



Pesticide Toxicity Index—A tool for assessing potential toxicity of pesticide mixtures to freshwater aquatic organisms



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HIGHLIGHTS

- Pesticide Toxicity Index (PTI) is a tool to assess pesticide mixtures.
- PTI is an indicator of potential toxicity of pesticide mixtures to aquatic life.
- The PTI was updated and expanded to include 440 pesticides and 52 degradates.
- A new type of PTI provides a more sensitive indicator of potential toxicity.
- From published field data, daphnid mortality occurred above PTI values of 0.1 to 1.

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ABSTRACT

Pesticide mixtures are common in streams with agricultural or urban influence in the watershed. The Pesticide Toxicity Index (PTI) is a screening tool to assess potential aquatic toxicity of complex pesticide mixtures by combining measures of pesticide exposure and acute toxicity in an additive toxic-unit model. The PTI is determined separately for fish, cladocerans, and benthic invertebrates. This study expands the number of pesticides and degradates included in previous editions of the PTI from 124 to 492 pesticides and degradates, and includes two types of PTI for use in different applications, depending on study objectives. The Median-PTI was calculated from median toxicity values for individual pesticides, so is robust to outliers and is appropriate for comparing relative potential toxicity among samples, sites, or pesticides. The Sensitive-PTI uses the 5th percentile of available toxicity values, so is a more sensitive screening-level indicator of potential toxicity. PTI predictions of toxicity in environmental samples were tested using data aggregated from published field studies that measured pesticide concentrations and toxicity to *Ceriodaphnia dubia* in ambient stream water. *C. dubia* survival was reduced to $\leq 50\%$ of controls in 44% of samples with Median-PTI values of 0.1–1, and to 0% in 96% of samples with Median-PTI values > 1 . The PTI is a relative, but quantitative, indicator of potential toxicity that can be used to evaluate relationships between pesticide exposure and biological condition.

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Abbreviations: CA, concentration addition; CCWQP, Central Coast Water Quality Preservation; CWA, Clean Water Act; DPR, California Department of Pesticide Regulation; EC50, 50 percent effective concentration; ECOTOX, ECOTOXicology database; HCP, pth percentile hazardous concentration; K_{ow} , octanol/water partition coefficient; LC50, 50 percent lethal concentration; maxTU, the maximum TU of any single component in a mixture; MOA, mode of action; msPAF, multi-substance Potentially Affected Fraction; MTC, median toxicity concentration; NAWQA, National Water-Quality Assessment Program; NOEC, no-observed-effect concentration; O/E, observed-to-expected ratio; OPP, Office of Pesticide Programs; PPDB, Pesticide Properties Database; PTI, Pesticide Toxicity Index; RA, response addition; SMAV, species mean acute value; SSD, species sensitivity distribution; STC, sensitive toxicity concentration; TC, toxicity concentration; TU, toxic unit; USEPA, U.S. Environmental Protection Agency; USGS, U.S. Geological Survey.

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1. Introduction

A major challenge when assessing the water quality of streams is to link contaminant data to effects on aquatic biota, particularly for pesticides, which tend to occur in streams as complex mixtures with strong seasonal and geographic patterns. In data collected during 1992–2001 by the U.S. Geological Survey's (USGS) National Water Quality Assessment (NAWQA) Program, U.S. streams with substantial agricultural and/or urban land within their watersheds had detections of 2 or more pesticides or degradates in water more than 90% of the time, 5 or more about 70% of the time, and 10 or more about 20% of the time (Gilliom et al., 2006). The most common way of directly assessing potential effects of pesticides is through the use of standardized laboratory toxicity tests that expose a single species to a single pesticide over a

range of concentrations for a specified period of time. Comparison of such toxicity test results among pesticides (or for a pesticide among test organisms) indicates the relative toxicity of these pesticides (or the relative toxicity of that pesticide to these test organisms) under standardized test conditions, but doesn't address many factors that are important in extrapolating to field conditions. Such factors include the dose–response relationship; mode(s) of action; endpoints selected; environmental factors (such as organic carbon, pH, and temperature); and potential for additive or interactive effects of contaminant mixtures. This paper addresses one of these factors—exposure to pesticide mixtures—and presents the Pesticide Toxicity Index (PTI) as a robust and readily applicable screening tool for interpreting the biological significance of concentration data for pesticide mixtures in hydrologic systems.

Procedures developed in previous studies to predict or evaluate the effects of pesticide mixtures using standardized single-species toxicity test data commonly share two steps: (1) environmental concentrations of individual pesticides are divided by compound-specific measures of toxicity or effect, and then (2) a model is applied to combine the contributions of individual pesticides in the mixture.

First, individual chemical concentrations have long been scaled to some measure of toxicity or effect (Bliss, 1939) as part of a toxic unit (TU) approach (Sprague and Ramsay, 1965). Studies have differed in key details, however, such as type(s) of toxicity measures used, taxonomic groups included, procedures for selecting one toxicity value to represent a compound when multiple values were available, and procedures for handling pesticides with no toxicity data available (examples cited below).

- TUs for pesticides have been calculated using acute 50-percent lethal or effective concentrations (LC50 or EC50, respectively) (Battaglin and Fairchild, 2002; Guy et al., 2011; Munn and Gilliom, 2001; Munn et al., 2006; Schäfer et al., 2011a, 2011b; Stenström, 2013), chronic no-observed-effect concentrations (NOECs) or LC50/EC50s multiplied by a safety factor to represent chronic effects (Anderson, 2008), water-quality standards (Stenström, 2013), and hazardous concentrations (HC) derived from species sensitivity distributions (SSD) (Guy et al., 2011; Maltby et al., 2005, 2009; Sala et al., 2012; Schäfer et al., 2013; Whiteside et al., 2008). A SSD entails fitting a statistical distribution to toxicity data for certain broad taxonomic groups, and SSDs can be constructed from acute LC50/EC50 values (Schäfer et al., 2013; Whiteside et al., 2008), chronic NOEC values, or any other selected toxicity criterion (Posthuma and de Zwart, 2006). From the concentration–effect relationship, a specific HC (HCp) can be derived that corresponds to effects on % of species.
- TUs have been calculated for a single taxon such as *Daphnia magna* (Guy et al., 2011; Liess and von der Ohe, 2005; Schäfer et al., 2011a, 2011b; Stenström, 2013), the most sensitive taxon (i.e., the taxon with the lowest LC50) (Schäfer et al., 2013), or taxonomic groups such as cladocerans (Munn et al., 2006). Similarly, SSDs may represent a fairly narrow taxonomic group such as arthropod invertebrates (Maltby et al., 2005, 2009; Sala et al., 2012), crustaceans (Whiteside et al., 2008), or broader groups such as freshwater invertebrates (Schäfer et al., 2013), aquatic animals (Etterson, 2011), or a combination of aquatic animals, aquatic macrophytes and algae (Maltby et al., 2009).
- When multiple toxicity values are available for a given pesticide, different studies selected the minimum value (Anderson, 2008), the median (Munn et al., 2006), the geometric mean (MacDonald et al., 2000; Sala et al., 2012), or the mean value (Battaglin and Fairchild, 2002). The U.S. Environmental Protection Agency's (USEPA) Office of Pesticide Programs (OPP) uses minimum values for ecological risk assessment and for calculating OPP aquatic-life benchmarks (http://www.epa.gov/oppefed1/ecorisk_ders/aquatic_life_benchmark.htm). The geometric mean is used by USEPA's Office of Water for computing Species Mean Acute Values (SMAV) in deriving USEPA water-quality

criteria for protection of aquatic organisms (Stephan et al., 1985). The median and geometric mean values are more robust to outliers, which is important because toxicity values for a given compound may vary over several orders of magnitude, reflecting variability both among species and among tests for the same species.

- For pesticides with little or no experimental toxicity test data available, previous studies either omitted such pesticides from the index (Anderson, 2008; Munn et al., 2006) or estimated their toxicity values from structurally similar compounds (Battaglin and Fairchild, 2002; Schäfer et al., 2011b), compounds with the same MOA (Sala et al., 2012), or the octanol/water partition coefficient (K_{ow}) using quantitative-structure–activity relationships (Schäfer et al., 2011a, 2011b).

For the present study, as will be described, we selected the median and 5th percentile of all applicable acute toxicity values (LC50/EC50s) for a given compound and taxonomic group to compute TUs, in order to maximize the number of pesticides included in the index, while using a robust and readily transparent approach based on experimental toxicity data.

In the second step, after concentrations have been scaled to toxicity, a model is applied to assess the contribution of individual components of the mixture to overall toxicity. A number of approaches have been used in previous studies:

- The most common model for combining TUs of pesticide components of a mixture is concentration addition (CA), which entails summing TUs for individual compounds in a mixture (Bliss, 1939; Sprague and Ramsay, 1965). CA assumes that components of the mixture behave as concentrations or dilutions of one another, differing only in their potencies, and do not interact. In theory, CA applies to compounds with a similar mode of action (MOA) and dose–response curves.
- The response addition (RA) model (also called independent action) assumes that compounds in the mixture act independently, so the combined effect is calculated according to the probability of non-excluding processes (Bliss, 1939; de Zwart and Posthuma, 2005).
- In the maximum TU (maxTU) approach, only the most toxic component of a mixture is considered, and potential contributions from other compounds in the mixture are assumed to be negligible (Liess and von der Ohe, 2005; Schäfer et al., 2011a, 2011b, 2012).
- For complex pesticide mixtures, de Zwart and Posthuma (2005) combined CA (for compounds with the same MOA) and RA (for compounds with different MOAs) in a two-stage model called the multi-substance Potentially Affected Fraction (msPAF) model. The msPAF model has been applied to assess assemblage metrics for fish and invertebrates (Posthuma and de Zwart, 2006, 2012; Schäfer et al., 2013).

For the present study, as will be described, we used the CA model. Studies of mixtures have shown that for compounds with a similar MOA, the CA model generally provides accurate predictions and the RA model may underestimate effects; but for compounds with different MOAs, the RA model provides more accurate predictions and the CA model may overestimate toxicity (Backhaus and Faust, 2012; Belden et al., 2007; Faust et al., 2000, 2003; Junghans et al., 2006). However, several studies of pesticide mixtures have shown that toxicity predicted using the CA model was within a factor of 2 or 3 of the observed toxicity, regardless of the MOA of the components (Belden et al., 2007; Faust et al., 2003; Warne, 2003). Moreover, application of the RA model, or a two-stage combination of CA and RA models, requires additional data on the MOA for each compound (which may be unknown or may vary for organisms in different trophic levels) and on dose–response curves for compounds with dissimilar MOAs (which are usually required to assess the effect that each compound would have if it were applied singly at the concentration at which it is present in the mixture) (Backhaus and Faust, 2012). For field samples, which may contain a large number of pesticides with similar, dissimilar, and unknown MOAs, CA appears to

be a slightly conservative, but broadly applicable model with a relatively small likelihood of underestimating effects (Backhaus and Faust, 2012; Belden et al., 2007).

A limitation of the CA and RA models is that they do not account for potential interactive effects among pesticides in a mixture. In general, interactive effects have been reported to occur in 10–30% of studies (Deneer, 2000; Faust et al., 1994; Warne and Hawker, 1995), were fairly evenly divided between synergism and antagonism, and typically did not deviate much from additivity (Warne and Hawker, 1995). Most studies of pesticide interaction have evaluated only limited endpoints, such as acute mortality or immobilization for animals and simple short-term growth measures for plants (Belden et al., 2007). The incidence of interactive effects may depend on the endpoint measured and test duration (Cedergreen and Streibig, 2005), on the test chemicals (Deneer, 2000; Lydy et al., 2004), and possibly on test species (Cedergreen et al., 2008).

Schäfer et al. (2011a) preferred the maxTU over the summed TUs (CA model) because the sum of TUs exhibited a stronger dependency on the number of compounds measured and could overestimate the toxicity of compounds with a dissimilar mode of action. Because the maxTU approach considers only the component with the highest toxic unit value, it does not require additional data on MOA or dose–response. The observation that RA models may underestimate toxicity for mixtures of similarly acting compounds (Backhaus and Faust, 2012) suggests that the same may be the case for the maxTU approach, which includes only the most toxic component of a mixture. However, the sum of TUs and the maxTU were highly correlated with one another, and both had a similar relationship to macroinvertebrate abundance in streams in Australia and Europe (Schäfer et al., 2011a).

The PTI approach presented in this paper is a variation of a risk-based scoring system described by Kimerle et al. (1997). The PTI was first developed by Munn and Gilliom (2001) for use with data on pesticides in stream water collected as part of the NAWQA Program, and was updated in 2006 (Munn et al., 2006). The PTI is a TU procedure that follows the CA model. This approach was selected because CA is a slightly conservative (protective), broadly applicable model that requires only standard toxicity data for individual pesticides, and is suitable as a robust screening tool for application to complex environmental mixtures of pesticides with similar, dissimilar, or unknown MOAs. Thus, the PTI approach allows us to maximize the number of pesticides included in the mixtures index, using only experimental data. As originally developed, PTI values are derived separately for fish, cladocerans, and benthic invertebrates by using the median of acute LC50s and EC50s for appropriate test species in standardized tests. Because only experimental data are used, any pesticides without standardized test data available for organisms in a given taxonomic group are omitted from the PTI calculations for that taxonomic group. The PTI may under-represent the relative acute toxicity of pesticides in a water sample because PTI calculations are limited to pesticides that (i) are measured and (ii) have toxicity data available. Because it is based on median toxicity values, the original PTI is relatively robust to outliers, but it would not necessarily be indicative of possible effects on all species in all life stages that may be exposed in hydrologic systems.

The PTI can be applied to pesticide mixtures detected in ambient water samples from hydrologic systems and used to interpret data in a number of ways. PTI values for individual samples can be used to rank sites or samples according to their expected relative toxicity due to pesticides, to assess changes in potential toxicity over time at a single site, or to predict which of the pesticides detected may be the greatest contributor to potential toxicity for a site or sample. PTI values for samples, seasons, or sites have been used as explanatory variables in multivariate analyses designed to determine which environmental variables best explain spatial patterns in the structure of a biological community (Fuhrer et al., 2004; Waite et al., 2006). As described further in Section 3.2, the PTI originally was designed to be an indicator of relative toxicity, and was not necessarily appropriate as a sensitive tool for

predicting whether pesticide mixtures in water samples are likely to be toxic to aquatic organisms.

The present paper updates and modifies the PTI, which initially was developed for 75 pesticides (Munn and Gilliom, 2001) and later expanded to 124 pesticides analyzed by the NAWQA program (Munn et al., 2006). The objectives of this paper are to (1) expand the target list of pesticides to all pesticides used in agriculture in the U.S. from 1992 to 2011; (2) update and expand experimental toxicity data using additional data sources and acceptable endpoints; (3) investigate the possibility of basing the PTI on more sensitive toxicity values; and (4) apply the PTI method to past field studies to test how well the PTI predicted toxicity in these studies and to illustrate its application to interpret water-quality data.

2. Methods

The PTI methodology used in the present study follows that of previous editions (Munn and Gilliom, 2001; Munn et al., 2006), except for some important modifications. The target analyte list was expanded to include nearly 500 pesticides from an agricultural use database for the U.S. (described below). Toxicity data sources and endpoints were expanded to increase the number of pesticides with experimental toxicity data, including the use of the LC50 as well as the EC50 (immobilization) endpoints for cladocerans; use of non-standard data for those pesticides that have no standard test data available; and addition of toxicity data from OPP risk assessment documents and the Pesticide Properties Database (PPDB). Finally, the PTI from previous editions (here called “Median-PTI”) was supplemented with a second type of PTI (“Sensitive-PTI”) that was calculated using more sensitive toxicity concentrations. These changes are described further in Sections 2.1–2.3.

2.1. Toxicity data sources

A master list of 484 pesticides was compiled from agricultural pesticide use lists for 1992 to 2011 (GfK Kynetec, 2009; personal communication, Melissa Sims, Senior Business Development Manager Americas, GfK Kynetec, June 1, 2012), and was composed of 197 herbicides; 135 insecticides, acaricides and nematocides; 105 fungicides; 9 fumigants; 4 adjuvants and synergists; and 34 other miscellaneous pesticides. In addition, a master list of 104 pesticide degradates included high priority (Tier 1) degradates identified during a pesticide prioritization effort by the U.S. Geological Survey (Norman et al., 2012).

For these 484 pesticides and 104 degradates, the following sources were searched for experimental toxicity test data: USEPA's ECOTOXology (ECOTOX) database (U.S. Environmental Protection Agency, 2012a); USEPA registration and risk assessment documents cited in support of the OPP aquatic-life benchmarks (U.S. Environmental Protection Agency, 2012b); and the PPDB (University of Hertfordshire, 2013). The ECOTOX database was queried for toxicity data for pesticides on July 5, 2012 and degradates on February 20, 2013. Toxicity data from OPP documents and the PPDB were compiled during September–October 2012 for pesticides and May 2013 for degradates. Details about each of these data sources are provided in Appendix A of the online supplementary information that accompanies this paper. Toxicity test data were selected as follows, in order of priority:

- Standardized toxicity test data from the ECOTOX database (U.S. Environmental Protection Agency, 2012a). Standardized data are defined as meeting the screening criteria in Table 1;
- Toxicity test data from core or supplemental studies underlying OPP aquatic-life benchmarks or summarized in registration documents cited by USEPA (2012b);
- Non-standard toxicity test data from the ECOTOX database—i.e., tests that did not meet the screening criteria in Table 1, or that had

Table 1

Standard criteria for toxicity test duration, endpoint, and measured effect, by taxonomic group. [EC50, 50 percent effective concentration; LC50, 50 percent lethal concentration].

Taxonomic group:	Fish or benthic invertebrates	Cladocerans
Duration (hours)	96	48
Endpoint	LC50	LC50 or EC50
Effect	Mortality	Mortality or immobilization
Value	Discrete	Discrete

unbounded toxicity values (i.e., reported as “greater than” or “less than” a specified concentration, such as >100 µg/L);

- Toxicity values compiled from the PPDB (University of Hertfordshire, 2013).

Bioassay data used in this paper are for fish and invertebrates only. When querying the ECOTOX database, specific species were not queried by name; the species list of aquatic animals included crustaceans, fish, insects/spiders, other invertebrates, and molluscs. Most species in the data query output are uniquely found in freshwater environments; however, a limited number of species spend part of their life cycle in the freshwater environment (e.g., salmon), or are known to inhabit marine or estuarine habitats but were tested under freshwater conditions. Bioassay data records were removed for sponges, microcrustaceans such as rotifers and ostracods, worms such as turbellarians and oligochaetes, protozoans, and ciliates, which were not retained with the invertebrates. In addition, bioassays were limited to laboratory tests conducted in freshwater, which the ECOTOX database defines as having salinity of 4 parts per thousand or below (U.S. Environmental Protection Agency, 2011). Subsequently, ECOTOX test data were subdivided by taxonomic group, and classified as standard (Table 1) or non-standard as described in Section 2.2.

For pesticides without standardized test data in ECOTOX, but that have acute OPP aquatic-life benchmarks for fish and/or invertebrates, toxicity values associated with those benchmark values (U.S. Environmental Protection Agency, 2012b) were compiled from the cited source document. Toxicity values obtained from OPP documents were the cited LC50 or EC50 values, and were not equivalent to the acute OPP benchmarks themselves, which include an additional safety factor of 2. Some OPP benchmark values are unbounded because the EC50 or LC50 was greater than the highest concentration tested or less than the lowest concentration tested. Unbounded LC50 or EC50 values were considered non-standard data (Table 1; Section 2.2).

Aquatic toxicity values for pesticide compounds that did not have data available from ECOTOX or OPP documents were obtained from the PPDB (University of Hertfordshire, 2013), when available. Data included experimental 96-h LC50 values for fish (usually rainbow trout), 48-h EC50 values for aquatic invertebrates (usually *D. magna*), and 96-h LC50 values for sediment dwelling organisms (usually the midge, *Chironomus riparius*). In the present study, toxicity values from the PPDB were limited to those with (1) standard test durations and (2) confidence scores of 5 (verified with regulatory purpose), 4 (verified), or 3 (unverified but known source). For LC50 values, the effect (mortality) can be inferred, but for cladoceran EC50 values, the effect was not reported in the PPDB but was accepted for the present paper as likely referring to immobilization, and therefore standard. Toxicity values from the PPDB were considered standard if the test duration, endpoint, and species were appropriate and the values were discrete; if the reported toxicity value was unbounded, the endpoint was non-standard, or the species was not reported, then the toxicity value was considered to be non-standard.

2.2. Data screening process

The criteria for standardized test data in Table 1 are identical to those used in previous editions of the PTI, with one exception—for

cladocerans, the inclusion of the LC50 values is new, as only EC50s based on immobilization were used previously (Munn and Gilliom, 2001; Munn et al., 2006). Both endpoints are widely used and it is difficult to ascertain organism mortality with certainty without examining the organism under a dissecting microscope for a heartbeat (Jonczyk and Gilron, 2005). Standardized methods are available for both immobilization (Commission of the European Communities, 1992; Organization for Economic Cooperation and Development, 2004; U.S. Environmental Protection Agency, 1996) and mortality (U.S. Environmental Protection Agency, 2002). Inclusion of LC50 values for cladocerans may increase the median toxicity value for cladocerans to some degree; however, this represents only one of many sources of uncertainty in experimental toxicity test data (Section 3.4). The added uncertainty from combining LC50 with EC50 values is countered by the increase in the number of data records: Of the 2064 toxicity values for cladocerans in the present study, 739 (36%) were LC50 values, allowing determination of toxicity concentrations for an additional 24 pesticides for which EC50 values for immobilization were not available. The rationale for including LC50 values for cladocerans is discussed further in Appendix A of the supplementary information.

For pesticide compounds that did not have standardized test data (Table 1) available, non-standard test data were selected using the screening procedure below. Non-standard toxicity values were characterized by the direction of bias where possible.

- Concentration: If non-standard tests with discrete values were available, then only these tests were used, and any tests with results reported as unbounded or as concentration ranges were omitted. If no available studies reported discrete values, then toxicity values that were unbounded or ranges were retained, subject to further screening below. A toxicity value expressed as a greater-than concentration (e.g., >100 µg/L) was considered to overestimate toxicity, and a result expressed as a less-than concentration to underestimate toxicity. For test results expressed as a concentration range (e.g., >100 µg/L but <1000 µg/L), the unbounded low end of the range was retained (this only applied to one test).
- Effect: For fish studies, the only acceptable endpoint was mortality. For cladocerans and benthic invertebrates, if one or more studies had immobilization or mortality measured, all tests with other effects measured (such as population growth or hatching success) were omitted; if all invertebrate tests had non-standard effects measured, then these tests were retained, subject to screening for duration. Typically the direction of bias in such cases was rated as unknown.
- Duration: If all available studies had non-standard exposure periods, then the tests with the duration closest to the standard duration were selected; if test durations were both shorter and longer (to a comparable degree) than the standard test duration, then the longer period was selected to be more protective. For example, if the standard duration is 4 days, and there were data for 1, 2, 5, and 21 days, then the 5-day study was selected. In occasional cases where the longer exposure periods did not have the lowest (most sensitive) toxicity values, then all discrete toxicity values associated with all acute test durations were included. A study with long exposure duration was considered to overestimate toxicity, and a study with short exposure duration to underestimate toxicity.

2.3. Development of the Pesticide Toxicity Index

The PTI is determined separately for three taxonomic groups: fish, cladocerans, and benthic invertebrates. Cladocerans are treated separately from benthic invertebrates because of differences in physiology, habitat and functional group. In addition, their long history of use in toxicity testing has resulted in a large cladoceran dataset with a relatively small number of genera.

The PTI_t is the sum of toxicity quotients for each pesticide compound (*i*) measured in ambient water from a hydrologic system, for each taxonomic group (*t*):

$$PTI_t = \sum_{i=1}^n (E_i/TC_{i,t}) \quad (1)$$

where E_i is the concentration of pesticide *i*, *n* is the number of detected pesticides in an environmental sample, and $TC_{i,t}$ is the toxicity concentration for the pesticide *i* for the taxonomic group *t*. *E* and *TC* are expressed in the same units.

The PTI of Munn and Gilliom (2001) and Munn et al. (2006) was computed from Eq. (1) using the median of toxicity values available for a given pesticide. The median was selected because, for pesticides, there is a large difference in sensitivities of different taxonomic groups (Maltby et al., 2005, 2009; Sala et al., 2012; van den Brink et al., 2006). Even under standardized test conditions within a taxonomic group, there is considerable variability in toxicity values for many pesticides. In data compiled for the present study, the distribution of standard toxicity values for a given pesticide within a taxonomic group spanned up to 3–4 orders of magnitude for pesticides with 20 or more toxicity test values available, and extreme values—defined as more than three orders of magnitude from the median—occurred for 17 to 41% of these pesticides, depending on the taxonomic group. Factors that may contribute to variability include differences between test organisms, test conditions, and pesticide test material (see Section 3.4).

On the other hand, the use of median toxicity values in the PTI means that the concentration selected for a given pesticide may not be representative of some sensitive species or life stages. For certain applications of the PTI, a more sensitive indicator of toxicity may be preferable. Therefore, for the present study, the PTI method was modified to include two types of PTI values for a given taxonomic group, which are computed by using two different types of *TC* values in Eq. (1):

- The Median-PTI is calculated from Eq. (1) using the median toxicity concentration (MTC), which is defined as the median of the toxicity values available for each compound towards the taxonomic group. The Median-PTI is equivalent to the original PTI of Munn et al. (2006), except for some modifications to the kinds of toxicity data that are acceptable (Sections 2.1 and 2.2).
- The Sensitive-PTI is calculated from Eq. (1) using the sensitive toxicity concentration (STC). The STC is either the 5th percentile or (if fewer than 13 values were available) the minimum toxicity value for each compound towards the taxonomic group (Section 2.4).

MTC values are robust to outliers and provide a more consistent representation of relative toxicity values among pesticides, whereas STC values provide a more sensitive indication of potential toxicity to aquatic organisms. Because extreme values were fairly prevalent in the toxicity test dataset, care was taken to minimize the likelihood of selecting an outlier as the STC (Section 2.4 and Supplemental Appendix A).

2.4. Determination of STC values

The 5th percentile of the available toxicity values was selected as the STC because it is a sensitive indicator of relative toxicity, yet it is more robust to outliers than the minimum value is. Species that have a greater number of toxicity tests available will have more influence on the STC than other species. The most frequently tested species tended to be among the more sensitive species within this dataset (Supplemental Appendix A); therefore, STCs tended to be biased low (i.e., to overestimate toxicity), which was considered acceptable because the objective of the STC was to establish a sensitive indicator of potential toxicity to aquatic life.

The toxicity value percentiles were determined for each pesticide compound within a taxonomic group using the Cunnane (1978) plotting position of 0.4, as recommended by Helsel and Hirsch (1992), and linear extrapolation. The *quantile.default* function in Spotfire S + 8.1 (TIBCO Software Inc., 2008) was used to perform the computations.

For pesticide compounds with 20 or more toxicity values available, the 5th percentile was used as the STC. For pesticide compounds with 13 to 19 toxicity values available, the STC was the 5th percentile as determined using linear extrapolation, with the two lowest ranked toxicity values used to extrapolate near or below the minimum value. One consequence of this extrapolation method is that if the lowest two toxicity values were a tie, then the 5th percentile value was equal to the minimum. For pesticide compounds with 12 or fewer toxicity values, the minimum value was used as the STC in lieu of the 5th percentile. Monte Carlo simulations (detail in Supplemental Appendix A) showed that, for pesticides with 12 or fewer toxicity values available, the probability was less than 50% that the minimum toxicity value was a statistical outlier, defined as within the lowest 5th percentile of a hypothetical large population of toxicity values.

As described in Supplemental Appendix A, an alternative approach was evaluated in which toxicity tests were first aggregated by species and SMAVs were computed as the geometric mean of all tests for a given species and compound; the STC then was determined to be either the lowest SMAV or (for compounds with more than 12 species) the 5th percentile of the SMAVs. The resulting species-weighted STCs were not only highly correlated with the unweighted STCs, although species weighting raised STC values for many pesticides, but also (for those pesticides whose lowest SMAV was based on a single test) increased the likelihood of selecting a possible low outlier as the STC (see Supplemental Appendix A.3 for further discussion and examples). For compounds with multiple tests available for only a single species, selecting the lowest SMAV as the STC resulted in a value that was similar to (or even greater than) the MTC, rather than a more conservative, sensitive value. In theory, it would be preferable to weight species equally when determining the STC; however, in practice this was problematic because of the unevenness and scarcity of the toxicity test dataset (Supplemental Appendix A.3). Although the unweighted STC is more influenced by results for some species than for others, a species-weighted STC would be more influenced by results of some individual tests than of others.

2.5. Application of the Pesticide Toxicity Index to interpret water-quality data

To test the PTI model and to illustrate its application to water-quality data, published studies were compiled that reported concurrent data on pesticide concentrations and aquatic toxicity. These studies reported raw data for both (1) concentrations of a substantial number of pesticides in ambient stream water and (2) organism survival in toxicity tests conducted in the laboratory with undiluted ambient water. Studies included a series of reports and memoranda on pesticides and toxicity in California streams published by the California Department of Pesticide Regulation (DPR) (Ensminger et al., 2009; Ganapathy, 1999a, b; Gill, 2002; Jones, 2000; Kim et al., 1999a, b; Nordmark, 1999, 2000; Walters et al., 2000a, 2000b, 2000c, 2000d, 2000e, 2000f, 2000g, 2000h, 2000i, 2000j, 2000k, 2000l, 2000m, 2001a, 2001b, 2001c, 2001d, 2001e, 2001f, 2001g, 2001h, 2001i, 2001j) and the Central Coast Water Quality Preservation (CCWQP) (2008, 2009). Some independent studies also provided suitable data (Kuivila and Foe, 1995; Phillips et al., 2012).

These studies measured acute toxicity to *Ceriodaphnia dubia* in 4 to 8-day laboratory exposures to undiluted ambient stream water, so results were compared to the PTI for cladocerans (Section 3.3.1). When data were aggregated from these studies, the total number of individual samples was 479 (Table 3). Samples from CCWQP (2008, 2009) and Kuivila and Foe (1995) were exposed for 6–8 days (total *n* = 174 samples), whereas the remaining samples in the aggregated dataset were

exposed for 4 days ($n = 305$ samples). Each study analyzed between 8 and 27 parent pesticides, and collected water samples between 1993 and 2009 (Table 3). All aggregated samples were from California and most studies focused on organophosphate insecticides, which were responsible for placement of some California streams on the Clean Water Act (CWA) §303(d) list (Central Coast Water Quality Preservation Inc., 2008, 2009; Ensminger et al., 2009).

The results from one study in Table 3 that analyzed multiple classes of pesticides—a study of dormant spray pesticides by the California DPR (Gill, 2002; Nordmark, 1999, 2000)—are described in more detail to illustrate application of the PTI to interpret water-quality data (Section 3.3.2). For the purposes of discussion, the term “toxic” is defined in this paper as reduction of *C. dubia* survival to $\leq 50\%$ of that in control samples. Although not statistically based, the 50% value in this definition corresponds to the EC50/LC50 values from which PTI values are calculated. Spearman rank correlations and least-square regressions were performed using DataDesk 6.3.1.

Invertebrate community condition in streams across the U.S. was assessed as part of the USGS NAWQA Program during 1993–2005 (Bryant and Carlisle, 2012; Carlisle and Meador, 2007; Carlisle et al., 2013; Yuan et al., 2009). Invertebrate communities were characterized by quantifying the deviation of a site's measured macroinvertebrate composition from the expected composition, expressed as the ratio of observed/expected (O/E) taxa. The O/E index provides a way to compare across sites because it is standardized by each site's potential for species composition and richness. Expected invertebrate assemblage compositions (E) for each site were the sum of predicted probabilities of capturing common species at a given site, derived from predictive models developed from regional reference sites (Carlisle and Hawkins, 2008; Carlisle and Meador, 2007; Yuan et al., 2008).

Based on data compiled for Carlisle et al. (2013), NAWQA sites were identified that had both (1) an invertebrate community sample with an O/E ratio and (2) data for 26 pesticides analyzed in water at all sites at least 3 times during the 90 days prior to the invertebrate biological sample, with at least 2 samples occurring in different calendar months. A total of 128 stream sites sampled between 1993 and 2004 met these conditions. The potential toxicity of pesticides at these sites was characterized using the maximum Median-PTI for benthic invertebrates that occurred in samples within the 90-day time period. The relationship between O/E ratio and the Median-PTI was evaluated using least-squares regression.

3. Results and discussion

3.1. Toxicity concentrations – MTC and STC

For pesticides and degradates combined, MTCs and STCs were based on toxicity values from a total of 10,837 bioassays for 559 species in

three taxonomic groups. The number of unique species was 228 for fish, 47 for cladocerans, and 284 for benthic invertebrates. Table 2 summarizes the number of compounds with toxicity values and the number of underlying bioassays for pesticides and degradates, by taxonomic group. MTC and STC values for individual pesticides and degradates in each taxonomic group are provided in Supplemental Appendix B for use in calculating PTI values from pesticide concentrations measured in environmental samples. MTC and STC values are listed for each taxonomic group separately—for fish (Table B.1), cladocerans (Table B.2), and benthic invertebrates (Table B.3)—including the number of toxicity tests, number of species, type and source of toxicity data, and any applicable bias for pesticides and degradates with non-standard toxicity data.

3.1.1. Fish

MTC and STC values for fish were determined for a total of 435 pesticides and 48 degradates. Many more bioassays were available for pesticides (6685) than for degradates (173; Table 2). Of the total bioassays, most (98%; Table 2) met the definition of standard data from Table 1. The median number of standardized bioassays per compound was four for pesticides and one for degradates; the median number for non-standard bioassays was one for both pesticides and degradates. The use of non-standard data for pesticide compounds that did not have standard data available increased the total number of compounds with MTC and STC values by 78 (22% increase) for pesticides and 18 (60% increase) for pesticide degradates (Tables 2 and B.1). Just over half (53%) of the pesticides and most (83%) of the degradates with non-standard data for fish have only a single toxicity value, which means that the MTC and STC are the same for these pesticides. Overall, the standard dataset for fish includes toxicity data from 6692 bioassays representing 228 different species, including warm and cold water fish, but about half of the bioassays are for one of three species: rainbow trout (23%), bluegill (18%), and fathead minnow (11%) (Appendix C, Table C.1).

3.1.2. Cladocerans

MTC and STC values for cladocerans were determined for a total of 423 pesticides and 47 degradates; a total of 2063 bioassays were available for pesticides and 116 for degradates (Tables 2 and B.2). The median number of bioassays per compound was one, for both standard and nonstandard data. Addition of the non-standard dataset for cladocerans increased the number of compounds with MTC and STC values by 68 (19%) for pesticides, and 19 (68%) for degradates (Tables 2 and B.1). There are 17 genera and 47 species represented in the standard dataset (Tables C.1 and C.2); however, most tests used *D. magna* (63%), *C. dubia* (12%), *Daphnia pulex* (5%), *Daphnia longispina* or *Simocephalus serrulatus* (3% each). The non-standard cladoceran data set includes only 5 species,

Table 2

Summary of the number of compounds and bioassays in the standard and non-standard datasets for the three taxonomic groups: fish, cladocerans, and benthic invertebrates. [*b*, number of bioassays].

Bioassay data by taxonomic group	Pesticides			Pesticide degradates		
	Fish	Cladocerans	Benthic invertebrates	Fish	Cladocerans	Benthic invertebrates
No. of total compounds	435	423	205	48	47	15
No. of total bioassays (<i>b</i>)	6685	2063	1734	173	116	66
Overall median <i>b</i> per compound	3	1	3	1	1	2
<i>Standard data</i>						
No. of compounds	357	355	139	30	28	10
No. of bioassays	6540	1978	1571	152	97	53
Median <i>b</i> per compound	4	1	4	1	1	2
<i>Non-standard data</i>						
No. of compounds	78	68	66	18	19	5
No. of bioassays	145	85	162	21	19	13
Median <i>b</i> per compound	1	1	1	1	1	2
Increase in no. of compounds by adding non-standard data	22%	19%	47%	60%	68%	50%

Table 3
Studies included in the aggregated dataset used to plot survival from *Ceriodaphnia dubia* toxicity tests as a function of Median-PTI and Sensitive-PTI (Fig. 1) and to determine the optimum Median-PTI and Sensitive-PTI thresholds (Fig. 2). All toxicity tests were conducted with undiluted ambient stream water. [CA DPR, California Department of Pesticide Regulation; Carb, carbamate; CCWQP, Central Coast Water Quality Preservation; d, days; Herb, herbicide; I, insecticides; No., number; OP, organophosphate; Pyr, pyrethroid; Ref, reference; Tox, toxicity].

Author or agency	Study area	No. sites	No. samples	Sampling dates	Number of parent pesticides analyzed						Tox test duration (d)	General sampling design	Ref	
					OP-I	Carb-I	Pyr-I	Other-I	Herb	Total				
CA DPR	Sacramento River watershed (Wadsworth Canal)	1	42	1998–2000	8	2	–	–	9	19	4	Baseline sample in Dec, then sample twice weekly through dormant spray season	1	
		1	24	2000–2001	8	2	–	–	9	19	4		2	
CA DPR	San Joaquin River watershed (Orestimba Creek)	1	18	1997–1998	8	2	–	–	9	19	4	Baseline sample in Dec, then sample twice weekly through dormant spray season	3	
		1	20	1998–1999	8	2	–	–	9	19	4		4	
		1	20	1999–2000	8	2	–	–	9	19	4		5	
CA DPR	Del Puerto and Orestimba Creeks	2	21	2007–2008	10	–	8	–	–	18	4	Monthly samples from Dec–June; 3 storm samples in Dec, Jan, Feb	6	
CA DPR	Orange County	3	8	1999	8	–	–	–	–	8	4	Rainfall runoff sampling	7	
		12	90	1999–2000	8	–	1	3	–	12	4	Monthly sampling; Feb 2000 samples coincided with rainfall	8	
		8	38	2000	8	–	1	3	–	12	4	Rainfall runoff sampling	9	
		2	14	2000–2001	5	–	1	3	–	9	4	Monthly sampling; no rainfall runoff	10	
CCWQP	Lower Salinas and Santa Maria watersheds	18	18	2007	19	–	–	–	–	19	6–8 d	Sample during dry season	11	
		4	6	2008	14	6	–	–	–	7	27	6–8 d	Sample during dry season	11
		21	21	2008	19	–	–	–	–	19	6–8 d	Sample during dry season	11	
		23	94	2006–2007	19	–	–	–	–	19	6–8 d	Sample twice per year in dry season and twice in winter/wet season	12	
Kuivila	San Francisco Estuary	2	35	1993	^a 4	^a 1	–	–	^a 3	8	7 d	Daily samples for 3 weeks; included some rainfall events	13	
Phillips	Santa Maria River and Oso Flaco Creek watersheds	10	10	2009	6	–	9	–	–	15	4	Bimonthly for 1 year; full pesticide analysis on 1 sample per site	14	

^a 19 pesticides analyzed but only those detected were identified

1. Nordmark (1999, 2000)
2. Gill (2002)
3. Ganapathy (1999a)
4. Ganapathy (1999b)
5. Jones (2000)
6. Ensminger et al. (2009)
7. Kim et al. (1999a)

8. Kim et al. (1999b); Walters et al. (2000c); Walters et al. (2000e, f, g, h, i, j, k, m)
9. Walters et al. (2000l)
10. Walters et al. (2000a, b, d, 2001a, b, c, d, e, f, g, h, i, j)
11. Central Coast Water Quality Preservation Inc. (2009)
12. Central Coast Water Quality Preservation Inc. (2008)
13. Kuivila and Foe (1995)
14. Phillips et al. (2012)

and *D. magna* accounts for 90% of the non-standard bioassays (Table C.1).

3.1.3. Benthic invertebrates

MTC and STC values for benthic invertebrates were determined for 205 pesticides and 15 degradates from a total of 1734 bioassays for pesticides and 66 bioassays for degradates (Tables 2 and B.3). Addition of the non-standard data for benthic invertebrates increased the number of compounds with MTC and STC values by 66 (47%) for pesticides and 5 (50%) for degradates. The standard dataset had 3 or more bioassays for 59% of compounds and represented 259 species, with amphipods of the genus *Gammarus* making up nearly 20% of the standard bioassays (Tables B.3 and C.1). The non-standard dataset for benthic invertebrates included toxicity data from 44 different species (Table C.1), with the greatest number of bioassays for the midge, *C. riparius* (16% of the total), followed by the mosquito, *Aedes aegypti* (9%) and crayfish, *Procambarus clarkii* (8%). Collectively, the standard and non-standard benthic invertebrate datasets include data for 284 species and 172 genera. Benthic crustaceans account for 25% of the genera and 33% of the species; insects/spiders account for 47% and 38%, and molluscs account for 28% and 28% of the genera and species, respectively (Table C.2).

3.2. PTI values for a water sample

MTC and STC values for individual pesticide compounds in Supplemental Appendix Tables B.1 to B.3 can be used with Eq. (1) to calculate Median-PTI and Sensitive-PTI values, respectively, for a sample. Pesticide compounds with non-standard MTC and STC values have greater uncertainty compared to compounds for which standardized toxicity test data are available, but the decision to exclude that pesticide from the PTI because it has a non-standard MTC or STC is equivalent to treating it as a non-detection in the sample. For some applications, it may be preferable to include an uncertain or biased toxicity value (i.e., non-standard MTC or STC value) for a given pesticide, in lieu of leaving that pesticide out of the PTI. However, potential bias of pesticides with non-standard data should be considered when applying the PTI to interpret water-quality data, especially if a pesticide with a non-standard MTC that is biased low (i.e., overestimates toxicity) tends to dominate the PTI of a water sample.

MTC and STC values are significantly correlated with one another ($p < 0.001$) within a taxonomic group (R^2 of 0.84 to 0.93). The ratio of MTC to STC values for a given pesticide reflects the degree of variability in the toxicity data available for that pesticide. The MTC/STC ratios vary the least for cladocerans (coefficient of variation, CV = 400%), the group containing the smallest number of unique species. Fish are

intermediate ($CV = 500\%$) and benthic invertebrates, which is the most diverse taxonomic group, have the most variable MTC/STC ratios ($CV = 1,100\%$). The MTC/STC ratio is 10 or higher for 9% of pesticides for cladocerans, 15% of pesticides for fish, and 22% of pesticides for benthic invertebrates. The Median-PTI dampens differences in toxicity values among taxa, tests, and test conditions, and provides a measure of central tendency for comparing the toxicity of one pesticide to another towards a given taxonomic group.

When MTCs (medians) are used, the purpose of the index is to represent the relative toxicity of sites, samples, or individual pesticides—MTC values may be higher than effect concentrations for some species and life stages in a given hydrologic system, but they are relatively robust to outliers. When the STC values are used, the index is better suited for use as a screening level, because it is a more conservative (protective) indicator of the potential for toxicity. Although the 5th percentile of the available toxicity values was selected as the basis for the Sensitive-PTI (as discussed in Section 2.4), it does not necessarily protect 95% of species and it is not equivalent to a 5th-percentile hazardous concentration (HC5) from a SSD (Etterson, 2011). The PTI methodology computes the 5th (and 50th) percentiles of all suitable independent toxicity tests, whether tests are based on the same species or on different species. It is intended to prevent low outlier values, such as from singularly sensitive species, statistically anomalous results, or unusual test conditions, from skewing the results.

3.3. Example applications of the PTI to interpret water-quality data

3.3.1. Validation test of the PTI using aggregated data from published studies

Data aggregated from published studies were used to test how well the PTI approach predicted the incidence of observed toxicity, (i.e., reduction in *C. dubia* survival). The available studies (Table 3), which analyzed different numbers and classes of pesticides at different analytical reporting levels, are not considered to represent a robust test of the PTI model. However, these results provide some indication of PTI values at which toxicity has been observed in field-collected water samples.

Based on aggregated data from published studies (Table 3 and Supplemental Appendix D), Fig. 1 shows survival of *C. dubia* in 4–8 day toxicity tests in ambient stream water as a function of the Median-PTI (Fig. 1A) and the Sensitive-PTI (Fig. 1B). With some exceptions (those symbols shown on y-axis), survival remained near 100% until the Median-PTI reached about 0.1. Survival rates decreased sharply at Median-PTI values between about 0.1 and 0.5, with highly variable % survival within this range. Of samples with Median-PTI values > 1 , 97% had *C. dubia* survival reduced to $\leq 50\%$ relative to controls, and 96% had 0% survival. Percent survival declined to 50% well below the theoretical value of 1 for the Median-PTI. The same studies plotted in relation to the Sensitive-PTI look very similar (Fig. 1B), except that most results are shifted to the right, so that *C. dubia* survival appears to drop at Sensitive-PTI values between 0.5 and 1. Results were similar among studies and no single study dominated the pattern shown.

The pattern shown in Fig. 1 was the same for studies that exposed *C. dubia* to ambient water for 4 days vs. 6–8 days and also for studies that measured only organophosphate insecticides vs. multiple classes of pesticides (not shown). Organophosphates accounted for over half of the Median-PTI in 71% of samples overall, and in 86% of samples with *C. dubia* survival reduced to $\leq 50\%$. This occurred in part because organophosphates are highly toxic, but also because most of the published studies had focused on organophosphate insecticides because of CWA §303(d) listing of streams. Where organophosphates were the only pesticides analyzed (141 samples) or the only pesticides detected (110 additional samples), the PTI model is indistinguishable from an organophosphate TU model, in which only pesticides with this common mode of action (acetylcholinesterase inhibition) are included. However, of 228 samples with multiple pesticide classes detected, 81 samples had

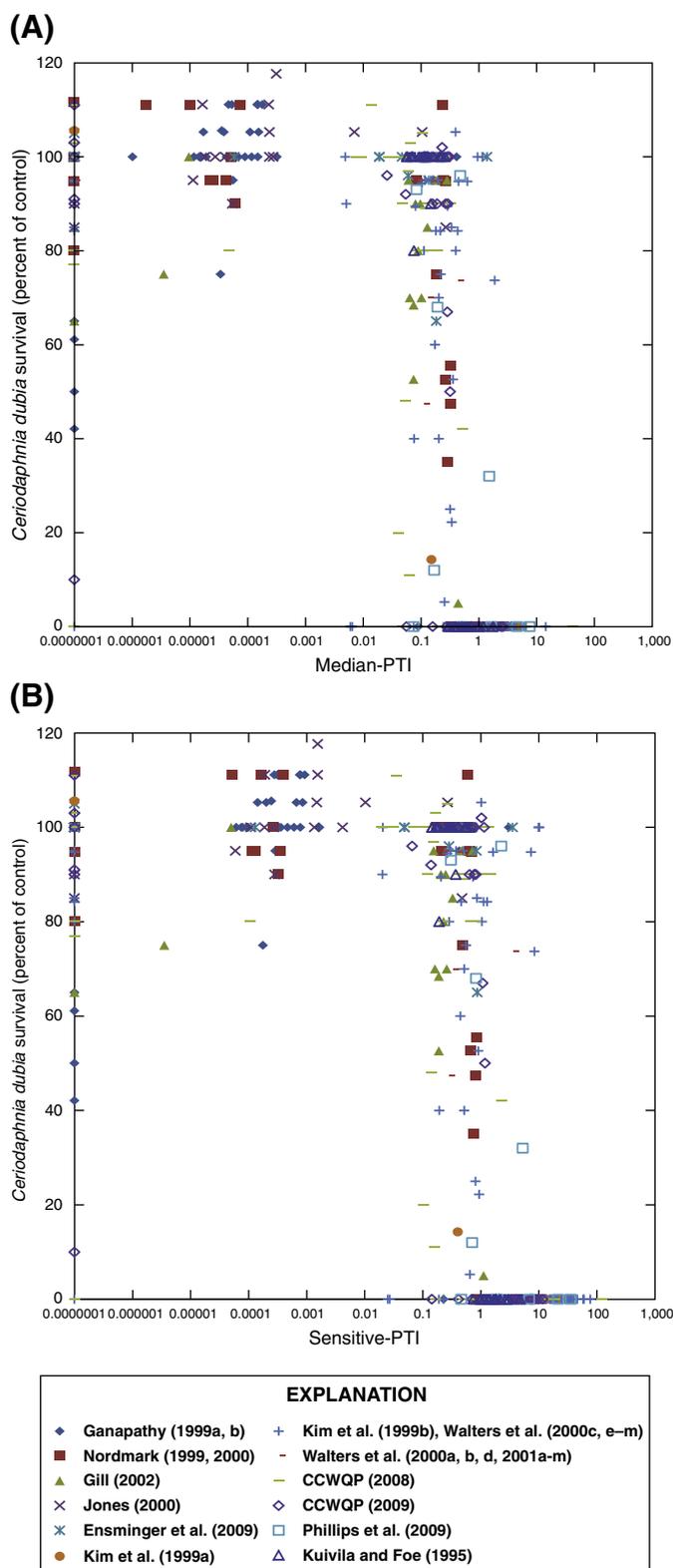


Fig. 1. *Ceriodaphnia dubia* survival on 4–8 day exposure to ambient water, as a function of Median-Pesticide Toxicity Index (PTI) (A) and Sensitive-PTI for cladocerans (B), computed for pesticides detected in the sample. Each symbol represents a single sample ($n = 479$ samples). Results are aggregated from published studies in Table 3.

C. dubia survival reduced to $\leq 50\%$. In these 81 “toxic” samples, organophosphates accounted for less than half of the Median-PTI in 32% of samples, and less than one quarter of the Median-PTI in 17% of

samples—indicating that other pesticides besides organophosphates may have contributed to the observed toxicity in these samples.

The incidence of toxicity in samples with Median-PTI values < 1 is expected because the lowest toxicity values were excluded by use of the median, pesticides may be present at concentrations below the analytical detection level, and contaminants may have been present that were not analyzed in the sample or that were not included (because toxicity data were not available) in the PTI. In addition, variability is expected because of uncertainty in MTCs used to compute PTI values, deviation from the CA model that underlies the PTI approach, and environmental factors affecting toxicity in the sample. Lack of toxicity at Median-PTI values > 1 is more difficult to explain, but this occurred in only 3 samples from 2 studies, out of a total of 479 samples. Possible explanations include the fact that dose–response curves vary for different pesticides and mortality occurs at concentrations below the EC50 or LC50 endpoints used to derive MTCs; use of the MTC to represent a given pesticide, which may underestimate toxicity to some organisms and overestimate toxicity to others; the assumption that toxicity is additive with no chemical interaction for pesticides from multiple classes with different modes of action; and the fact that the PTI does not consider environmental factors (e.g., organic carbon, temperature) that may affect bioavailability and/or toxicity.

In summary, although data available to test the PTI model are limited, *C. dubia* survival was reduced to ≤ 50% of controls in 44% of samples with Median-PTI values in the range of 0.1 to 1, and to 0% in 96% of samples with Median-PTI values > 1. For the Sensitive-PTI, *C. dubia* survival was reduced to ≤ 50% of controls in 81% of samples in the range of 0.1 to 1, and in 89% of samples with Sensitive-PTI values > 1.

In theory, for TUs that are based on acute LC50/EC50 data, the CA model predicts 50% mortality at a theoretical threshold of 1, and theoretically based thresholds were proposed in previous studies by Battaglin and Fairchild (2002) and Anderson (2008) for classifying samples according to the probability of toxicity from pesticide mixtures. In contrast, an empirical threshold was previously developed for contaminant mixtures in sediment (Ingersoll et al., 2001; MacDonald et al., 2000) on the basis of validation data from field studies, which indicated a high probability of toxicity at values > 0.5 for an additive sediment-quality index called the Mean Probable Effect Concentration Quotient. Similarly, in the present study, the validation dataset consisting of field data on pesticides and *C. dubia* survival (Table 3 and Supplemental Appendix D) can be used to determine an apparent empirical threshold at which the PTI model best explains the incidence of toxicity observed in the reviewed studies, but in this case the “threshold” is actually based on a 50%-mortality response. An empirical threshold of toxicity should result in the correct classification of as many samples as possible—so that all or most of the samples above the empirical threshold are toxic, and all or most of the samples below the threshold are nontoxic—however “toxic” may be defined.

Fig. 2 shows the percentage of samples in the aggregated dataset that were correctly classified as “toxic” or “nontoxic,” as a function of various possible 50%-mortality threshold values for Median-PTI and Sensitive-PTI. In the analysis shown in Fig. 2, toxicity was defined as reducing *C. dubia* survival to ≤ 50% of controls, or 50% mortality, because this corresponds to the definition of LC50 and EC50 on which the cladoceran Median-PTI and Sensitive-PTI are based. Correspondingly, “nontoxic” is used to indicate survival > 50% relative to control. Correct classification is defined as the sum of (i) the number of samples with PTI above a potential 50%-mortality threshold and also toxic (as defined above), plus (ii) the number of samples with PTI below the threshold and nontoxic, as a percentage of (iii) the total number of samples. It is important to acknowledge that the potential 50%-mortality thresholds under discussion designate index levels at which *C. dubia* survival was reduced to ≤ 50%, whereas in actuality, mortality and sublethal toxicity will occur at levels below LC50 and EC50 values.

In Fig. 2, each symbol represents all the data ($n = 479$ samples) assessed relative to the corresponding possible 50%-mortality threshold

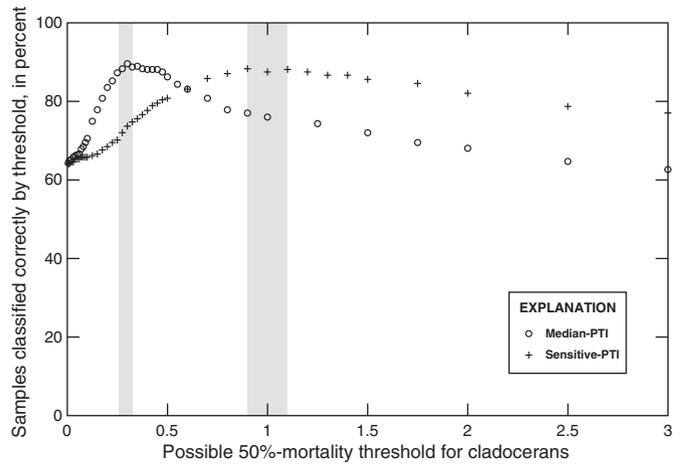


Fig. 2. Effectiveness of various 50%-mortality thresholds for the Pesticide Toxicity Index (PTI) in correctly classifying samples in the aggregated dataset as nontoxic or toxic. Each symbol shows the percentage of samples in the entire dataset ($n = 479$) that was correctly classified using the threshold value on the x-axis. The gray-shaded areas indicate the optimum 50%-mortality threshold range (x-axis value corresponding to the maximum y-axis value) for the Sensitive-PTI and Median-PTI.

on the x-axis. The x-axis represents the possible 50%-mortality threshold values ranging from 0.01 to 3, and the y-axis indicates how well each threshold value performed in explaining *C. dubia* mortality in the aggregated dataset. At low 50%-mortality threshold values, the correct classification rate is low because a large proportion of samples above these low thresholds were nontoxic. As increasingly higher values for the 50%-mortality threshold values are considered, fewer of the samples above the threshold are nontoxic, so the percentage of samples that were correctly classified increases to a maximum. As potential 50%-mortality threshold values continue to increase above the level associated with this maximum, the number of toxic samples below the threshold begins to increase, causing the frequency of correct classification to decline. The optimum 50%-mortality threshold for correct classification of samples in this dataset—that is, the value resulting in the maximum percentage of correctly classified samples—is 0.3 for the Median-PTI and 0.9–1.1 for the Sensitive-PTI (Fig. 2).

The 50%-mortality thresholds of 0.3 and about 1 for Median-PTI and Sensitive-PTI, respectively, are empirical thresholds that apply only to the studies on which they were based. Although in this analysis, toxicity was defined as reduction of *C. dubia* survival to ≤ 50% of controls during a 4–8 day exposure, the results were similar when toxicity was defined as reducing survival to ≤ 70% of that of controls (the optimum 70%-mortality threshold for the Median-PTI was still 0.3) or ≤ 40% of that of controls (the optimum 40%-mortality threshold for the Median-PTI was 0.35). This indicates robustness in the analysis, in that an empirical “mid-level” mortality threshold was not strongly influenced by the exact percentage survival used to define a “toxic” sample. Because the empirical 50%-mortality thresholds for the Median-PTI and Sensitive-PTI predict about 50% mortality, it is likely that some toxicity—some degree of reduced survival, sublethal effects, and/or adverse effects on invertebrate communities—will occur at lower index levels.

Whether the empirical 50%-mortality thresholds derived here would successfully predict mortality in future studies of large numbers of pesticides from multiple classes is uncertain because (1) this analysis is based on a limited number of samples (479); (2) the studies on which this analysis is based each determined a relatively small number of pesticides (8 to 27 per study); (3) many of these studies were focused on organophosphate insecticides, which share a common mode of action and have been shown to exhibit additive toxicity to cladocerans (Bailey et al., 1997); and (4) concentrations of organophosphate insecticides have decreased in streams (Ryberg et al., 2010; Sullivan et al., 2009) since USEPA restricted their uses in both agricultural and urban applications (U.S. Environmental Protection Agency, 2000, 2001),

whereas concentrations of insecticides in other classes, including carbaryl, pyrethroids, and fipronil, have increased (Ryberg et al., 2010).

3.3.2. Determination of potentially toxic pesticides in a watershed

Results from one of the studies in Table 3 (and included in the aggregated dataset in Section 3.3.1 and Appendix D) are used as an example to illustrate the application of the PTI to interpret water quality data. In this study of dormant-spray pesticides and associated water toxicity conducted by the California DPR, water samples were collected from the Sacramento River watershed at Wadsworth Canal, a tributary of the Sutter Bypass, which receives inflow from dormant spray areas and has no major inputs from municipal or industrial sources (Gill, 2002; Nordmark, 1999, 2000). Samples were collected in December, prior to the onset of dormant spray season, and again during January to March, continuing until dormant spray had ceased, for three consecutive years (1998–2001). The parent pesticides analyzed were 8 organophosphate insecticides, 2 carbamate insecticides, and 9 herbicides. Acute toxicity to *C. dubia* was measured in 4-day static renewal tests.

Median-PTI and Sensitive PTI values for cladocerans were calculated using Eq. (1) and the pesticide concentrations reported in the California DPR study. Data from the first two years (the first 42 of the 66 total samples) of the Wadsworth Canal study (Nordmark, 1999, 2000) are shown in Fig. 3. Total pesticide concentrations (Fig. 3A) tended to be low in December, and the herbicide diuron was the only pesticide detected. The number of unique pesticides detected increased to seven during the spray season, with total pesticide concentrations peaking in late January and February (Fig. 3A). Median-PTI values also peaked in late January and February (Fig. 3B) and were dominated by diazinon, with minor contributions from methidathion in some samples. The same pattern was observed for Sensitive-PTI (Fig. 3C), except that the scale of PTI values was higher and the contribution of methidathion was barely visible. Although the herbicides simazine, hexazinone, diuron and bromacil combined to account for a third to half of the summed concentrations of pesticides detected during the dormant spray season, organophosphates accounted for over 99% of the Median-PTI for samples in which *C. dubia* survival was $\leq 50\%$. Toxicity was observed in samples with Median-PTI greater than about 0.3 and Sensitive-PTI greater than about 0.8. The percent survival of *C. dubia* at the Wadsworth Canal site over the 3-year study (Fig. 3D) was significantly correlated with both the Median-PTI and the Sensitive-PTI ($p < 0.0001$, Spearman rho = -0.67), which each explained 43% of the variability in % survival in separate least-squares regressions. The relationship also was significant for the summed TUs for organophosphate insecticides only ($p < 0.0001$, Spearman rho = -0.68), which explained 37% of the variability in % survival. PTI analysis, such as that shown here, can aid in determining which sites, samples, or pesticides are the most important in terms of potential toxicity to aquatic organisms.

3.3.3. Invertebrate community condition in streams across the U.S.

Median-PTI values for benthic invertebrates were calculated for pesticide concentrations detected in water at 128 stream sites across the U.S. sampled during 1993–2004 by the USGS NAWQA Program. MTC values were available for 20 of the 26 pesticides analyzed in these samples. Macroinvertebrate community condition, characterized by the O/E ratio at the same 128 stream sites, was analyzed relative to the maximum Median-PTI for benthic invertebrates occurring within the 90-days prior to the date of the invertebrate sample, as described in Section 2.5. The O/E ratio is a measure of biodiversity. An O/E ratio of 1 indicates that the measured invertebrate composition is equal to the expected composition (U.S. Environmental Protection Agency, 2006), with ratios < 0.8 indicating biological degradation (Carlisle and Meador, 2007). There was a significant inverse relationship between O/E and the Median-PTI ($p = 0.01$, not shown), but the Median-PTI explained only 5% of the variability in O/E ratios (biodiversity). This small amount of variability explained is not unexpected because invertebrate community condition in streams is affected by many factors in

addition to pesticide contamination, including other chemical stressors (e.g., nutrients, salinity, acidification, hydrophobic contaminants in sediment), physical stressors (e.g. sediment, temperature, flow alteration, habitat degradation) and biological stressors (e.g. invasive or non-native species), as frequently noted in the literature (e.g., Bryant and Carlisle, 2012; Carlisle et al., 2013; Tate and Heiny, 1995; U.S. Environmental Protection Agency, 2006).

3.4. Limitations

The PTI has several limitations, which must be carefully considered in applications:

- The PTI is a relative ranking system that indicates that one sample is likely to be more or less toxic than another sample, but does not indicate that toxicity will necessarily occur.
- Toxicity values are based on short-term laboratory experiments with EC50 (nonlethal response) or LC50 (mortality) endpoints; the PTI does not reflect long-term/chronic exposure or (except for immobilization in cladocerans) incorporate sublethal endpoints.
- The PTI does not account for environmental factors, such as dissolved organic carbon, particulates, pH, and temperature, which can affect the toxicity and bioavailability of pesticides.
- The PTI assumes that pesticide toxicity is additive and there is no chemical interaction (synergism or antagonism), which may not be the case for complex mixtures of pesticides from different chemical classes and with different MOAs across all taxonomic groups and life stages.
- The PTI does not take into account the dose–response curves of either single-chemical or mixtures exposures.
- The PTI is limited to pesticides measured in the water column; hydrophobic pesticides may be underrepresented in terms of potential toxicity, especially to benthic organisms.
- Uncertainty in the relative toxicity of compounds is high for pesticides with relatively few bioassays available. The 10,837 bioassays in this data set are divided among 440 pesticides and 52 degradates, 559 different species, and three taxonomic groups, making the number in each group relatively small. Although this does not preclude the use of the data as the best available, it demonstrates the sparseness of available data on the toxicity of many currently used pesticides.
- Most data are for standard test species (rainbow trout, bluegill, and fathead minnow for fish, and daphnids for invertebrates). Analysis of SSDs for pesticides showed that values for the three typical OPP test species (rainbow trout, bluegill, and *D. magna*) typically resulted in smaller HC5 values (i.e., indicating greater toxicity) compared to random subsets of three toxicity values for the available test species (Etterson, 2011).
- Many factors contribute to the high variability observed in laboratory toxicity tests, including formulation of the pesticide; test species, age and condition of individual organisms used; water conditions (pH, temperature, concentration and type of dissolved organic carbon); testing environment (flow-through or static); and whether test organisms are fed during exposure (Jonczyk and Gilron, 2005; Persoone et al., 2009). For applications in which certain individual compounds are particularly important, special attention should be given to the variability in toxicity test results for those compounds.

4. Conclusions

The PTI is a relative, but quantitative, indicator of potential toxicity that can be used in study design or to interpret water quality data, relate pesticide exposure to biological condition, and prioritize future assessments. The PTI uses toxicity concentrations available for 440 pesticides (out of a total of 484 pesticides with reported agricultural use during 1992 to 2011) and 52 pesticide degradates. Combined, the toxicity data for pesticides and

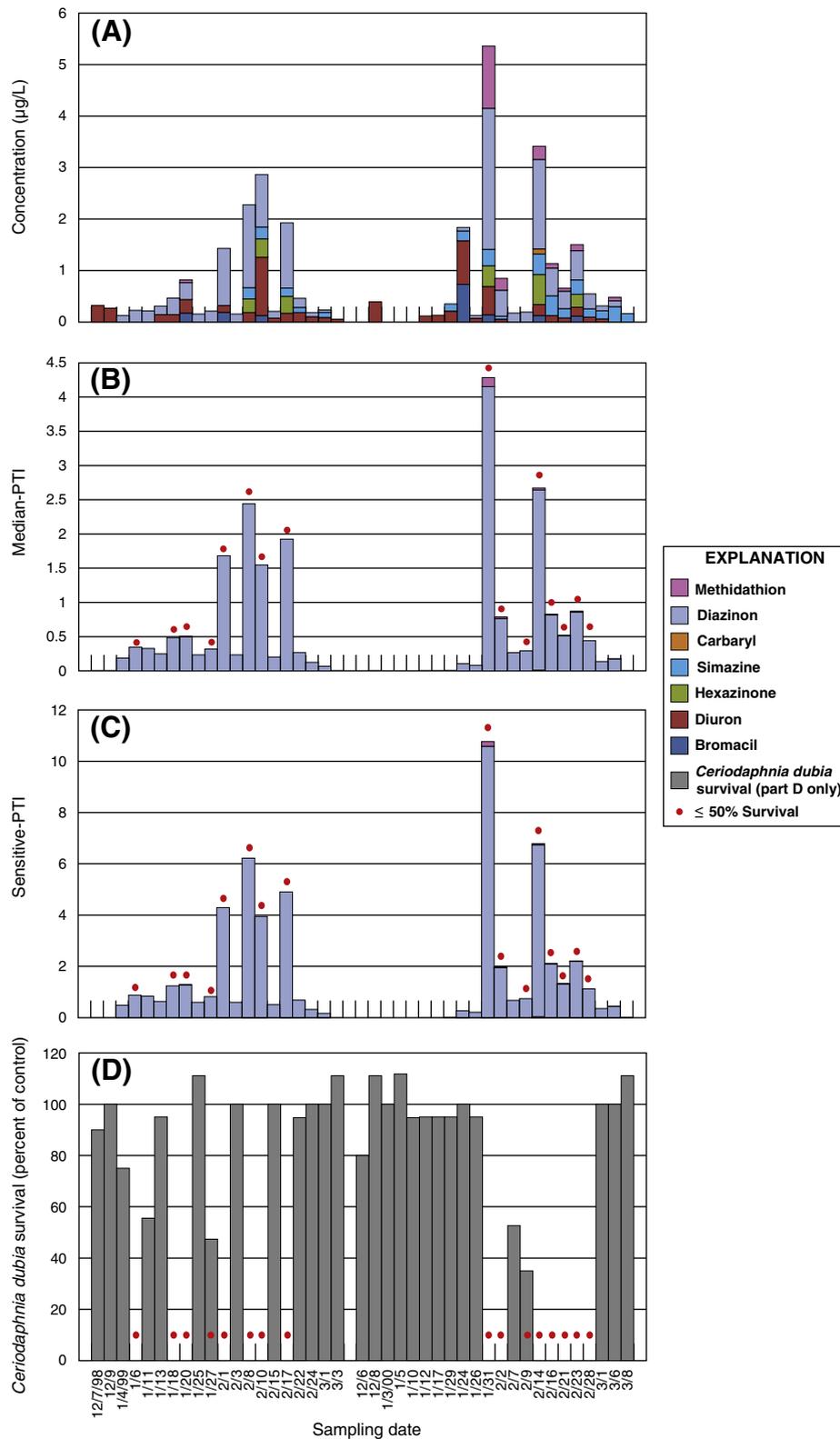


Fig. 3. Summed pesticide concentrations (A), Median-Pesticide Toxicity Index (PTI) (B), Sensitive-PTI (C), and *Ceriodaphnia dubia* survival as percentage of control for samples from the Wadsworth Canal during the 1999 and 2000 dormant spray seasons (D). Pesticide concentrations and *C. dubia* survival are from Nordmark (1999, 2000). The height of each segment in the stacked bar indicates the corresponding pesticide's contribution to the total. Circles above bars indicate that these samples were toxic to *C. dubia*, defined as having survival reduced to $\leq 50\%$ relative to control after 4-day exposure.

degradates include 10,837 bioassays representing 559 different species in three taxonomic groups. Toxicity endpoints include LC50 for fish, EC50 or LC50 for cladocerans, and LC50 or EC50 for benthic invertebrates.

Two types of PTI, as described herein, can be calculated for a field sample. The Median-PTI is calculated using median toxicity concentrations for each pesticide in a sample mixture. The Sensitive-PTI uses the 5th percentile of toxicity concentrations (except that the minimum is

substituted for pesticides with small data sets). The Median-PTI approach is more robust to outliers, whereas the Sensitive-PTI may be appropriate for applications in which a conservative comparison to a screening level is needed.

To test the PTI model, data on concurrent pesticide concentrations and toxicity to *C. dubia* in ambient stream water were aggregated from published field studies. PTI values were calculated, and empirical 50%-mortality thresholds for the Median-PTI and Sensitive-PTI were determined to be about 0.3 and 1, respectively. These 50%-mortality thresholds correctly predicted toxicity (defined as $\geq 50\%$ mortality) or nontoxicity ($< 50\%$ mortality) in about 90% of samples in the reviewed studies. Although field data are not available to validate the PTI model for a comprehensive range of pesticides, the analysis of available data described here indicates that substantial mortality may occur at Median-PTI values in the range of 0.1 to 1—based on the observation that $\geq 50\%$ mortality to *C. dubia* occurred in only 6% of samples from the aggregated dataset with Median-PTI values < 0.1 , but in 44% of samples in the 0.1 to 1 range. Mortality likely would be high at Median-PTI values > 1 , where 96% of samples in the aggregated validation data set showed 100% mortality to *C. dubia*. For the Sensitive-PTI, the percentage of samples with $\geq 50\%$ mortality was 5% in samples with Sensitive-PTI values < 0.1 , 19% in the 0.1 to 1 range, and 89% at Sensitive-PTI values > 1 .

The PTI can be applied to pesticide mixtures detected in ambient water samples, and PTI values for individual water samples then can be used to rank sites according to their expected relative potential toxicity caused by pesticides, to assess changes in potential toxicity over time at a single site, or to predict which of the pesticides detected may be the biggest contributor to potential toxicity for the sample or site. PTI values for samples, seasons, or sites also can be used as explanatory variables in multivariate analysis designed to determine which environmental variables best explain spatial patterns in the structure of a biological community. A more rigorous test of the PTI model is needed, but this will require the availability of data for pesticides from multiple classes and modes of action, concurrent with data on aquatic toxicity and(or) ecological condition.

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Disclosure statement

None of the authors have any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work.

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References

- Anderson A-M. Development of an aquatic pesticide toxicity index for use in Alberta. Edmonton, Alberta: Alberta Environment, Environmental Assurance; 2008W0803 [30 pp.].
- Backhaus T, Faust M. Predictive environmental risk assessment of chemical mixtures: a conceptual framework. *Environ Sci Technol* 2012;46:2564–73.
- Bailey H, Miller J, Miller M, Wiborg L, Deanovic L, Shed T. Joint acute toxicity of diazinon and chlorpyrifos to *Ceriodaphnia dubia*. *Environ Toxicol Chem* 1997;16:2304–8.
- Battaglin W, Fairchild J. Potential toxicity of pesticides measured in Midwestern streams to aquatic organisms. *Water Sci Technol* 2002;45:95–103.
- Belden JB, Gilliom RJ, Lydy MJ. How well can we predict the toxicity of pesticide mixtures to aquatic life? *Integr Environ Assess Manag* 2007;3:364–72.
- Bliss CI. The toxicity of poisons applied jointly. *Ann Appl Biol* 1939;26:585–615.
- Bryant WL, Carlisle DM. The relative importance of physicochemical factors to stream biological condition in urbanizing basins: evidence from multimodel inference. *Freshw Sci* 2012;31:154–66.
- Carlisle DM, Hawkins CP. Land use and the structure of western US stream invertebrate assemblages: predictive models and ecological traits. *JN Am Benthol Soc* 2008;27:986–99.
- Carlisle DM, Meador MR. A biological assessment of streams in the eastern United States using a predictive model for macroinvertebrate assemblages. *J Am Water Resour Assoc* 2007;43:1197–207.
- Carlisle DM, Meador MR, Short TM, Tate CM, Gurtz ME, Bryant WL, et al. The quality of our Nation's waters—ecological health in the Nation's streams, 1993–2005. Circular. Reston, VA: U.S. Geological Survey; 20131391 [120 pp.].
- Cedergreen N, Streibig JC. Can the choice of endpoint lead to contradictory results of mixture-toxicity experiments? *Environ Toxicol Chem* 2005;24:1676–83.
- Cedergreen N, Christensen AM, Kamper A, Kudsik P, Mathiassen SK, Streibig JC, et al. A review of independent action compared to concentration addition as reference models for mixtures of compounds with different molecular target sites. *Environ Toxicol Chem* 2008;27:1621–32.
- Central Coast Water Quality Preservation Inc.. Phase I follow-up water quality monitoring: organophosphate pesticide sampling final data report. Central Coast Region Conditional Ag Waiver Cooperative Monitoring Program; 2008 [37 pp.].
- Central Coast Water Quality Preservation Inc.. Supplemental water quality monitoring for organophosphate pesticides and aquatic toxicity. Central Coast Region Conditional Ag Waiver Cooperative Monitoring Program; 2009 [24 pp.].
- Commission of the European Communities. Commission Directive 92/69/EEC of 31 July 1992 adapting to technical progress for the 17th time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. C.2. Acute toxicity test *Daphnia*. Commission of the European Communities; 1992.
- Cunnane C. Unbiased plotting positions—a review. *J Hydrol* 1978;37:205–22.
- de Zwart D, Posthuma L. Complex mixture toxicity for single and multiple species: proposed methodologies. *Environ Toxicol Chem* 2005;24:2665–76.
- Deneer JW. Toxicity of mixtures of pesticides in aquatic systems. *Pest Manag Sci* 2000;56:516–20.
- Ensminger M, Bergin R, Spurlock F. Pesticide concentrations in water and sediment and associated invertebrate toxicity in Del Puerto and Orestimba Creeks, California. Sacramento, CA: California Environmental Protection Agency, Department of Pesticide Regulation; 2009 [47 pp.].
- Etterson M. Analyses of sensitivity distributions for estimation of acute hazard concentrations to aquatic animals. Appendix C of USEPA, review of methods for characterizing effects of pesticides and other chemical stressors to aquatic organisms. U.S. Environmental Protection Agency; 2011.
- Faust M, Altenburger R, Boedeker W, Grimme LH. Algal toxicity of binary combinations of pesticides. *Bull Environ Contam Toxicol* 1994;53:134–41.
- Faust M, Altenburger R, Backhaus T, Bodeker W, Scholze M, Grimme LH. Predictive assessment of the aquatic toxicity of multiple chemical mixtures. *J Environ Qual* 2000;29:1063–8.
- Faust M, Altenburger R, Backhaus T, Blanck H, Boedeker W, Gramatica P, et al. Joint algal toxicity of 16 dissimilarly acting chemicals is predictable by the concept of independent action. *Aquat Toxicol* 2003;63:43–63.
- Fuhrer GJ, Morace JL, Johnson HM, Rinella JF, Ebbert JC, Embrey SS, et al. Water quality in the Yakima River basin, Washington, 1999–2000. Circular. Reston, VA: U.S. Geological Survey; 20041237 [34 pp.].
- Ganapathy C. Preliminary results of acute chronic toxicity testing of surface water monitored in the San Joaquin River watershed, winter 1997–1998. Memorandum from Carissa Ganapathy to Donald Weaver, January 5, 1999. Sacramento, CA: California Department of Pesticide Regulation; 1999a [27 pp.].
- Ganapathy C. Preliminary results of acute chronic toxicity testing of surface water monitored in the San Joaquin River watershed, winter 1998–1999. Memorandum from Carissa Ganapathy to Donald Weaver, July 20, 1999. Sacramento, CA: California Department of Pesticide Regulation; 1999b [22 pp.].
- GfK Kynetec. Unpublished proprietary agricultural pesticide use data for 2009, 2009.
- Gill S. Preliminary results of pesticide residue analysis and acute and chronic toxicity testing of surface water monitored in the Sacramento River watershed, winter 2000–2001 (study number 199). Memorandum from Sheryl Gill to Kean Goh, January 22, 2002. Sacramento, CA: California Department of Pesticide Regulation; 2002 [25 pp.].
- Gilliom RJ, Barbash JE, Crawford CG, Hamilton PA, Martin JD, Nakagaki N, et al. The quality of our Nation's waters—pesticides in the Nation's streams and ground water, 1992–2001. Circular. Reston, VA: U.S. Geological Survey; 20061291 [172 pp.].
- Guy M, Singh L, Mineau P. Using field data to assess the effects of pesticides on crustacea in freshwater aquatic ecosystems and verifying the level of protection provided by water quality guidelines. *Integr Environ Assess Manag* 2011;7:426–36.
- Helsel DR, Hirsch RM. Statistical methods in water resources. New York: Elsevier Science; 1992.
- Ingersoll C, MacDonald D, Wang N, Crane J, Field L, Haverland P, et al. Predictions of sediment toxicity using consensus-based freshwater sediment quality guidelines. *Arch Environ Contam Toxicol* 2001;41:8–21.
- Jonczyk E, Gilron G. Acute and chronic toxicity testing with *Daphnia* sp. In: Blaise C, Féraud J-F, editors. Small-scale Freshwater Toxicity Testing for freshwater Environments. Small-scale Freshwater Toxicity Investigations. Toxicity Test MethodsSpringer; 2005. p. 337–93.
- Jones D. Preliminary results of acute and chronic toxicity testing of surface water monitored in the San Joaquin River watershed, winter 1999–2000. Memorandum from

- DeeAn Jones to Patricia Dunn, October 3, 2000. Sacramento, CA: California Department of Pesticide Regulation; 2000 [23 pp].
- Jungthans M, Backhaus T, Faust M, Scholze M, Grimme LH. Application and validation of approaches for the predictive hazard assessment of realistic pesticide mixtures. *Aquat Toxicol* 2006;76:93–110.
- Kim D, Walters J, Sava R. Preliminary background results of organophosphate analysis and acute toxicity testing of surface water monitored for the red imported fire ant project in Orange County, March and April, 1999. Memorandum from Dave Kim, Johanna Walters, and Roger Sava to Kean S Goh, November 29, 1999. Sacramento, CA: California Department of Pesticide Regulation; 1999a [7 pp.].
- Kim D, Walters J, Sava R. Preliminary results of pesticide analysis and acute toxicity testing of monthly surface water monitoring for the red imported fire ant project in Orange County, May and June, 1999. Memorandum from Dave Kim, Johanna Walters, and Roger Sava to Kean S Goh, December 2, 1999. Sacramento, CA: California Department of Pesticide Regulation; 1999b [8 pp.].
- Kimerle R, Barnthouse L, Brown R, de Beyssac BC, Gilbertson M, Monk K, et al. Ecological effects, Chap. 4 in Chemical ranking and scoring—guidelines for relative assessments of chemicals. In: Swanson MB, Socha AC, editors. Proceedings of the Pellston Workshop on Chemical Ranking and Scoring, 12–16 February, 1995, Sandestin, FL. SETAC special publication series Pensacola, FL: SETAC Press; 1997. p. 89–111.
- Kuivila KM, Foe CG. Concentrations, transport and biological effects of dormant spray pesticides in the San Francisco Estuary, California. *Environ Toxicol Chem* 1995;14:1141–50.
- Liess M, von der Ohe PC. Analyzing effects of pesticides on invertebrate communities in streams. *Environ Toxicol Chem* 2005;24:954–65.
- Lydy M, Belden J, Wheelock C, Hammock B, Denton D. Challenges in regulating pesticide mixtures. *Ecol Soc* 2004;9:1. [online, <http://www.ecologyandsociety.org/vol9/iss6/art1/>].
- MacDonald DD, Ingersoll CG, Berger TA. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. *Arch Environ Contam Toxicol* 2000;39:20–31.
- Maltby L, Blake N, Brock TCM, van den Brink PJ. Insecticide species sensitivity distributions: importance of test species selection and relevance to aquatic ecosystems. *Environ Toxicol Chem* 2005;24:379–88.
- Maltby L, Brock TCM, van den Brink PJ. Fungicide risk assessment for aquatic ecosystems: importance of interspecific variation, toxic mode of action, and exposure regime. *Environ Sci Technol* 2009;43:7556–63.
- Munn MD, Gilliom RJ. Pesticide Toxicity Index for freshwater aquatic organisms. Water-Resources Investigations Report 01–4077. Reston, VA: U.S. Geological Survey; 2001. [55 pp.].
- Munn MD, Gilliom RJ, Moran PW, Nowell LH. Pesticide Toxicity Index for freshwater aquatic organisms. Scientific Investigations Report 2006–5148. 2nd ed. Reston, VA: U.S. Geological Survey; 2006. [81 pp.].
- Nordmark C. Preliminary results of acute and chronic toxicity testing of surface water monitored in the Sacramento River watershed, winter 1998–1999. Memorandum from Craig Nordmark to Don Weaver, May 26, 1999. Sacramento, CA: California Department of Pesticide Regulation; 1999 [32 pp.].
- Nordmark C. Preliminary results of acute and chronic toxicity testing of surface water monitored in the Sacramento River watershed, winter 1999–2000. Memorandum from Craig Nordmark to Don Weaver, September 15, 2000. Sacramento, CA: California Department of Pesticide Regulation; 2000 [29 pp.].
- Norman JE, Kuivila KM, Nowell LH. Prioritizing pesticide compounds for analytical methods development. Scientific Investigations Report 2012–5045. Reston, VA: U.S. Geological Survey; 2012. [206 pp.].
- Organization for Economic Cooperation and Development. Test No. 202: *Daphnia* sp. Acute immobilisation test, OECD guidelines for testing of chemicals, Section 2. OECD; 2004 [12 pp.].
- Persoone G, Baudo R, Cotman M, Blaise C, Thompson KC, Moreira-Santos M, et al. Review on the acute *Daphnia magna* toxicity test – evaluation of the sensitivity and the precision of assays performed with organisms from laboratory cultures or hatched from dormant eggs. *Knowl Manag Aquat Ecosyst* 2009;393:29.
- Phillips BM, Anderson BS, Hunt JW, Siegler K, Voorhees JP, Tjeerdema RS, et al. Pyrethroid and organophosphate pesticide-associated toxicity in two coastal watersheds (California, USA). *Environ Toxicol Chem* 2012;31:1595–603.
- Posthuma L, de Zwart D. Predicted effects of toxicant mixtures are confirmed by changes in fish species assemblages in Ohio, USA, rivers. *Environ Toxicol Chem* 2006;25:1094–105.
- Posthuma L, de Zwart D. Predicted mixture toxic pressure relates to observed fraction of benthic macrofauna species impacted by contaminant mixtures. *Environ Toxicol Chem* 2012;31:2175–88.
- Ryberg KR, Vecchia AV, Martin JD, Gilliom RJ. Trends in pesticide concentrations in urban streams in the United States, 1992–2008. Scientific Investigations Report 2010–5139 2010–5139. Reston, VA: U.S. Geological Survey; 2010. [101 pp.].
- Sala S, Migliorati S, Monti GS, Vighi M. SSD-based rating system for the classification of pesticide risk on biodiversity. *Ecotoxicology* 2012;21:1050–62.
- Schäfer RB, Pettigrove V, Rose G, Allinson G, Wightwick A, von der Ohe PC, et al. Effects of pesticides monitored with three sampling methods in 24 sites on macroinvertebrates and microorganisms. *Environ Sci Technol* 2011a;45:1665–72.
- Schäfer RB, von der Ohe PC, Kühne R, Schüürmann G, Liess M. Occurrence and toxicity of 331 organic pollutants in large rivers of north Germany over a decade (1994 to 2004). *Environ Sci Technol* 2011b;45:6167–74.
- Schäfer RB, von der Ohe PC, Rasmussen J, Kefford BJ, Beketov MA, Schulz R, et al. Thresholds for the effects of pesticides on invertebrate communities and leaf breakdown in stream ecosystems. *Environ Sci Technol* 2012;46:5134–42.
- Schäfer RB, Gerner N, Kefford BJ, Rasmussen JJ, Beketov MA, de Zwart D, et al. How to characterize chemical exposure to predict ecologic effects on aquatic communities? *Environ Sci Technol* 2013;47:7996–8004.
- Sprague JB, Ramsay BA. Lethal levels of mixed copper–zinc solutions for juvenile salmon. *J Fish Res Board Can* 1965;22:425–32.
- Stenström JR. Mixture toxicity of pesticides and biological effects in agricultural streams. LicentiateUppsala, Sweden: Faculty of Natural Resources and Agricultural Sciences, Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences; 201343.
- Stephan CE, Mount DJ, Hanson DJ, Gentile JH, Chapman GA, Brungs WA. Guidelines for deriving numerical National Water Quality Criteria for the protection of aquatic organisms and their uses. EPA PB85–227049. Duluth, MN: U.S. Environmental Protection Agency, Environmental Research Laboratories; 1985.
- Sullivan DJ, Vecchia AV, Lorenz DL, Gilliom RJ, Martin JD. Trends in pesticide concentrations in corn-belt streams, 1996–2006. Scientific Investigations Report 2009–5132. Reston, VA: U.S. Geological Survey; 2009. [75 pp.].
- Tate CM, Heiny JS. The ordination of benthic invertebrate communities in the South Platte River Basin in relation to environmental factors. *Freshw Biol* 1995;33:439–54.
- TIBCO Software Inc.. TIBCO Spotfire S + 8.1 for Windows, Revision Date 11/4/2008; 2008.
- University of Hertfordshire. The Pesticide Properties DataBase (PPDB) developed by the Agriculture & Environment Research Unit (AERU) at the University of Hertfordshire, 2006–2013. Accessed September–October, 2012, and May 2013, at <http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm>.
- U.S. Environmental Protection Agency. Ecological effects test guidelines. OPPTS 850.1010 aquatic invertebrate acute toxicity test, freshwater daphnids, public draft. EPA 712-C-96-114. Washington, DC: U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances; 1996 [10 pp.].
- U.S. Environmental Protection Agency. Chlorpyrifos revised risk assessment and agreement with registrants. Fact sheet. Washington, DC: U.S. Environmental Protection Agency, Prevention, Pesticides and Toxic Substances; 2000 [4 pp.].
- U.S. Environmental Protection Agency. Diazinon revised risk assessment and agreement with registrants. Fact sheet. Washington, DC: U.S. Environmental Protection Agency, Prevention, Pesticides and Toxic Substances; 2001 [4 pp.].
- U.S. Environmental Protection Agency. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. EPA 821-R-02-0125th ed. Washington, DC: U.S. Environmental Protection Agency, Office of Water; 2002 [266 pp.].
- U.S. Environmental Protection Agency. Wadeable streams assessment – a collaborative survey of the Nation's streams. EPA 841-B-06-002. Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development, Office of Water; 2006 [113 pp.].
- U.S. Environmental Protection Agency. ECOTOX user guide: ECOTOXicology Database System, Version 4.0. Duluth, MN: U.S. Environmental Protection Agency, Office of Research and Development and the National Health and Environmental Effects Research Laboratory's Mid-Continent Ecology Division; 2011 [96 pp.].
- U.S. Environmental Protection Agency. ECOTOXicology Database System, Version 4.0. U.S. Environmental Protection Agency, Office of Research and Development and the National Health and Environmental Effects Research Laboratory's Mid-Continent Ecology Division; 2012a [Updated June 15, 2012, Accessed July 5, 2012, at <http://www.epa.gov/ecotox/>].
- U.S. Environmental Protection Agency. Office of Pesticide Programs' aquatic life benchmarks (freshwater). U.S. Environmental Protection Agency, Office of Pesticide Programs; 2012b [Updated May 30, 2102, Accessed September–October, 2012, at http://www.epa.gov/oppfed1/ecorisk_ders/aquatic_life_benchmark.htm].
- van den Brink PJ, Blake N, Brock TCM, Maltby L. Predictive value of species sensitivity distributions for effects of herbicides in freshwater ecosystems. *Hum Ecol Risk Assess* 2006;12:645–74.
- Waite JR, Sobieszky S, Carpenter KD, Arnsberg AJ, Johnson HM, Hughes CA, et al. Effects of urbanization on stream ecosystems in the Willamette River basin and surrounding area, Oregon and Washington. Scientific Investigations Report 2006-5101-D. Reston, VA: U.S. Geological Survey; 2006. [63 pp.].
- Walters J, Kim D, Goh KS. Preliminary results of pesticide analysis and acute toxicity testing of monthly surface water monitoring for the red imported fire ant project in Orange County, August 2000 (Study 183). Memorandum from Johana Walters, Dave Kim, and Kean S. Goh to John S. Sanders, October 5, 2000. Sacramento, CA: California Department of Pesticide Regulation; 2000a [10 pp.].
- Walters J, Kim D, Goh KS. Preliminary results of pesticide analysis and acute toxicity testing of monthly surface water monitoring for the red imported fire ant project in Orange County, July 2000 (Study 183). Memorandum from Johana Walters, Dave Kim, and Kean S. Goh to John S. Sanders, October 5, 2000. Sacramento, CA: California Department of Pesticide Regulation; 2000b [10 pp.].
- Walters J, Kim D, Goh KS. Preliminary results of pesticide analysis and acute toxicity testing of monthly surface water monitoring for the red imported fire ant project in Orange County, June 2000 (Study 183). Memorandum from Johana Walters, Dave Kim, and Kean S. Goh to John S. Sanders, September 1, 2000. Sacramento, CA: California Department of Pesticide Regulation; 2000c [9 pp.].
- Walters J, Kim D, Goh KS. Preliminary results of pesticide analysis and acute toxicity testing of monthly surface water monitoring for the red imported fire ant project in Orange County, September 2000 (Study 183). Memorandum from Johana Walters, Dave Kim, and Kean S. Goh to John S. Sanders, December 22, 2000. Sacramento, CA: California Department of Pesticide Regulation; 2000d [12 pp.].
- Walters J, Kim D, Sava R, Goh KS. Preliminary results of pesticide analysis and acute toxicity testing of monthly surface water monitoring for the red imported fire ant project in Orange County, April 2000 (Study 183). Memorandum from Johana Walters, Dave Kim, Roger Sava, and Kean S. Goh to John S. Sanders, July 6, 2000. Sacramento, CA: California Department of Pesticide Regulation; 2000e [10 pp.].
- Walters J, Kim D, Sava R, Goh KS. Preliminary results of pesticide analysis and acute toxicity testing of monthly surface water monitoring for the red imported fire ant project

- in Orange County, December 1999 (Study 183). Memorandum from Johana Walters, Dave Kim, Roger Sava, and Kean S. Goh to John S. Sanders, April 12, 2000. Sacramento, CA: California Department of Pesticide Regulation; 2000f [8 pp.].
- Walters J, Kim D, Sava R, Goh KS. Preliminary results of pesticide analysis and acute toxicity testing of monthly surface water monitoring for the red imported fire ant project in Orange County, February 2000 (Study 183). Memorandum from Johana Walters, Dave Kim, Roger Sava, and Kean S. Goh to John S. Sanders, May 17, 2000. Sacramento, CA: California Department of Pesticide Regulation; 2000g [9 pp.].
- Walters J, Kim D, Sava R, Goh KS. Preliminary results of pesticide analysis and acute toxicity testing of monthly surface water monitoring for the red imported fire ant project in Orange County, January 2000 (Study 183). Memorandum from Johana Walters, Dave Kim, Roger Sava, and Kean S. Goh to John S. Sanders, April 7, 2000. Sacramento, CA: California Department of Pesticide Regulation; 2000h [8 pp.].
- Walters J, Kim D, Sava R, Goh KS. Preliminary results of pesticide analysis and acute toxicity testing of monthly surface water monitoring for the red imported fire ant project in Orange County, March 2000 (Study 183). Memorandum from Johana Walters, Dave Kim, Roger Sava, and Kean S. Goh to John S. Sanders, July 5, 2000. Sacramento, CA: California Department of Pesticide Regulation; 2000i [10 pp.].
- Walters J, Kim D, Sava R, Goh KS. Preliminary results of pesticide analysis and acute toxicity testing of monthly surface water monitoring for the red imported fire ant project in Orange County, October 1999 (Study 183). Memorandum from Johana Walters, Dave Kim, Roger Sava, and Kean S. Goh to John S. Sanders, February 4, 2000. Sacramento, CA: California Department of Pesticide Regulation; 2000j [8 pp.].
- Walters J, Kim D, Sava R, Goh KS. Preliminary results of pesticide analysis and acute toxicity testing of monthly surface water monitoring for the red imported fire ant project in Orange County, September 1999 (Study 183). Memorandum from Johana Walters, Dave Kim, Roger Sava, and Kean S. Goh to John S. Sanders, January 31, 2000. Sacramento, CA: California Department of Pesticide Regulation; 2000k [8 pp.].
- Walters J, Kim D, Sava R, Goh KS. Results of pesticide analysis and acute toxicity testing of rain runoff monitoring for the red imported fire ant project in Orange County, January 2000 (Study 183). Memorandum from Johana Walters, Dave Kim, Roger Sava, and Kean S. Goh to John S. Sanders, May 30, 2000. Sacramento, CA: California Department of Pesticide Regulation; 2000l [10 pp.].
- Walters J, Kim D, Sava R, Goh KS. Revised-preliminary results of pesticide analysis and acute toxicity testing of monthly surface water monitoring for the red imported fire ant project in Orange County, May 2000 (Study 183). Memorandum from Johana Walters, Dave Kim, Roger Sava, and Kean S. Goh to John S. Sanders, September 29, 2000. Sacramento, CA: California Department of Pesticide Regulation; 2000m [9 pp.].
- Walters J, Kim D, Goh KS. Preliminary results of pesticide analysis and acute toxicity testing of monthly surface water monitoring for the red imported fire ant project in Orange County, August 2001 (Study 183). Memorandum from Johana Walters, Dave Kim, and Kean S. Goh to John S. Sanders, November 8, 2001. Sacramento, CA: California Department of Pesticide Regulation; 2001a [11 pp.].
- Walters J, Kim D, Goh KS. Preliminary results of pesticide analysis and acute toxicity testing of monthly surface water monitoring for the red imported fire ant project in Orange County, December 2000 (Study 183). Memorandum from Johana Walters, Dave Kim, and Kean S. Goh to John S. Sanders, April 26, 2001. Sacramento, CA: California Department of Pesticide Regulation; 2001b [10 pp.].
- Walters J, Kim D, Goh KS. Preliminary results of pesticide analysis and acute toxicity testing of monthly surface water monitoring for the red imported fire ant project in Orange County, February 2001 (Study 183). Memorandum from Johana Walters, Dave Kim, and Kean S. Goh to John S. Sanders, May 3, 2001. Sacramento, CA: California Department of Pesticide Regulation; 2001c [9 pp.].
- Walters J, Kim D, Goh KS. Preliminary results of pesticide analysis and acute toxicity testing of monthly surface water monitoring for the red imported fire ant project in Orange County, January 2001 (Study 183). Memorandum from Johana Walters, Dave Kim, and Kean S. Goh to John S. Sanders, April 27, 2001. Sacramento, CA: California Department of Pesticide Regulation; 2001d [10 pp.].
- Walters J, Kim D, Goh KS. Preliminary results of pesticide analysis and acute toxicity testing of monthly surface water monitoring for the red imported fire ant project in Orange County, July 2001 (Study 183). Memorandum from Johana Walters, Dave Kim, and Kean S. Goh to John S. Sanders, November 1, 2001. Sacramento, CA: California Department of Pesticide Regulation; 2001e [9 pp.].
- Walters J, Kim D, Goh KS. Preliminary results of pesticide analysis and acute toxicity testing of monthly surface water monitoring for the red imported fire ant project in Orange County, June 2001 (Study 183). Memorandum from Johana Walters, Dave Kim, and Kean S. Goh to John S. Sanders, September 7, 2001. Sacramento, CA: California Department of Pesticide Regulation; 2001f [10 pp.].
- Walters J, Kim D, Goh KS. Preliminary results of pesticide analysis and acute toxicity testing of monthly surface water monitoring for the red imported fire ant project in Orange County, March 2001 (Study 183). Memorandum from Johana Walters, Dave Kim, and Kean S. Goh to John S. Sanders, July 13, 2001. Sacramento, CA: California Department of Pesticide Regulation; 2001g [10 pp.].
- Walters J, Kim D, Goh KS. Preliminary results of pesticide analysis and acute toxicity testing of monthly surface water monitoring for the red imported fire ant project in Orange County, May 2001 (Study 183). Memorandum from Johana Walters, Dave Kim, and Kean S. Goh to John S. Sanders, July 17, 2001. Sacramento, CA: California Department of Pesticide Regulation; 2001h [9 pp.].
- Walters J, Kim D, Goh KS. Preliminary results of pesticide analysis and acute toxicity testing of monthly surface water monitoring for the red imported fire ant project in Orange County, November 2000 (Study 183). Memorandum from Johana Walters, Dave Kim, and Kean S. Goh to John S. Sanders, February 6, 2001. Sacramento, CA: California Department of Pesticide Regulation; 2001i [10 pp.].
- Walters J, Kim D, Goh KS. Revised preliminary results of pesticide analysis and acute toxicity testing of monthly surface water monitoring for the red imported fire ant project in Orange County, April 2001 (Study 183). Memorandum from Johana Walters, Dave Kim, and Kean S. Goh to John S. Sanders, September 7, 2001. Sacramento, CA: California Department of Pesticide Regulation; 2001j [8 pp.].
- Warne M. A review of the ecotoxicity of mixtures, approaches to, and recommendations for, their management. In: Langgley A, Gilbey B, Kennedy B, editors. Proceedings of the Fifth National Workshop on the Assessment of Site Contamination. Adelaide, Australia: National Environment Protection Council Service Corp.; 2003.
- Warne M, Hawker D. The number of components in a mixture determines whether synergistic and antagonistic or additive toxicity predominate: the funnel hypothesis. *Ecotoxicol Environ Saf* 1995;31:23–8.
- Whiteside M, Mineau P, Morrison C, Knopper LD. Comparison of a score-based approach with risk-based ranking of in-use agricultural pesticides in Canada to aquatic receptors. *Integr Environ Assess Manag* 2008;4:215–36.
- Yuan LL, Hawkins CP, Van Sickle J. Effects of regionalization decisions on an O/E index for the US national assessment. *J N Am Benthol Soc* 2008;27:892–905.
- Yuan LL, Pollard AI, Carlisle DM. Using propensity scores to estimate the effects of insecticides on stream invertebrates from observational data. *Environ Toxicol Chem* 2009;28:1518–27.