonia was examined in *C. dubia* using 48 h acute toxicity tests with dosed water and effluents containing both stressors (Bailey et al. 2001). The results indicated a less than additive response for the binary mixture in both laboratory-dosed and effluent samples. In a separate study using *C. dubia*, Banks et al. (2003) found less than additive responses for binary mixtures of diazinon and copper. In other studies, greater than additive responses have been noted. For example, several researchers have found that triazine herbicides can potentiate the effects of some OPs (Pape-Lindstrom and Lydy 1997, Belden and Lydy 2000, Anderson and Lydy 2002, Lydy and Linck 2004). The greater than additive responses noted in these studies actually represent a potentiation effect, because the herbicides were not acutely toxic to the study organisms. The magnitude of this potentiation depends greatly on the concentration and type of triazine herbicide and OP tested. In the terrestrial system, atrazine increased the toxicity of chlorpyrifos to the earthworm *Eisenia fetida* by a factor of seven (Lydy and Linck 2004), whereas, in aquatic systems, 200 μg/L of atrazine increased the toxicity of chlorpyrifos up to a factor of four (Belden and Lydy 2000).

Environmental studies rarely investigate the toxicokinetic and toxicodynamic processes involved in the joint toxicity of pesticides. An exception is the effort to better understand the mechanism by which triazine herbicides potentiate OP toxicity. Pape-Lindstrom and Lydy (1997) suggested that atrazine increased the biotransformation of OPs by converting them into more toxic O-analog metabolites. Organophosphorothioate insecticides require oxidative activation by cytochrome P450 enzymes to their corresponding oxon analogs, which are much more potent AChE inhibitors than the parent compound. These authors further suggested that atrazine might be accomplishing this metabolic activation by inducing the cytochrome P450 enzymes responsible for the conversion. Previous studies have supported this hypothesis by demonstrating that biotransformation enzyme complexes can be induced by atrazine exposure in a variety of invertebrate and vertebrate species (Egaas et al. 1993). Miota et al. (2000) further validated this hypothesis when they showed the induction of a 45 kDa protein in atrazine-treated midges, and Belden and Lydy (2000) did the same by demonstrating that atrazine-treated midges transform a greater amount of chlorpyrifos to the oxon form than do unexposed midges. The intensity of this atrazine-induced protein was representative of the proteins associated with the heme-thiolate membrane within the 45 kDa molecular-weight enzyme system. This

enzyme system plays a key role in the metabolism of a wide variety of endogenous and exogenous substances in insects (Miota et al. 2000). It is clear from these studies that toxicokinetic processes such as biotransformation are important in determining the toxicity of some pesticide mixtures.

When large numbers of chemicals are included in the mixture experiments, an additive response is typically found (Broderius and Kahl 1985, Altenburger et al. 2000). For example, Broderius and Kahl (1985) found additivity using the concentration-addition model when they examined the acute toxicity of large mixtures of organic chemicals in fathead minnows (*Pimephales promelas*). Currently, regulatory agencies support the concentration addition model when assessing the joint acute toxicity of large numbers of chemical mixtures on aquatic biota because additivity is assumed, which simplifies the calculations.

This review has so far focused on the effects of chemical stressors in combination with other chemical stressors. However, aquatic ecosystems exhibit a myriad of physical and biological variables that would need to be included along with the chemical stressors to more accurately model real environmental exposure scenarios. For example, using a bacterial bioluminescence inhibition assay, Benson and Long (1991) showed that humic acids significantly reduced the toxicity of some AChE inhibitors, e.g., azinophos-methyl, chlorpyrifos, and carbofuran, while enhancing the toxicity of others such as methyl parathion and carbaryl (Benson and Long 1991). Temperature has been demonstrated to have an inverse effect on pyrethroid toxicity, which increases at lower temperatures (Mahboob et al. 1999, Motomura and Narahashi 2000). Conversely, Lydy et al. (1990) reported increases as high as 100-fold in the toxicity of the OP parathion at higher temperatures. Herbranson et al. (2003*a*,*b*) examined the effects of suspended solids on carbofuran toxicity to *Daphnia magna* and found no measurable toxicity associated with exposure to suspended solids at a wide range of concentrations. However, when exposed to a constant concentration of carbofuran, the number of affected organisms increased with increasing concentration of suspended solids. The authors speculated that the suspended solids were either decreasing the caloric intake of the *D. magna* because of a dilution effect or that ingestion of the solids was causing the *D. magna* to sink, which forced them to expend significantly more energy to maintain proper buoyancy. In turn, this increased energy expenditure made the *D. magna* more susceptible to the carbofuran toxicity.

Many toxicity studies are performed with an excess food source, which can significantly affect the experimental outcome. It is possible that low food density may result in increased toxicity. Barry et al. (1995) showed that esfenvalerate toxicity to *D. carinata* increased significantly with decreasing food concentration. The converse is also possible in that increased food density can result in decreased toxicity. For example, Herbrandson et al. (2003*a*,*b*) showed that increased food availability significantly reduced carbofuran toxicity to *D. magna*. However, the mechanism behind these observations is difficult to interpret, because the observed effects could be the result of changes in either organism fitness or toxicant concentration caused by sorption to the food source. Taken together, these results show that the experimental effects of changing food concentrations cannot be easily predicted even though they can significantly affect toxicity. Consequently, attempts to perform toxicity studies that model realistic environmental exposure scenarios should account for variables such as temperature, food availability, etc.

The inclusion of multiple variables can make data interpretation difficult. Although the joint action of pesticides is often additive, there are many reports of less and greater than additive toxicity. When interpreting data involving this joint action, it is important to understand that the magnitude of the deviation from additivity is also important. For instance, in some studies, pesticide interactions were reported when changes in toxicity of less than 30% occurred. This deviation may be real, but it is not significant if this much variation is likely to exist between different cultures of the same organism (intratest variability). However, when toxicity changes by larger factors, the assumption of additivity could lead to poor estimates of environmental impact. Because of the complex interaction of pesticides within an organism, nearly every combination would deviate from additivity to some degree if enough statistical power were used. Currently, no consensus has been reached regarding the magnitude of deviation from additive that is important.

It is not realistic to physically test every combination of pesticides found in the environment. For example, with a simple mixture of only 20 pesticides, there are 190 pairs and more than a million possible

combinations involving pairs, triples, etc. Consequently, there is a need for simple models that can easily predict the toxicity of complex mixtures. As previously discussed, several mixture models that take concentration addition and independent action into account are available to accomplish this task. However, these models are based on statistical concepts of interaction. Other models have been based on the physical and chemical properties of the pesticides, such as the octanol/water partition coefficient, and derive toxicological end points, e.g., impaired reproduction or death, through a quantitative structure activity relationship (QSAR) approach (Hansch et al. 1995, Comber et al. 2003). The use of QSARs can significantly reduce the amounts of time and resources spent on toxicity studies. However, an important limitation in many of the QSAR models generated to date is that they were developed using homogeneous mixtures (Escher and Hermens 2002). It is unrealistic to expect QSAR models to be able to predict the toxicity of chemical mixtures across multiple classes. Their usefulness is limited to examining relationships among compounds with a high degree of structural similarity and/or similar mechanisms of action. Future QSAR studies on chemical mixtures should address the effects of realistic exposure scenarios and multiple species upon toxicity. There are numerous articles in the literature that examine the use of QSARs to predict toxicity, but it is beyond the scope of this paper to review them all (Blum and Speece 1990, Nendza and Russom 1991, Nendza et al. 1995, Yang et al. 1998, Gramatica et al. 2001, Altenburger et al. 2003, Bradbury et al. 2003, Vighi et al. 2003).

REGISTRATION PROCESS UNDER FIFRA

Prior to reaching most world markets, pesticides must be legally registered to ensure that they are safe. In the United States, the U.S. Environmental Protection Agency, hereafter referred to as the EPA, registers pesticides under the *Federal Insecticide, Fungicide and Rodenticide Act* (FIFRA) and the *Federal Food, Drug and Cosmetic Act.* The registration process includes product efficacy assessments, assessments of risks to human health, and, more relevant to this paper, ecological risk assessments (EPA 1997). During the process of conducting ecological risk assessments (ERAs), the expected environmental concentration of a single pesticide is compared to a broad spectrum of toxicological end points for several organisms. Currently, the EPA does not formally assess pesticide mixtures or any form of multiple stressors within ERAs.

Evaluating the effects of pesticide mixtures in ERAs will prove to be challenging. First, as previously discussed, we have a limited understanding of the joint action of pesticides even within a single species. Understanding the joint action of pesticides that may occur for the diversity of organisms protected by an ERA, which may involve entire ecosystems, is an immense task. Second, before evaluating the joint action of pesticide mixtures, it will be necessary to define which chemicals are present, the concentrations at which they are detected, and the types of temporal trends expected in their occurrence. With regard to the regulation of pesticides, two situations occur: initial registration, i.e., the evaluation of new pesticides, and the re-registration of pesticides registered prior to November 1984 (EPA 2000*a*).

When new pesticides enter the market, usage patterns and environmental occurrence may only be projected. Actual testing of all pesticide combinations would be a daunting if not impossible task that is not economically feasible. However, the landscapes where the pesticide is expected to be applied may help to determine the potential mixtures that may occur. These landscapes would best be evaluated at a regional scale using spatial and temporal occurrences to estimate the probabilities of co-occurrence. Testing of likely mixtures may then be conducted prior to initial registration. In some landscapes, this may be a manageable task. For instance, a new pesticide applied to corn may have a limited number of co-occurring pesticides in a landscape with primarily corn/soybean rotation. However, the same pesticide applied to vegetables in the San Joaquin Valley in California could involve many more co-occurring pesticides (Gronberg et al. 1998). This approach may be the most feasible way to assign priorities to pesticide mixtures.

The re-registration process also considers the effects of pesticides on human health and the environment. For most compounds in the re-registration process, including atrazine and many organophosphate insecticides (OPs), a large amount of research has been conducted on their environmental fate and effects. Because of the availability of larger databases on these pesticides, re-registration may provide an opportunity to assess the toxicity resulting from potential chemical interactions. In other words, for an existing pesticide undergoing re-registration, occurrence data from

monitoring programs such as the National Water Quality Assessment Program and from environmental fate studies available to the EPA could be used to characterize the mixtures associated with the regional or crop-based landscapes for that pesticide. Subsequently, mixture studies could be conducted and used in the re-registration process. Re-registration may provide an opportunity to update pesticide test procedures to include some relevant mixture toxicity testing. Because the re-registration process has several possible outcomes, e.g., the EPA can order reduced application rates, mandatory best management practices, or changes in the pesticide formulation, pesticide registration could then be adjusted to take into account the potential impacts of pertinent mixtures.

To date, few pesticide registrations have included the potential effects of pesticide mixtures. In the conditional registration of acetochlor, a chloroacetanilide herbicide, the EPA required an overall reduction in herbicide usage on corn for the product's registration to continue (see http://www.epa.gov/oppefed1/aceto/index.htm). Although this technique helps prevent the addition of extra pesticide loads into the environment, it does not directly link the registration of a compound to mixture toxicity.

Additionally, the EPA has set a precedent in evaluating pesticide mixtures in terms of human health assessments. In response to the 1996 *Food Quality Protection Act* (EPA 2003), the EPA is conducting cumulative risk assessments of several groups of pesticides. Each group has been defined based on specific “common mechanisms of toxicity.” The EPA uses the concept of a “risk cup” to determine the acceptable amount of risk associated with the use of a class of pesticides. The determined risk for a compound such as an OP is determined and “placed” into the risk cup. Each accepted registration of a compound with a similar mechanism of action, e.g., additional OPs, is then added to the cup. Once the cup is full, no new registrations are allowed. Currently identified groups for which the risk cup is being used include OPs, selected triazine herbicides including atrazine and related metabolites and herbicides, N-methyl-carbamates, thio- and dithiocarbamates, and chloroacetanilide herbicides including metolachlor, alachlor, and related compounds. In the current approach, each compound is assigned a hazard index that is a measure of the relative potency factor of each OP. This technique is similar to the toxic unit (TU) approach previously discussed, and additive results are assumed. In addition, safety factors are imposed based on the number of pesticides involved and the potential sensitivity of children. Although this process provides a first step in evaluating exposure to pesticide mixtures, only tightly defined groups are considered to have joint action, e.g., only OPs. This approach might not be very effective in coping with more complex mixtures in the environment, because joint exposures to pesticides often have less than or greater than additive effects, regardless of the mode of toxic action.

Ultimately, each of these regulation methods suffers because our poor understanding of the mechanisms of chemical mixtures results in poor predictive ability. More research devoted to the mode of toxic action of individual compounds as well as chemical mixtures and their effects on nontarget organisms is needed to develop better techniques for predicting the joint action of pesticides. Until we have improved techniques that enable us to evaluate the joint action of pesticides prior to usage, our only ecological protection from mixtures depends on testing surface water for toxicity.

REGULATORY AND RESEARCH ACTIONS FOR ADDRESSING PESTICIDE MIXTURES

The federal *Clean Water Act* (CWA) was enacted with the objectives of “restoring the chemical, physical and biological integrity of the Nation's waters.” The CWA prohibits the discharge of pollutants in toxic amounts. Although the CWA initially resulted in the regulation of point-source discharges, the act has recently become important for nonpoint-source contamination as well. Based on the stated goals of the CWA, the U.S. Environmental Protection Agency (EPA) and individual states take three approaches to protecting water quality. These approaches include chemical-specific limits for the 126 priority pollutants, toxicity testing, and biological criteria/bioassessment (EPA 1991). In addition to introducing each of these approaches to the reader, we will examine their potential for assessing mixtures. A key distinction between the CWA and the *Federal Insecticide, Fungicide and Rodenticide Act* (FIFRA) is that the CWA does not consider benefit when examining risk, whereas FIFRA makes a risk/benefit-based decision.

The chemical-specific approach involves the development of water quality criteria (WQC) for each

chemical and is designed to protect most of the tested species most of the time. WQC are developed based on the results of both acute and chronic toxicity testing with the specified numbers and types of aquatic species following standard EPA water quality guidelines (Stephan et al. 1985). Although the EPA has developed water quality criteria for priority pollutants as required under CWA Section 304, only nine of the priority pollutants are pesticides, and those include mostly organochlorine insecticides of limited current use, such as dichlorodiphenyltrichloroethane (DDT) and endosulfan. However, these guidelines will not protect against mixtures of pesticides with unknown interactions or chemicals that do not have any chemical-specific criteria. For this reason, it may be necessary to add conservative safety factors in areas where there are gaps in the data. The EPA is currently in the process of updating and revising its 1985 *Guidelines for Deriving Aquatic Life Criteria.* It is expected that these new and revised guidelines will cover several new issues such as nontraditional end points, threatened and endangered species, and bioaccumulative chemicals. Chemical mixtures are one of the issues that the guidelines do not currently address, but they may be incorporated in the near future. These limitations demonstrate the importance of toxicity testing and bioassessment assessments in the overall evaluation of aquatic resources.

Toxicity testing can be used to assess the effects of all the chemical stressors in aqueous samples such as effluents, receiving waters, or stormwater runoff. This allows the effect of a mixture to be evaluated, rather than the toxic responses of individual chemicals. Toxicity tests can be used to assess ambient water bodies such as receiving water, making them an effective tool in the assessment of small and large watersheds (de Vlaming et al. 2000). For example, the State of California has successfully used an ambient toxicity testing approach to identify and regulate frequently occurring toxic chemicals. The approach includes pinpointing integral sampling locations and collecting ambient waters to be assessed using both acute and chronic toxicity tests (Foe and Sheipline 1993, Foe 1995, Kuivila and Foe 1995, de Vlaming et al. 2000). If toxicity is detected at a site, additional samples are collected to determine the spatial and temporal toxicity patterns. The EPA's Toxicity Identification Evaluation (TIE) protocols are then used to identify the causative toxicant or toxicants (Nordberg-King et al. 1992, EPA 1993*a*,*b*). The goal of a TIE is to identify the chemical(s) causing toxicity in an aqueous sample. This approach has led to the listing of chemicals in addition to the 126 priority pollutants commonly tested; one such addition is the pesticide diazinon, which is not a priority pollutant (State Water Resources Control Board 2000). Therefore, the approach of toxicity testing in conjunction with TIE analysis may be used to check for chemicals that are greater than additive in nature.

The primary advantage of the bioassessment approach is that it integrates effects from both physical and biological stressors on aquatic biota. Biological assessments are based upon the premise that the structure and function of an aquatic biological community can provide critical information about the quality of the surface water. The bodies of water being evaluated are assessed and compared to predetermined criteria for impairment and nonattainment of a designated use. The use of the stressor identification process is a method for identifying biological and physical stressors of the impaired body of water (EPA 2000*b*).

If a body of water is impaired as measured by any of these three approaches, e.g., the WQC are not attained, the CWA requires that the impaired bodies of water be listed on the State's 303(d) list and that a total maximum daily load (TMDL) be developed to address the pollutant(s) causing the impairment. The TMDL is a written, quantitative assessment of water quality problems and pollution sources. For waters that do not meet state water quality standards, it specifies the total concentration by which each pollutant must be reduced to meet the standard for that body of water. This provides the basis for the actions to be taken to restore the water to its designated use. The TMDL may require additional actions such as a discussion of alternative pesticides and/or the development of best management practices (BMPs) that may involve buffer strips, constructed wetlands, vegetated drainage ditches, etc., to minimize off-site movement of pesticides. BMPs such as drainage ditches are important for reducing not only the targeted pesticide for the TMDL development but other stressors of aquatic organisms as well, such as nutrients, sediments, and other pesticides (Moore et al. 2001, Cooper et al. 2002). The consideration of chemical mixtures is important, because regulatory TMDLs are typically developed for a single chemical in a body of water, although it is likely that a mixture of chemicals exists. Potential effects of chemical mixtures in bodies of water could be considered during the numeric target

selection, margin of safety components of the TMDL development, and/or the implementation phase.

CONCLUSIONS

In this paper, we have attempted to summarize some of the issues related to the toxicity of pesticide mixtures. In general, mixture studies are difficult to perform and are further complicated by the fact that the observed interactions are often not predictable given our current knowledge of the toxicokinetic and toxicodynamic processes involved. The combined effects of pesticides within the same class can be predicted fairly well based on our understanding of the mechanism of toxic action of these pesticides. However, the effects of across-class mixtures of pesticides, such as the triazine herbicides and organophosphate insecticides, are more difficult to predict and understand. The issues involved in examining the effects of chemical mixtures are going to continue to increase in complexity as additional chemicals are introduced. New generations of pesticides such as spinosyns (Sparks et al. 2001) and genetically modified organisms are being developed that will further complicate the issue of chemical mixtures.

We have also attempted to highlight some of the regulatory issues associated with pesticide mixtures. Current regulations do not adequately allow for greater than additive toxicity, and even the risk cup approach of the *Food Quality Protection Act*, which was designed to address mixtures of pesticides with a similar mechanism of action, fails to address chemical synergism and the effects of mixtures of pesticides from multiple classes. It will most likely fall upon researchers to determine the limitations of current toxicity testing paradigms before regulatory agencies are able to act.

It never will be practical to perform toxicity studies on every combination of pesticides under all exposure scenarios. Therefore, it is important that studies be designed for maximum data extraction. This might be achieved through the use of quantitative structure activity relationships (QSARs) that relate chemical properties to toxicity and allow extrapolation to untested chemicals. However, the current level of QSAR models indicates that this approach is not suitable for estimations across chemical classes or for chemicals of dissimilar chemical structure. Further developments will most likely be needed in QSAR methods before these models for predicting the toxicity of chemical mixtures become widely applicable. In addition, full concentration-response relationships should be reported, even in single-compound toxicity tests. As mixture models improve, more precise data throughout the toxicity range could be required. Finally, more research on pesticide mode of toxic action and secondary physiological effects caused by pesticides would provide a platform for understanding the physiology of mixture effects, lead to better predictive models, and allow for rational experimental design. The recent increase in the number of papers addressing this issue shows that the scientific community is aware of the problem, and we expect to see an even greater number of studies in the future addressing issues raised in this paper. It is expected that future studies will continue to highlight the importance of examining the toxicity of chemical mixtures in addition to single compounds. We believe that these types of studies are critical for realistic estimations of toxicity, because rarely are organisms exposed to only a single chemical in the field.

WHAT ARE THE MAJOR CHALLENGES IN REGULATING PESTICIDE MIXTURES?

We conclude with a list of what we consider to be the major challenges in working with pesticide mixtures. This does not represent a comprehensive list of challenges, but will hopefully stimulate additional dialogue among scientists.

1. If two compounds have an interaction, which chemical is to blame? For instance, in the triazine work, atrazine increases the toxicity of chlorpyrifos. Should this be considered in registration decisions for both compounds?
2. Is the assumption of additivity protective for most bodies of water, most of the time? What degree of deviation from additivity is important?
3. Guidelines in human health assessments recommend combining similarly acting compounds into a single risk cup. What are the criteria for “similar” modes of toxic action?
4. Pharmacologically, we have reason to believe that similarly acting compounds would be best modeled using concentration addition techniques and that dissimilarly acting compounds would be best modeled using independent action techniques. However, because the true toxicological mode of toxic

action is rarely known for a given mixture or even for most of its components, we are often uncertain which model to use. What steps are necessary to choose the correct model? Are either of these models appropriate, or do we need a new model that encompasses both techniques?

5. The order of exposure to each pesticide can affect the toxic response, e.g., organophosphate pesticides and triazine herbicide mixtures. We must not only recognize that a mixture has occurred, but we must also understand the dynamics of the chemicals in the system and how temporal variations influence the toxicity of mixtures.
6. The large numbers of chemicals and varying exposure routes in the environment make testing each possible exposure scenario unreasonable. Is it possible to assess the mixture effects of a few high-priority mixtures and develop extrapolation models for the remainder using the available data on the mixture components or similar mixtures?
7. What is the priority for choosing the chemical mixtures to test? Should we pick those chemicals with high environmental occurrence, such as atrazine, or choose diverse compounds that will help us understand how to model mixture effects?
8. For many mixtures that exhibit greater than additive toxicity, the mechanism is a change in toxicokinetics induced by one compound. Based on this information, could we develop screening assays for compounds to identify their potential role in this type of interaction?

The ultimate challenge in aquatic toxicology is understanding the dynamic world that organisms encounter. Beyond pesticides, they are exposed to many other chemical stressors, and have to survive biological and physical stressing agents at the same time. It is naïve to assume that these stressors do not interact. However, until we better understand the biology of aquatic systems, from the molecular to the ecosystem level, we will continue to struggle to predict the existence and significance of chemical interactions.

Responses to this article can be read online at: http://www.ecologyandsociety.org/vol9/iss6/art1/responses/index.html

Acknowledgments:

This work was supported in part by a grant from the CALFED Bay-Delta Program (Award # 99-NO8) and by NIEHS Grant R37 ES02710, NIEHS Superfund Grant P42 ES04699, NIEHS Center for Environmental Health Sciences Grant P30 ES 05707, and NIH Grant AI58267. C. E. W. was supported by NIH postdoctoral training grant T32 DK07355-22.

LITERATURE CITED

Altenburger, R., T. Backhaus, W. Boedeker, M. Faust, M. Scholze, and L. H. Grimme. 2000. Predictability of the toxicity of multiple chemical mixtures to *Vibrio fischeri*: mixtures composed of similarly acting chemicals. *Environmental Toxicology and Chemistry* **19**:2341-2347.

Altenburger, R., M. Nendza, and G. Schuurmann. 2003. Mixture toxicity and its modeling by quantitative structure-activity relationships. *Environmental Toxicology and Chemistry* **22**:1900-1915.

Anderson, T. D., and M. J. Lydy. 2002. Increased toxicity to invertebrates associated with a mixture of atrazine and organophosphate insecticides. *Environmental Toxicology and Chemistry* **21**:1507-1514.

Ando, T., L. O. Ruzo, J. L. Engel, and J. Casida. 1983. 3-(3,3-Dihalo-2-propenyl) analogues of allethrin and related pyrethroids: synthesis, biological activity, and photostability. *Journal of Agriculture and Food Chemistry* **31**:250-253.

Bailey, H., L. Deanovic, E. Reyes, T. Kimball, K. Larson, K. Cortright, V. Connor, and D. Hinton. 2000. Diazinon and chlorpyrifos in urban waterways in northern California, USA. *Environmental Toxicology and Chemistry* **19**:82-87.

Bailey, H., C. DiGiorgio, K. Kroll, J. Miller, D. Hinton, and G. Starrett. 1996. Development of procedures for identifying pesticide toxicity in ambient waters: carbofuran, diazinon, chlorpyrifos. *Environmental Toxicology and Chemistry* **15**:837-845.

Bailey, H., J. Miller, M. Miller, L. Wiborg, L. Deanovic, and T. Shed. 1997. Joint acute toxicity of diazinon and chlorpyrofios to *Ceriodaphnia dubia*. *Environmental Toxicology and Chemistry* **16**:2304-2308.

Bailey, H. C., J. R. Elphick, R. Krassoi, and A. Lovell. 2001. Joint acute toxicity of diazinon and ammonia to *Ceriodaphnia dubia. Environmental Toxicology and Chemistry* **20**:2877-2882.

Banks, K., S. Wood, C. Matthews, and K. Theusen. 2003. Joint acute toxicity of diazinon and copper to *Ceriodaphnia dubia. Environmental Toxicology and Chemistry* **22**:1562-1567.

Baron, R. L. 1991. Carbamate insecticides. Pages 1125-1190 *in* W. J. Hayes and E. R. Laws, editors. *Handbook of pesticide toxicology.* Academic Press, San Diego, California, USA.

Barry, M. J., D. C. Logan, J. T. Ahokas, and D. A. Holdway. 1995. Effect of algal food concentration on toxicity of two agricultural pesticides to *Daphnia carinata. Ecotoxicology and Environmental Safety* **32**:273-279.

Belden, J. B., and M. J. Lydy. 2000. Impact of atrazine on organophosphate insecticide toxicity. *Environmental Toxicology and Chemistry* **19**:2266-2274.

Benson, W. H., and S. F. Long. 1991. Evaluation of humic-pesticide interactions on the acute toxicity of selected organophosphate and carbamate insecticides. *Ecotoxicoly and Environmental Safety* **21**:301-307.

Berenbaum, M. 1985. The expected effect of a combination of agents: the general solution. *Journal of Theoretical Biology* **114**:413-431.

Bliss, C. 1939. The toxicity of poisons applied jointly. *Annuals of Applied Biology* **26**:585-615.

Blum, D. J. W., and R. E. Speece. 1990. Determining chemical toxicity to aquatic species: the use of QSARS and surrogate organisms. *Environmental Science and Technology* **24**:284-293.

Bradbury, S. P., C. L. Russom, G. T. Ankley, T. W. Schultz, and J. D. Walker. 2003. Overview of data and conceptual approaches for derivation of quantitative structure-activity relationships for ecotoxicological effects of organic chemicals. *Environmental Toxicology and Chemistry* **22**:1789-1798.

Broderius, S., and M. Kahl. 1985. Acute toxicity of organic chemical mixtures to the fathead minnow. *Aquatic Toxicology* **6**:307-322.

Carlock, L. L., W. L. Chen, E. B. Gordon, J. C. Killeen, A. Manley, L. S. Meyer, L. S. Mullin, K. J. Pendino, A. Percy, D. E. Sargent, L. R. Seaman, N. K. Svanborg, R. H. Stanton, C. I. Tellone, and D. L. Van Goethem. 1999. Regulating and assessing risks of cholinesterase-inhibiting pesticides: divergent approaches and interpretations. *Journal of Toxicology and Environmental Health B: Critical Reviews* **2**:105-160.

Casida, J. 1970. Mixed function oxidase involvement in the biochemistry of insecticide synergists. *Journal of Agriculture and Food Chemistry* **18**:753-772.

Comber, M. H., J. D. Walker, C. Watts, and J. Hermens. 2003. Quantitative structure-activity relationships for predicting potential ecological hazard of organic chemicals for use in regulatory risk assessments. *Environmental Toxicology and Chemistry* **22**:1822-1828.

Cooper, C. M., M. T. Moore, E. R. Bennett, S. Smith, and J. L. Farris. 2002. Innovative uses of vegetated drainage ditches for reducing agricultural runoff. Pages 119-126 *in* Proceedings of the Sixth International Conference on Diffuse Pollution (Amsterdam, 2002). International Water Association, Amsterdam, The Netherlands.

Corbel, V., F. Chandre, F. Darriet, F. Lardeux, and J. M. Hougard. 2003. Synergism between permethrin and propoxur against *Culex quinquefasciatus* mosquito larvae. *Medical and Vetinary Entomology* **17**:158-164.

de Vlaming, V., V. Connor, C. DiGiorgio, H. C. Bailey, L. Deanovic, and D. Hinton. 2000. Application of whole effluent toxicity test procedures to ambient water quality assessment. *Environmental Toxicology and Chemistry* **19**:42-62.

Denton, D., C. Wheelock, S. Murray, L. Deanovic, B. Hammock, and D. Hinton. 2003. Joint acute toxicity of esfenvalerate and diazinon to larval fathead minnows (*Pimephales promelas*). *Environmental Toxicology and Chemistry* **22**:336-341.

Donaldson, D., T. Kiely, and A. Grube. 2002. *Pesticide industry sales and usage, 1998 and 1999 market estimates.* Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, D.C., USA.

Douglas, W. S., A. McIntosh, and J. C. Clausen. 1993. Toxicity of sediments containing atrazine and carbofuran and to larvae of the midge *C. tentans. Environmental Toxicology and Chemistry* **12**:847-853.

Egaas, E., J. U. Skaare, N. O. Svendsen, M. Sandvik, J. G. Falls, W. C. Dauterman, T. K. Collier, and J. Netland. 1993. A comparative study of effects of atrazine on xenobiotic metabolizing enzymes in fish and insects and of the in vitro phase II atrazine metabolism in some fish, insects, mammals and one plant species. *Comparative Biological Physiology C* **106**:141-149.

EPA. 1991. *Technical support document for water quality-based toxics control.* Office of Water, U.S. Environmental Protection Agency, Washington, D.C.

EPA. 1993*a. Methods for aquatic toxicity identification evaluations. Phase II: Toxicity identification procedures for samples exhibiting acute and chronic toxicity.* EPA/600/R-92/081. U.S. Environmental Protection Agency, Duluth, Minnesota, USA.

EPA. 1993*b. Methods for aquatic toxicity identification evaluations. Phase III: Toxicity identification procedures for acutely and chronically toxic samples.* EPA/600/R-92/080. U.S. Environmental Protection Agency, Duluth, Minnesota, USA.

EPA. 1997. *The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and Federal Food, Drug, and Cosmetic Act (FFDCA) as amended by the Food Quality Protection Act (FQPA) of August 3, 1996.* Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, D.C., USA.

EPA. 2000*a. Pesticide registration facts.* Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, D.C., USA. Available online at USEPA Pesticide Registration.

EPA. 2000*b. Stressor identification guidance document.* EPA/822/B-00/025. U.S. Environmental Protection Agency, Washington, D.C., USA.

EPA. 2000*c. Supplementary guidance for conducting health risk assessment of chemical mixtures.* EPA/630/R-00/002. U.S. Environmental Protection Agency, Washington, D.C. USA.

EPA. 2003. *Food Quality Protection Act (FQPA) of 1996.* U.S. Environmental Protection Agency, Washington, D.C., USA. Available online at: USEPA FQPA.

Escher, B. I., and J. L. Hermens. 2002. Modes of action in ecotoxicology: their role in body burdens, species sensitivity, QSARs, and mixture effects. *Environmental Science and Technology* **36**:4201-4217.

Faust, M., R. Altenburger, T. Backhaus, H. Blanck, W. Boedeker, P. Gramatica, V. Hamer, M. Scholze, M. Vighi, and L. H. Grimme. 2003. Joint algal toxicity of 16 dissimilarly acting chemicals is predictable by the concept of independent action. *Aquatic Toxicology* **63**:43-63.

Faust, M., R. Altenburger, W. Boedeker, and L. Grimme. 1993. Additive effects of herbicide combinations on aquatic non-target organisms. *Science of the Total Environment* (Supplement): 941-952.

Faust, M., R. Altenburger, W. Boedeker, and L. Grimme. 1994. Algal toxicity of binary combinations of pesticides. *Bulletin of Environmental Contamination and Toxicology* **53**:134-141.

Foe, C. G. 1995. *Insecticide concentrations and invertebrate bioassay mortality in agricultural return water from the San Joaquin Basin.* California Regional Water Quality Control Board, Central Valley Region, Sacramento, California, USA.

Foe, C. G., and R. Sheipline. 1993. *Pesticides in surface water from applications on orchards and alfalfa during the winter and spring of 1991-92.* California Regional Water Quality Control Board, Central Valley Region. Sacramento, California, USA.

Gallo, M. A., and N. J. Lawryk. 1991. Organic phosphorus pesticides. Pages 917-1123 *in* W. J. Hayes and E. R. Laws, editors. *Handbook of pesticide toxicology.* Academic Press, San Diego, California, USA.

George, T. K., K. Liber, K. R. Solomon, and P. K. Sibley. 2003. Assessment of the probabilistic ecological risk assessment-toxic equivalent combination approach for evaluating pesticide mixture toxicity to zooplankton in outdoor microcosms. *Archives of Environmental Contamination and Toxicology* **45**:453-461.

Gilliom, R. J., J. E. Barbash, D. W. Kolpin, and S. J. Larson. 1999. Testing water quality for pesticide pollution. *Environmental Science and Technology* **33**: 16A-169A.

Gramatica, P., M. Vighi, F. Consolaro, R. Todeschini, A. Finizio, and M. Faust. 2001. QSAR approach for the selection of congeneric compounds with a similar toxicological mode of action. *Chemosphere* **42**:873-883.

Gronberg, J. M., N. M. Dubrovsky, C. R. Kratzer, J. L. Domagalski, L. R. Brown, and K. R. Burow. 1998. *Environmental setting of the San Joaquin-Tulare Basins, California.* Water Resources Investigation Report 97-4205. U.S. Geological Society, Sacramento, California, USA.

Hansch, C., D. Hoekman, A. Leo, L. Zhang, and P. Li. 1995. The expanding role of quantitative structure-activity relationships (QSAR) in toxicology. *Toxicology Letters* **79**:45-53.

Herbrandson, C., S. P. Bradbury, and D. L. Swackhamer. 2003*a*. Influence of suspended solids on acute toxicity of carbofuran to *Daphnia magna.* I. Interactive effects. *Aquatic Toxicology* **63**:333-342.

Herbrandson, C., S. P. Bradbury, and D. L. Swackhamer. 2003*b*. Influence of suspended solids on acute toxicity of carbofuran to *Daphnia magna*. II. An evaluation of potential interactive mechanisms. *Aquatic Toxicology* **63**:343-355.

Kuivila, K., and C. G. Foe. 1995. *Concentrations, transport and biological effects of dormant spray pesticides in the San Francisco Estuary, California.* Staff report. California Regional Water Quality Control Board, Central Valley Region, Sacramento, California, USA.

Leahey, J. 1985. *The pyrethroid insecticides.* Taylor and Francis, London, UK.

Lydy, M. J., and K. R. Austin. 2004. Assessment of pesticide mixtures from the Sacramento-San Joaquin delta using *Chironomus tentans. Archives of Environmental Contamination and Toxicology* **46**, *in press.*

Lydy, M. J., and S. L. Linck. 2004. Assessing the impact of triazine herbicides on organophosphate insecticide toxicity to the earthworm *Eisenia fetida. Archives of Environmental Contamination and Toxicology* **45**:343-349.

Lydy, M. J., T. W. Lohner, and S. W. Fisher. 1990. Influence of pH, temperature and sediment type on the toxicity, accumulation, and degradation of parathion in aquatic systems. *Aquatic Toxicology* **17**:27-44.

Mahboob, S. M., A. J. Howlader, and R. M. Shahjahan. 1999. Effect of temperature on the toxicity of three insecticides against the fourth instar larvae of *Culex quinquefasciatus say* (Diptera: Culicidae). *Bangladesh Journal of Zoology* **27**:185-189.

Matsumura, F. 1975. *Toxicology of insecticides.* Plenum Press, New York, New York, USA.

Miota, F., B. D. Siegfried, M. E. Scharf, and M. J. Lydy. 2000. Atrazine induction of P-450 in *Chironomus tentans* larvae. *Chemosphere* **40**:285-291.

Moore, M. T., E. R. Bennett, C. M. Cooper, S. Smith, F. D. Shields, J. L. Farris, and C. D. Milam. 2001. Transport and fate of atrazine and lambda-cyhalothrin in an agricultural drainage ditch in the Mississippi Delta, USA. *Agriculture, Ecosystems and Environment* **87**:309-314.

Moreby, S. J., S. Southway, A. Barker, and J. M. Holland. 2001. A comparison of the effect of new and established insecticides on nontarget invertebrates of winter wheat fields. *Environmental Toxicology and Chemistry* **20**:2243-2254.

Motomura, H., and T. Narahashi. 2000. Temperature dependence of pyrethroid modification of single sodium channels in rat hippocampal neurons. *Journal of Membrane Biology* **177**:23-39.

Nendza, M., and C. L. Russom. 1991. QSAR modelling of the ERL-D fathead minnow acute toxicity database. *Xenobiotica* **21**:147-170.

Nendza, M., A. Wenzel, and G. Wienen. 1995. Classification of contaminants by mode of action based on in vitro assays. *SAR QSAR Environmental Research* **4**:39-50.

Norberg-King, T. J., E. J. Durhan, G. T. Ankley, and E. Robert. 1991. Application of toxicity identification evaluation procedures to the ambient waters of the Colusa basin drain, California. *Environmental Toxicology and Chemistry* **10**:891-900.

Norberg-King, T., D. Mount, J. Amato, D. Jensen, and J. Thompson. 1992. *Toxicity identification evaluation: characterization of chronically toxic effluents, phase I.* EPA/600/6-91/005. U.S. Environmental Protection Agency. Washington, D.C., USA.

Pape-Lindstrom, P. A., and M. J. Lydy. 1997. Synergistic toxicity of atrazine and organophosphate insecticides contravenes the response addition mixture model. *Environmental Toxicology and Chemistry* **16**:2415-2420.

Soderlund, D. M., J. M. Clark, L. P. Sheets, L. S. Mullin,

V. J. Piccirillo, D. Sargent, J. T. Stevens, and M. L. Weiner. 2002. Mechanisms of pyrethroid neurotoxicity: implications for cumulative risk assessment. *Toxicology* **171**:3-59.

Sparks, T. C., G. D. Crouse, and G. Durst. 2001. Natural products as insecticides: the biology, biochemistry and quantitative structure-activity relationships of spinosyns and spinosoids. *Pest Management Science* **57**:896-905.

State Water Resources Control Board. 2000. *2000 California 305(b) report on water quality.* Available online at www.swrcb.ca.gov.

Stephan, D. E., D. I. Mount, D. J. Hansen, J. H. Gentile, G. A. Chapman, and W. A. Brungs. 1985. *Guidance for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses.* Office of Research and Development, U.S. Environmental Protection Agency, Duluth, Minnesota, USA.

Tripathi, A. M., and R. A. Agarwal. 1998. Molluscicidal and anti-AChE activity of tertiary mixtures of pesticides. *Archives of Environmental Contamination and Toxicology* **34**:271-274.

U.S. Department of Agriculture. 2003. *Agricultural chemical usage.* USDA, National Agricultural Statistics Service, Washington, D.C., USA.

U.S. Geological Survey. 1998. *Pesticides in surface and ground water of the United States: summary of the results of the National Water Quality Assessment Program (NAWQA).* U.S. Geological Survey, Washington, D.C., USA.

Vighi, M., R. Altenburger, A. Arrhenius, T. Backhaus, W. Bodeker, H. Blanck, F. Consolaro, M. Faust, A. Finizio, K. Froehner, P. Gramatica, L. H. Grimme, F. Gronvall, V. Hamer, M. Scholze, and H. Walter. 2003. Water quality objectives for mixtures of toxic chemicals: problems and perspectives. *Ecotoxicology and Environmental Safety* **54**:139-150.

Yang, R. S., R. S. Thomas, D. L. Gustafson, J. Campain, S. A. Benjamin, H. J. Verhaar, and M. M. Mumtaz. 1998. Approaches to developing alternative and predictive toxicology based on PBPK/PD and QSAR modeling. *Environmental Health Perspectives* **106** (Supplement 6):1385-1393.