

Potential toxicity of pesticides measured in midwestern streams to aquatic organisms

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Abstract Society is becoming increasingly aware of the value of healthy aquatic ecosystems as well as the effects that man's activities have on those ecosystems. In recent years, many urban and industrial sources of contamination have been reduced or eliminated. The agricultural community also has worked towards reducing off-site movement of agricultural chemicals, but their use in farming is still growing. A small fraction, estimated at <1 to 2% of the pesticides applied to crops are lost from fields and enter nearby streams during rainfall events. In many cases aquatic organisms are exposed to mixtures of chemicals, which may lead to greater non-target risk than that predicted based on traditional risk assessments for single chemicals. We evaluated the potential toxicity of environmental mixtures of 5 classes of pesticides using concentrations from water samples collected from ~50 sites on midwestern streams during late spring or early summer runoff events in 1989 and 1998. Toxicity index values are calculated as the concentration of the compound in the sample divided by the EC50 or LC50 of an aquatic organism. These index values are summed within a pesticide class and for all classes to determine additive pesticide class and total pesticide toxicity indices. Toxicity index values greater than 1.0 indicate probable toxicity of a class of pesticides measured in a water sample to aquatic organisms. Results indicate that some samples had probable toxicity to duckweed and green algae, but few are suspected of having significant toxicity to bluegill sunfish or chorus frogs.

Keywords Aquatic organisms; pesticides; streams; toxicity

Introduction and methods

Pesticide use 800 B.C. – 2000

Farmers have used methods such as hand cultivation and crop rotation to control weeds and other pests and to improve crop yields since the beginning of recorded history. Pesticide use was not common during this time, but Homer mentions the “pest-averting ability of sulfur” in 800 B.C.; Pliny proposed the use of arsenic as an insecticide in 50 A.D.; and arsenic sulfide use was reported in China by the 16th century. By 1900, about a dozen insecticide active ingredients were in use. Most of these were inorganic compounds like mercuric chloride, calcium arsenate, lead arsenate, copper sulfate, various selenium compounds, zinc, and fluorine. Some plant derivatives (e.g. pyrethrins, rotenone, Bordeaux mixture, and nicotine), natural oils, and salts were occasionally used to control weeds; however, the hoe and the hand were still the most utilized weed control tools.

During the twentieth century, farmers worldwide increased their reliance on pesticides and chemical fertilizer to improve crop yields (Mannion, 1995). Many of the early organic pesticides were developed for military applications. Later, pesticides like DDT, dieldrin, and other chlorinated hydrocarbons were used not only on crops but also on humans to control arthropod infestation – lice, ticks, and fleas. Many early pesticides were designed to be persistent. They have proven to be so; though most have now been banned in the United States (US), they are still found frequently in low concentrations in US waters (Gilliom, 1985; U.S. Geological Survey, 1999). These early pesticides soon gave way to an avalanche of new active ingredients and products. By 1965, between 250 and 400 active ingredients were used in 10,000 products. Pesticide usage in the US was estimated at

165 million kilograms, at a cost of 1 billion dollars (Shaw, 1966; Fleck, 1966; Edwards, 1970). By 1980, an estimated 575 active ingredients were found in 15,000 products. Amounts used in the US were estimated at 355 million kilograms at a cost of 5.8 billion dollars (Gianessi, 1992). By 2000, an estimated 900 active ingredients will be registered in 20,000+ products, with usage in the US approaching 500 million kilograms, at a cost of 8 billion dollars (Aspelin, 1999). The increase in pesticide use is not expected to continue into the next century largely due to the introduction of crops that are genetically altered to be insect resistant or herbicide tolerant. Some business analysts (Kline Group, 2000) are projecting a 6-million-kilogram-per-year reduction in insecticide use and a 20-million-kilogram-per-year reduction in herbicide use by 2009, and in the US, planting of genetically altered row crops is increasing (NASS, 2001). Although this trend may result in an overall reduction in pesticide use in the US, it does not consider the change from old pesticides to new pesticides, which are applied at lower rates and are active at lower concentrations.

Small fractions of the pesticides used in agricultural or urban settings end up moving with surface runoff into streams, rivers, and lakes (Clark *et al.*, 1999); leaching to the groundwater systems (Kolpin *et al.*, 2000); or volatilizing to the atmosphere (Majewski *et al.*, 2000; Foreman *et al.*, 2000). Usually less than 2 percent of the crop-applied pesticides ends up in streams, but larger percentages would be expected for some pesticides associated with specialized uses (e.g., flea dips) that are directly discharged into municipal sewage treatment systems and receiving water bodies during pet care activities. Once these pesticides leave their point of use they change from being “crop-protection” and “pest-control” chemicals to being environmental contaminants that are suspected sources of stress to aquatic plants and animals.

In spite of these concerns, there has been relatively little research directed at determining the risk of environmental mixtures of pesticides to non-target aquatic organisms. This research gap is due to several factors: (1) the difficulties arising from weather and the timing and rate of application in estimating exposures of organisms to various chemicals; and (2) the expense of conducting toxicity tests on the myriad of potential pesticides (and nutrient) mixtures found in the environment. One approach involves comparing actual measurements of pesticide residues in stream water with estimates of acute toxicity. The U.S. Geological Survey has collected data on pesticide occurrence in midwestern streams since 1989 (Thurman *et al.*, 1991) in order to examine relations between concentrations, land-use, pesticide use, geography, and climate of the associated drainage basins. Data collected in 1998 (Battaglin *et al.*, 2001) are used here to explore the hypothesis that aquatic communities are exposed to ecologically significant concentrations of pesticide mixtures. The specific objective of this study was to determine if the concentrations of pesticides measured in midwestern streams during spring and early-summer runoff events in 1998 were at levels that may be toxic to non-target aquatic organisms.

Toxicity estimates

The toxicity of 5 classes of pesticides are examined: (1) ALS-inhibiting herbicides (including sulfonylureas, sulfonamides, and imidazolinones), (2) triazine herbicides, (3) chloroacetamide herbicides, (4) organophosphate insecticides, and (5) carbamate pesticides. Acute toxicity data are primarily from the EPA'S ECOTOX database (U.S. EPA, 2001). but other sources were also used (Helfrich *et al.*, 1996; Fairchild *et al.*, 1997; 1998; Okamoto *et al.*, 1998; Sparling, 2000). Published EC50 values for two aquatic plants (duckweed, *Lemna gibba*; and green algae, *Selenastrum capricornutum*), and LC50 values for two aquatic vertebrates (bluegill sunfish, *Lepomis macrochirus*; and chorus frogs, *Pseudacris triseriata*) are used as toxicity metrics in this study (Table 1). Potential chronic

Table 1 Mean EC50 or LC50 values used to calculate toxicity indices and statistical summary of concentrations in water samples from midwestern streams (values in parentheses are the number of toxicity estimates used to calculate mean values; *, no data found; n/a, not applicable)

Chemical	Mean EC50 (µg/L)		Mean LC50 in µg/L		Concentration in µg/L		
	Duckweed	Green Algae	Bluegill sunfish	Chorus frog	minimum	median	maximum
ALS Inhibitor Herbicides							
Bensulfuron	430 (1)	1320 (2)	106,500 (2)	*	<0.010	<0.010	<0.010
chlormuron	0.27 (1)	*	1,500 (2)	*	<0.010	<0.010	0.300
chlorsulfuron	0.7 (1)	135 (1)	3,000 (1)	*	<0.010	<0.010	0.013
flumetsulam	3.1 (1)	3.2 (1)	300,000 (1)	*	<0.010	0.020	0.360
halosulfuron	0.042 (1)	4.1 (1)	69,500 (2)	*	<0.010	<0.010	0.067
imazapyr	24(1)	71,000 (1)	400,000 (3)	*	<0.010	<0.010	0.072
imazaquin	*	*	420,000 (1)	*	<0.010	<0.010	1.11
imazethapyr	*	59,200 (1)	423,000 (1)	*	<0.010	0.028	0.690
metsulfuron	0.38 (2)	237 (2)	150,000 (1)	*	<0.010	<0.010	<0.010
nicosulfuron	6.7 (1)	60 (1)	1,000,000 (2)	*	<0.010	0.010	0.266
primisulfuron	0.27 (1)	24 (1)	100,000 (3)	*	<0.010	<0.010	<0.010
prosulfuron	1.2 (1)	10 (1)	155,000 (1)	*	<0.010	<0.010	0.036
sulfometuron	0.47 (2)	4.6 (1)	58,500 (3)	*	<0.010	<0.010	0.018
thifensulfuron	1.45 (2)	15,000 (1)	*	*	<0.010	<0.010	0.015
triasulfuron	0.19 (1)	35 (1)	100,000(1)	*	<0.010	<0.010	<0.010
triflusaluron	2.82 (1)	*	*	*	<0.010	<0.010	<0.010
average for ALS inhibitors	33.7	11,310	234,785	n/a	n/a	n/a	n/a
Triazine Herbicides							
atrazine	99 (5)	115 (5)	35,450 (6)	410 ¹ (1)	0.013	3.77	224
cyanazine	416 (2)	17 (3)	22,500 (1)	*	<0.004	0.32	20
metribuzin	78 (3)	29 (4)	94,800 (4)	*	<0.004	0.020	1.76
prometon	*	98 (1)	29,670 (3)	*	<0.018	<0.018	0.346
prometryn	11.8 (1)	12 (1)	10,000 (2)	*	<0.05	<0.05	<0.05
simazine	153 (2)	670 (2)	67,850 (7)	*	<0.005	0.029	7.91
average for triazines	152	157	43,378	n/a	n/a	n/a	n/a
Chloroacetamide Herbicides							
acetochlor	3.4 (1)	1,430 (1)	1,470 (3)	*	<0.002	0.400	25.1
alachlor	340 (2)	5.9 (3)	5,500 (9)	*	<0.002	0.040	17.2
metolachlor	250 (3)	57 (3)	12,500 (2)	*	<0.002	1.67	143
propachlor	*	*	1,540 (2)	*	<0.007	<0.007	0.607
average for chloroacetamides	198	498	5,252	n/a	n/a	n/a	n/a
Organophosphate Insecticides							
azinphos-methyl	*	*	20.3 (15)	3,200 (1)	<0.001	<0.001	<0.001
chlorpyrifos	*	*	19.8 (11)	1.0 ² (1)	<0.004	<0.004	0.858
diazinon	*	3,700 (1)	225 (14)	*	<0.002	<0.002	0.071
disulfoton	*	*	125 (10)	*	<0.017	<0.017	<0.017
ethoprophos	*	*	3,760 (3)	*	<0.003	<0.003	0.045
fonofos	*	*	11.1 (9)	*	<0.003	<0.003	0.063
malathion	*	*	118 (13)	200 (1)	<0.005	<0.005	0.009
parathion	*	*	168 (13)	1,000 (1)	<0.004	<0.004	<0.004
parathion methyl	*	*	2,650 (7)	3,700 (1)	<0.006	<0.006	0.016
phorate	*	*	3.9 (7)	*	<0.002	<0.002	<0.002
terbufos	*	*	3.7 (6)	*	<0.013	<0.013	0.048
average for organophosphates	n/a	n/a	646	n/a	n/a	n/a	n/a
Carbamate Pesticides							
butylate	*	*	5,360 (5)	*	<0.002	<0.002	0.27
carbaryl	*	1,100 (2)	9,780 (23)	*	<0.003	<0.003	0.195
carbofuran	*	*	630 (9)	*	<0.003	<0.003	1.03
EPTC	7,512 (1)	3,070 (3)	25,225 (4)	*	<0.002	<0.002	0.076
molinate	3,300 (1)	220 (1)	13,980 (7)	*	<0.004	<0.004	0.049
pebulate	1,800(1)	230 (1)	7,100 (2)	*	<0.004	<0.004	<0.004
thiobencarb	770 (1)	17 (1)	1767 (6)	*	<0.002	<0.002	0.032
tri-allate	10,000 (1)	47 (1)	1677 (3)	*	<0.001	<0.001	<0.001
average for carbamate insecticides	4,676	781	8,190	n/a	n/a	n/a	n/a

¹ LC50 for *Rana catesbeiana*; ² LC50 for *Bufo americanus*

effects of low-level pesticide exposures or the non-cancer or mutagenic properties of pesticide exposures are not addressed in this study.

In many cases, more than one acute toxicity estimate was reported for a particular pesticide/aquatic organism combination (Table 1). The mean of the reported values was used for the calculations reported in this paper. It should be noted that in many cases, the lowest reported EC50 or LC50 values are about an order of magnitude lower than the mean. For example, the means of the reported EC50 values for atrazine and metolachlor on duckweed are 99 and 250 $\mu\text{g/L}$, respectively, while the lowest reported values are 37 and 48 $\mu\text{g/L}$, respectively. The means of the reported LC50 values for chlorpyrifos and carbaryl on bluegill sunfish are 19.8 and 9,780 $\mu\text{g/L}$, respectively, while the lowest reported values are 1.3 and 760 $\mu\text{g/L}$, respectively.

Acute toxicity estimates for herbicide degradates were not found in any of the listed sources. There are limited studies that show that herbicide degradates may or may not be as toxic as their corresponding parent compounds (Kolpin *et al.*, 2000). For this study, herbicide degradates were estimated to be one-half as toxic as the parent compounds.

Toxicity index values

A toxicity index (TI) value for pesticides in each sample was calculated as the concentration of the pesticide in the sample divided by the EC50 or LC50 value. All non-detects were treated as zeros for this calculation. Individual TI values were summed for all pesticides in a pesticide class. When an EC50 or LC50 value was not available (for example there was no reported EC50 value for imazaquin on duckweed), the mean value for the pesticide class was used. If less than 50% of the pesticides in a class had acute toxicity estimates, then a mean value was not calculated and a TI was not calculated for the pesticide with missing data.

The five pesticide class TI values were summed to calculate a total pesticide TI value for each sample. There is some debate over the validity of summing TI values for classes of compounds with different modes of action. Studies of mixtures of chemicals have generally concluded that an additive model is appropriate for estimating the toxicity of mixtures of chemicals with the same mode of action (Marking, 1985; Faust *et al.*, 2000). For mixtures of chemicals with differing modes of action, additive models may overestimate the mixture toxicity and independent action models may be more accurate (Faust *et al.*, 2000). Some recent research has also documented synergistic toxicity of mixtures of pesticides from different classes (Pape-Lindstrom and Lydy, 1997; Belden and Lydy, 2000).

The percentage of samples with TI values greater than 0.1, 0.5 and 1.0 for each class of pesticide and for total pesticides were calculated. Toxicity index values greater than 1.0 indicate probable toxicity of a class of pesticides to the subject aquatic organism. Toxicity index values greater than 0.5 indicate potential toxicity, while TI values greater than 0.1 indicate limited toxicity.

Water quality data

Water samples were collected from 71 sites on free-flowing streams in the Upper Mississippi, Missouri, and Ohio River basins (Battaglin *et al.*, 2001). Drainage areas for these sites ranged from 201 to 443,670 square kilometres. Two samples were collected at all but one site. The first samples were collected after pre-emergence pesticides were applied (May or June) and following a precipitation event that produced a significant increase in streamflow. The second samples were collected after post-emergence pesticides were applied (June or July) again following a precipitation event that produced an increase in streamflow. The intent of this timing of sample collection was to identify the peak concentrations of pre- and post-emergence herbicides expected during a typical year

(Battaglin and Goolsby, 1999). The peak concentrations of other pesticides such as insecticides or herbicide degradates are more likely to occur at other times of the year (Thurman *et al.*, 1994; Coupe *et al.*, 1995; Clark *et al.*, 1999; Crawford, 2001).

Samples were collected using protocols that are identical to those used for the collection of samples for low levels of other dissolved organic compounds (Shelton, 1994). All samples were analyzed for 16 ALS inhibitor herbicides (Furlong *et al.*, 2000), 46 pesticides and pesticide degradates (Zaugg *et al.*, 1995), and 13 herbicides and 10 herbicide degradates (Zimmerman and Thurman, 1999; Ferrer *et al.*, 1997).

Results

The percentage of samples with toxicity index values greater than 0.1, 0.5 and 1.0 for the five classes of pesticides and total pesticides on four organisms are shown on Figure 1. None of the samples had probable, potential, or limited toxicity from any of the five pesticide classes on bluegill sunfish (Table 1). Less than 10 percent of the samples showed probable or potential toxicity from ALS inhibitors to any of the four organisms (Figure 1a). Duckweed was the most susceptible species (Figure 1a). There was no ALS inhibitor toxicity data for chorus frogs (Table 1). Less than 10 percent of the samples showed probable or potential toxicity from triazine herbicides to any of the four organisms (Figure 1b). Green algae was slightly more susceptible than duckweed. A few samples had potential or limited toxicity from triazines to frogs. For this calculation the bullfrog (*R. catesbeiana*) with a reported LC50 of 410 µg/L was used as the test organism. LC50's for triazines on chorus frogs were not found. Reported LC50 values of atrazine on other frog species ranged from 220 to 127,000 µg/L (Birge *et al.*, 2000). More than 15% of samples showed potential toxicity from chloroacetamides to duckweed and 10% had TI values of 1.0 or more (Figure 1c). Green algae was less susceptible than duckweed, and there were no chloroacetamide toxicity data for chorus frogs. Only a few samples had limited or potential toxicity from organophosphate insecticides to frogs (Figure 1d), all as a result of the occurrence of chlorpyrifos. Chlorpyrifos toxicity to frogs is highly species dependent, but data for chorus frogs could not be found. For this calculation the American toad (*Bufo americanus*) with an LC50 of 1 µg/L was used. Other reported LC50 values for chlorpyrifos on frogs ranged from 10 to 3,000 µg/L (Cowman and Mazanti, 2000). EC50 values for most organophosphates to duckweed or green algae could not be found (Table 1). None of the samples had probable, potential, or limited toxicity from carbamates to the four organisms (Figure 1e). There were no carbamate toxicity data for frogs and limited data for duckweed and green algae.

The TI values for the 5 classes of pesticides were summed to estimate total pesticide TI values (Figure 1f). Duckweed was the most susceptible organism investigated; 17% of the samples had total pesticide TI values for duckweed greater than 1.0 and 27% had TI values greater than 0.5. For green algae, 8% of the samples had total pesticide TI values greater than 1.0, and 15% had TI values greater than 0.5. Only 1% of samples had total pesticide TI values for frogs greater than 0.5, but there were limited LC50 data for frogs (Table 1).

The effects of herbicide degradate occurrence on the potential toxicity of stream water to aquatic organisms have not been well studied. TI values for triazine and chloroacetamide herbicides and for total pesticides were recalculated using the assumption that herbicide degradates were one-half as toxic as their parent compounds to the four aquatic organisms. The addition of the triazine degradates had only a minor effect on the percentage of samples with TI values for duckweed or green algae that were greater than 0.1, 0.5, or 1.0 (Figures 1b; 2a). However, accounting for the chloroacetamide degradates added substantially to the percentages of samples with TI values for duckweed and green algae that were greater than 0.1, 0.5 or 1.0 (Figures 1c; 2b). The effect on the total pesticide TI values was also substantial. Twenty-six percent of the samples had total pesticide plus degradate TI values for

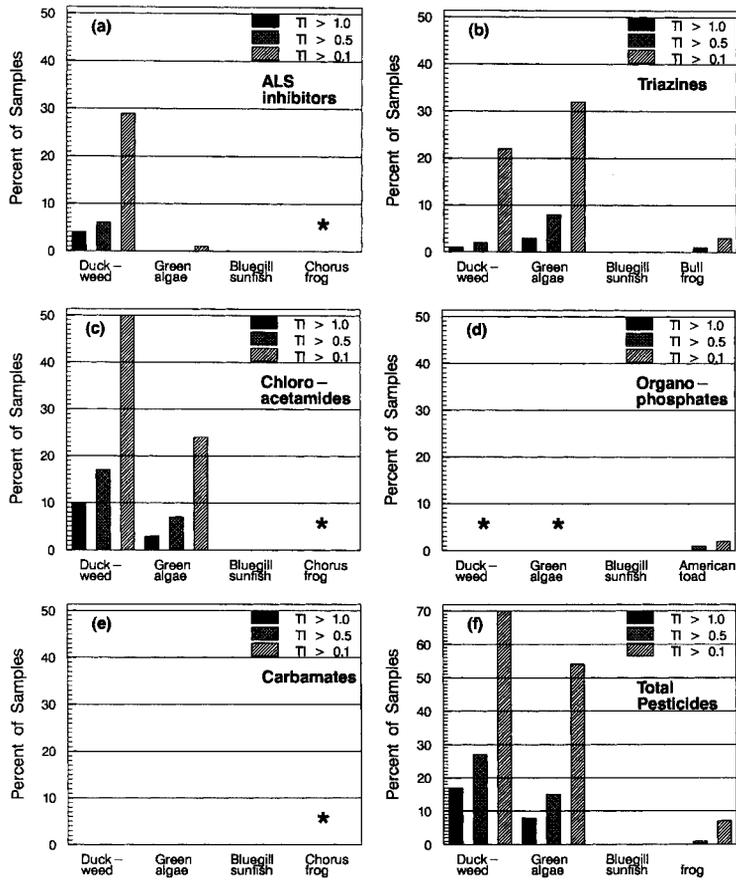


Figure 1 Percent of samples exceeding TI values of 0.1, 0.5, and 1.0 for (a) ALS inhibitors, (b) triazines, (c) chloroacetamides, (d) organophosphates, (e) carbamates, and (f) total pesticides (*, no toxicity data available)

duckweed greater than 1.0, and 50% had TI values greater than 0.5 (Figure 2c). Ten percent of the samples had total pesticide plus degradate TI values for green algae greater than 1.0, and 23% had TI values greater than 0.5.

Conclusions

Three conclusions can be drawn from the results of this investigation. First, water in mid-western streams during spring and early summer runoff events can contain pesticides in sufficient quantities to be toxic to non-target aquatic organisms. This result does not agree with Fairchild *et al.* (1999), who indicated that herbicides were unlikely to affect aquatic plants in the lower Missouri River. In this study, the concentrations of some pesticides did exceed concentrations thought to affect aquatic plants. Still, this data set may underestimate potential effects of pesticides on aquatic systems in smaller streams because peak concentrations are generally inversely related to stream size (Richards and Baker, 1993). Second, accounting for herbicide degradates can substantially increase the estimated toxicity of stream water to aquatic plants. More information is needed on both the occurrence of herbicide and insecticide degradates in streams and their toxicity to aquatic organisms. Finally, the quality of this analysis is limited by the lack of acute toxicity data for many of the pesticide-organism combinations.

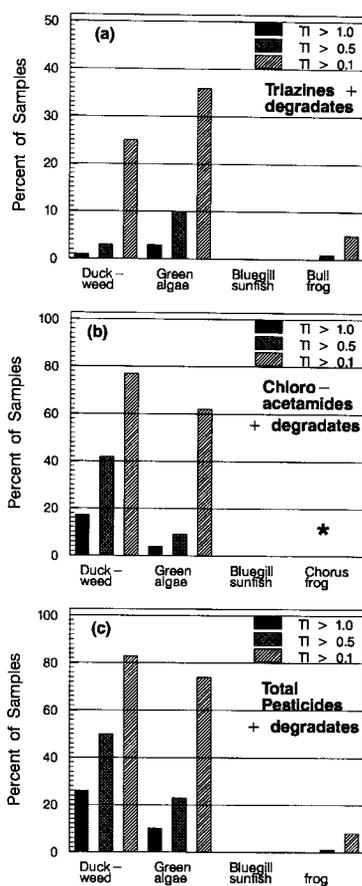


Figure 2 Percent of samples exceeding TI values of 0.1, 0.5, and 1.0 for (a) triazines plus degradates, (b) chloroacetamides plus degradates, and (c) total pesticides plus degradates (*, no toxicity data available)

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