EVALUATION OF HUMIC-PESTICIDE INTERACTIONS ON THE FATE AND EFFECTS OF AGRICULTURAL CHEMICALS

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Introduction

The majority of water quality standards presently are based on the toxicity of chemicals to organisms exposed in clean laboratory dilution water. This rationale does not take into account the interaction of chemicals with naturally occurring organic compounds found in aquatic ecosystems. Dissolved humic materials (DHMs) are ubiquitous components of the chemical matrix of freshwater ecosystems and can affect the availability and toxicity of chemicals in the environment.

Since humic-type compounds are ubiquitous in aquatic ecosystems, the toxicological consequences of chemical interaction with DHMs have been studied with a variety of compounds. DHMs may complex with inorganic as well as organic environmental contaminants, thereby affecting the physico-chemical state of these chemicals (Boehm and Quinn 1973; Benes et al. 1976; Poirrier et al. 1981). The mechanisms involved are not clearly understood and the binding strengths between the chemicals and humic materials will vary depending on the nature of the chemical and on the general composition of the aquatic matrix. Through complexation and binding, humic materials can enhance the water solubility of organic compounds (Gjessing and Berglind 1981; Carlberg and Martinsen 1982; Carter and Suffet 1983; Means and Wijayaratne 1982). For example, Chiou et al. (1987) demonstrated water solubility enhancements of polychlorinated biphenyls (PCBs) and DDT by aquatic humic materials as well as commercially available HA.

The toxicological consequences of humic-metal interactions have long been recognized. The influence of humic materials on the uptake, accumulation, and toxicity of heavy metals has been extensively studied over the past decade (Garvey et al. 1991; Giesy et al. 1977; Hung 1982; Winner 1986; Stackhouse and Benson 1988; Stackhouse and Benson 1989). On the other hand, studies on the influence of humics on the toxicity of organic environmental contaminants are sparse. It has been demonstrated that DHM reduced the bioconcentration of PAHs by the freshwater cladoceran, *Daphnia magna* (McCarthy and Jimenez 1985) and bluegill sunfish (Leversee et al. 1983). In addition, Carlberg et al. (1986) showed that natural humus water can reduce the bioavailability of selected trichlorophenols and lindane. Stewart (1984) has reported that DHM enhanced the toxicity of *o*-cresol, 2,4-dimethylphenol, and 2,3,6-trimethylphenol while reducing the toxicity of *p*-benzoquinone and quinoline. Recently, Benson and Long (1991) and Ortego and Benson (1992) reported that humic-toxicant interactions can alter the toxicity of pesticides and that the extent of alteration is dependent on the source of DHM.

The majority of humic-toxicant interactions have been modeled based on the results of studies with commercially available HAs. The advantages of using commercially available HAs relate to their cost and availability. However, Malcolm and MacCarthy (1986) reported that commercially available HAs differ chemically from DHMs isolated from soils and aquatic ecosystems. Based on chemical characterization via cross-polarization and magic-angle spinning ^{13}C nuclear magnetic resonance spectroscopy and elemental and infrared spectroscopy, it has been postulated that commercially available HAs are more hydrophobic as compared to their soil and aquatic counterparts. Consequently, commercially available HAs may differ significantly from soil and aquatic humic extracts in their ability to interact with toxicants. Several studies support this conclusion. For example, Chiou et al. (1987) demonstrated that commercially available HA enhanced the apparent water solubility of selected organic solutes to a greater extent than did dissolved organic matter of water and aquatic humic extracts. Servos et al. (1989) demonstrated that commercially available HAs affect the uptake rate constants of polychlorinated dioxins in rainbow trout differently than do dissolved organic materials from natural lake water. Commercially available HA reduced the uptake rate constant whereas HAs from natural lake water had little effect. As a result of chemical analyses, Malcolm and MacCarthy (1986) concluded that use of

commercially available HAs may not be suitable as analogues of soil and water humic substances because the increased hydrophobicity and a lack of information as to source, method of isolation, or other pretreatment of the DHMs.

Because of the broad acceptance and application of pesticides, concern of environmental exposure is warranted. Numerous studies have documented contamination of aquatic ecosystems nationally (Wiemeyer et al. 1988), as well as in the Mid-South (Smith et al. 1987), including Mississippi (Cooper et al. 1987). Clearly, an understanding of the influence of humic substances on the toxicity of pesticides becomes imperative if we are to maximize agricultural production, while instituting safeguards necessary to maintain reasonable and proper environmental health.

In view of the above considerations, an investigation was conducted to evaluate the toxicological consequences of humic-pesticide interactions on the fate and effects of agricultural chemicals used extensively in Mississippi and the South Atlantic-Gulf Region. The first specific task involved in the accomplishment of this objective was to evaluate the influence of humic materials on the acute toxicity of a suite of pesticides. A bioluminescent strain of bacteria (*Photobacterium phosphoreum*) was utilized as a sensitive, low cost, and standardized screening procedure to evaluate humic-pesticide interactions. This screening procedure has undergone extensive evaluation with a variety of environmental toxicants and results compare quite favorably with those obtained using freshwater invertebrate and vertebrate species (Bulich et al. 1981; Lebsack et al. 1981; Curtis et al. 1982; Qureshi et al. 1982; DeZwart and Slooff 1983). The second specific task was to examine the influence of different sources of DHM on the toxicity of pesticides.

Materials and Methods

Selection of Pesticides

Commercial pesticide formulations were provided by the Mississippi State Chemical Laboratory (Dr. Larry G. Lane, Director, Industrial and Agricultural Services). The selection of pesticides was based upon extensive use in Mississippi (Mississippi Cooperative Extension Service 1985) and the South Atlantic-Gulf Region (Gianessi et al. 1989) as well as toxicity to aquatic organisms (Johnson and Finley 1980).

Preparation of Dissolved Humic Material

Commercially available HA was obtained from Aldrich Chemical Company (sodium salt). Aldrich HA is reported to be of terrestrial origin (Chiou et al. 1986). The other DHMs used in this project were obtained from the International Humic Substances Society (IHSS) and were isolated from Suwanee River water. Final nominal concentrations of DHM tested were 0, 0.5, 5.0, 50, and 100 mg/L. The 0.5 mg/L HA concentration represents a pristine, but yet natural water source, while 5 mg/L is the average concentration of HA in surface waters (Neubecker and Allen 1983). The highest concentrations, 50 and 100 mg HA/L occur in "blackwater systems", such as swamps and marshes (Stewart 1984).

Bacterial Luminescence Inhibition

Bacterial bioluminescence assays were conducted essentially as described by Bulich and Isenberg (1981). Saline suspensions containing approximately 10^6 colony-forming units (CFU) of the luminescent bacterium *Photobacterium phosphoreum* (NRRL 811177) were exposed for up to 30 min at 15°C to selected concentrations of pesticide in the presence of 0, 0.5, 5.0, 50, and 100 mg DHM/L. Bioluminescence was monitored at 0-time and after 5, 15, and 30 min of exposure to pesticide using a Model 2055 Microtox Toxicity Analyzer. Such a procedure permitted examination of concentration- and time-toxicity relationships.

Analysis of Data

Estimates of the EC50 were obtained using linear regression analyses. The percent inhibition of light emitted at each humic-pesticide concentration and time point was converted to a gamma value which is defined as the ratio of light lost to light remaining (Johnson 1974). Values of gamma were calculated as a function of pesticide concentration and the EC50 was determined by the intersection of a best fit with gamma equal to 1.0. A mathematical procedure based on Fieller's theorem (Finney 1964) was used to calculate 95% confidence intervals for each estimate of the EC50 using a computer program (Microbics Corporation).

Results

Results are presented for experiments which focused on the evaluation of the influence of humic materials on the acute toxicity of a suite of pesticides. In addition, data are presented for investigations which focused on the influence of different sources of DHM on the toxicity of the pyrethroid insecticides, fenvalerate (Pydrin) and permethrin (Ambush).

Influence of DHM on the Toxicity of Pesticides

A combination of antagonistic and synergistic effects of commercially available HA was observed with organophosphate (Table 1) and carbamate insecticides (Table 2). For example, the 30 min EC50 value for azinphos-methyl increased from 0.409 mg/L (0 mg HA/L) to 0.783 mg/L in the presence of 100 mg

HA/L. Likewise, with chlorpyrifos, the respective EC50 values (30 min) for 0, 5.0, 50, and 100 mg HA/L were 5.84, 7.09, 10.1, and 9.13 mg/L. Conversely, the toxicity of methyl parathion was enhanced by the presence of humic material. The 30 min EC50 values for methyl parathion were 0.512, 0.293, and 0.372 mg/L in the presence of 0, 50, and 100 mg HA/L, respectively. As with methyl parathion, HA appeared to enhance toxicity of the carbamate insecticide, carbaryl, in a dose-dependent manner. However, the 30 min EC50 values for carbaryl at 0.5, 5.0, 50, and 100 mg/L were not significantly different from control (0 mg HA/L). The toxicity of carbofuran was reduced by low concentrations of HA (0.5 and 5.0 mg/L), however, no substantial differences were observed in the presence of 50 and 100 mg HA/L.

The pyrethroid insecticides exhibited a considerable degree of toxicity in the bacterial bioluminescence inhibition assay (Table 3). The 30 min EC50 values for fenvalerate and permethrin were 0.580 and 1.24 mg/L, respectively. A time-dependent decrease in toxicity of both pyrethroid insecticides to *Photobacterium phosphoreum* was observed. For example, the EC50 value for fenvalerate increased from 0.494 mg/L at 5 min to 0.580 mg/L by 30 min of exposure. Likewise, the EC50 value for permethrin increased from 0.980 mg/L (5 min) to 1.24 mg/L (30 min). Regarding the influence of commercially available HA on toxicity, HA significantly decreased toxicity of the pyrethroid insecticides in a dose-dependent manner. In the presence of 0, 50, and 100 mg HA/L, the 30 min EC50 values for fenvalerate were 0.580, 0.941, and 1.73 mg/L, respectively. For permethrin, the respective EC50 values (30 min) for 0, 50, and 100 mg HA/L were 1.24, 2.72, and 4.72 mg/L. Thus, the presence of HA (100 mg/L) reduced toxicity of the pyrethroid insecticides 3.0- to 3.8-fold.

Compared to the insecticides examined, the triazine herbicides were considerably less toxic to *Photobacterium phosphoreum* (Table 4). The 30 min EC50 values (0 mg HA/L) for atrazine, cyanazine, and simazine were 82.4, 82.2, and 242 mg/L. As with the pyrethroids, commercially available HA had a significant influence on toxicity of the triazines to *Photobacterium phosphoreum*. The presence of 50 mg HA/L reduced the toxicity of cyanazine and simazine by factors of 1.6 and 2.8, respectively. A concentration of 100 mg HA/L reduced the toxicity of atrazine, cyanazine, and simazine by factors of 3.4, 1.9, and 3.2, respectively.

The toxicity of glyphosate was significantly enhanced at 0.5 mg HA/L while a significant reduction in toxicity was observed at 50 and 100 mg HA/L (Table 5). The 30 min EC50 values for glyphosate were 25.5, 10.5, 106, and 112 mg/L in the presence of 0, 0.5, 50, and 100 mg HA/L, respectively. Commercially available HA had no significant influence on the toxicity of the herbicides, MSMA and pentachlorophenol, and trifluralin by 30 min exposure (Table 5).

Influence of Different Sources of DHM on Toxicity

Data on the effect of different sources of DHMs on the EC50 estimate for permethrin are presented in Table 6. Commercially available HA decreased the toxicity of permethrin by approximately 2.2- and 3.8-fold at 50 and 100 mg/L, respectively, after 30 min. Aquatic HA and FA significantly reduced the EC50 estimate of permethrin at all concentrations examined; however, the magnitude of toxicity reduction was not as great as with commercially-available HA. For aquatic HA (30 min) toxicity was reduced by 1.2-, 1.3-, and 1.4-fold for 0.5, 5.0, and 50 mg/L, respectively. Respective concentrations of aquatic FA decreased the toxicity of permethrin by 1.4-, 1.3-, and 1.3-fold (30 min).

Discussion

A combination of antagonistic and synergistic effects was observed with the organophosphate and carbamate insecticides. Similarly, in acute algal (4-hr) bioassays, Stewart (1984) reported antagonistic and synergistic effects of DHMs. When DHM was present, compounds in an aniline methylation series became more toxic (1.1- to 40-fold). In addition, DHM increased the toxicity of *o*-cresol, 2,4-dimethylphenol, and 2,3,6-trimethylphenol (2- to 5.4-fold) but reduced toxicity of *p*-benzoquinone and quinoline by factors of 6.1 and 1.2, respectively. In the present investigation, commercially available HA significantly decreased the toxicity of both azinphos-methyl and chlorpyrifos, while the toxicity of methyl parathion was enhanced in the presence of humic materials. On the other hand, a clear indication of reduced toxicity, in the presence of HA (100 mg/L), was observed with the pyrethroid insecticides (3.0- to 3.8-fold) and triazine herbicides (1.9- to 3.4-fold). As with the organophosphate and carbamate insecticides, a combination of antagonistic and synergistic effects was observed with glyphosate. In addition, the insecticides examined were shown to be considerably more toxic than the herbicides. This finding is consistent with information available in fish species (Johnson and Finley 1980).

Regarding pyrethroid insecticides, the humic-toxicant interactions appeared to be compound specific. For example, fenvalerate and permethrin reacted

differently to the same source and concentration of DHM; the reason this occurred is unclear. The compounds are similar in structure, as are all of the pyrethroids, but with some important differences. Fenvalerate possesses a cyano substituted phenoxyphenyl group and a chlorine substituted 1-methylethylbenzene group [(±)-α-cyano-3-phenoxybenzyl (±)-α-2-(4-chlorophenyl)-3-methylbutyrate]. Permethrin lacks a cyano group on the phenoxyphenyl group and has a dichlorovinyl dimethyl substituted cyclopropane instead of the chlorine substituted 1-methylethylbenzene group [3-phenoxybenzyl (±)cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate]. The differences in chemical structure result in differing biological effects and, thus, fenvalerate is classified as a type II pyrehroid and permethrin a type I pyrethroid. This classification is based on signs of poisoning. It is probable that this difference in chemical structure is responsible for the observed differences in humic-toxicant interaction.

Additionally, a time-dependent decrease in toxicity, as evidenced by an increased EC50 from 5 to 30 min, was observed with both fenvalerate and permethrin (Table 3). A possible explanation could be DHM-mediated hydrolysis or biodegradation of fenvalerate and permethrin. Liu et al. (1983) have reported FA-enhanced biodegradation of synthetic organics. Additional explanations could be the loss of volatile organics from the formulated pesticide or an increase of DHM-bound pesticide with time. Several studies have indicated that a temporal relationship exists between HA exposure and maximal humic-toxicant interaction. For example, Karickhoff (1980) proposed two types of binding of contaminants to sediments, a "fast" and a "slow" component. McCarthy and Jimenez (1985) indicated that "binding" of DHM to benzo[a]pyrene (B[a]P) was a type of "fast" association taking 5 to 10 min. However, these investigators could not rule out further interaction indicative of the "slow" type between B[a]P and DHM given a longer contact time. Indeed, Johnsen (1987) has indicated that the time for PAH-humic interactions to reach equilibrium may take longer than 70 d. Given the time-dependent toxicity reduction observed in the present investigation, within 15 min in some cases, it is possible that the reduction in toxicity was due entirely to the "fast" component of DHM-toxicant "binding". However, the "slow" component may have been responsible for the toxicity reduction observed at 30 min.

Contact time-dependent "binding" of toxicants with DHM may occur, in part, because such interaction is dependent upon the charge-transfer interaction (electron donor-acceptor complex formation) between DHM and toxicant. The charge-transfer type of interaction has been postulated for organic molecules, specifically chloranil, and humic materials, but was described as applicable to other organic molecules. Humic-chloranil interaction was reported to have continued over several weeks, again indicating a temporal relationship in humic-toxicant interactions (Melcer et al. 1987).

In a related study, the effect of natural water source on the toxicity of a series of substituted chlorophenols was studied also utilizing the Microtox assay (Cunningham et al. 1986). With three of the four compounds tested, the water source was responsible for a significant reduction in toxicity as evidenced by an increase in the EC50 estimate. The reduction in toxicity was traced to specific compound characteristics and the nature and concentration of the DOC in one of the water sources as determined by fluorescence spectroscopy and fingerprinting by pyrolytic gas chromatography/mass spectrometry. However, only a 15 min time point was used in the Microtox assay, so that any relationship between contact time and toxicity reduction could not be assessed.

Regarding the influence of different sources of DHM on toxicity, results indicate that humic-toxicant interactions can alter the toxicity of pyrethroid insecticides. Furthermore, the extent of alteration was dependent on the source of DHM. With permethrin, commercially available HA had a much greater influence on toxicity than either aquatic HA or FA. Garvey et al. (1991) found the same trend in a study investigating the effect of terrestrial HA, and aquatic HA and FA on copper toxicity in algae. When deflagellation of the algae was used as the indicator of toxicity, it was observed that terrestrial HA decreased toxicity by the greatest factor followed by aquatic HA. Aquatic FA had no effect on the toxicity of copper to the algae. Results from the present investigation support these findings. The inability of aquatic FA to affect the toxicity of the pyrethroid insecticides was expected. In a study to quantitatively measure the binding of PAHs and pesticides to DHMs, Carter and Suffet (1983) demonstrated that HAs showed a greater binding tendency than FAs. Results from the present investigation support this finding.

Tables

Table 1. Influence of commercially available humic acid (HA) on the toxicity of selected organophosphate insecticides

Insecticide	mg HA/L	Luminescence EC50 (mg/L) with 95% confidence limits		
		5 min	15 min	30 min
Azinphos-methyl	0	0.351 (0.327 - 0.377)	0.370 (0.341 - 0.402)	0.409 (0.375 - 0.446)
	0.5	0.454* (0.384 - 0.538)	.487 (0.398 - 0.597)	0.558* (0.465 - 0.670)
	5.0	0.485* (0.442 - 0.532)	0.498* (0.456 - 0.544)	0.549* (0.494 - 0.611)
	50	0.581* (0.518 - 0.651)	0.592* (0.515 - 0.681)	0.638* (0.552 - 0.738)
	100	0.754* (0.710 - 0.801)	0.752* (0.670 - 0.845)	0.783* (0.651 - 0.942)
Chlorpyrifos	0	3.86 (3.51 - 4.25)	4.61 (4.27 - 4.99)	5.84 (5.45 - 6.26)
	0.5	6.65* (5.62 - 7.85)	6.14 (4.86 - 7.77)	8.13 (6.04 - 10.9)
	5.0	6.35* (5.74 - 7.04)	6.33* (5.79 - 6.92)	7.09* (6.44 - 7.79)
	50	6.69* (6.23 - 7.18)	7.55* (6.99 - 8.15)	10.1* (9.29 - 11.1)
	100	6.37* (5.91 - 6.87)	7.57* (6.79 - 8.44)	9.13* (8.23 - 10.1)
Methyl parathion	0	0.543 (0.498 - 0.592)	0.505 (0.467 - 0.545)	0.512 (0.466 - 0.563)
	0.5	0.363* (0.323 - 0.408)	0.362* (0.318 - 0.412)	0.365* (0.320 - 0.416)
	5.0	0.395* (0.315 - 0.495)	0.403* (0.325 - 0.501)	0.379* (0.308 - 0.467)
	50	0.275* (0.244 - 0.309)	0.314* (0.270 - 0.365)	0.293* (0.254 - 0.338)
	100	0.342* (0.305 - 0.384)	0.379* (0.326 - 0.441)	0.372* (0.325 - 0.425)

* Significantly different from control (0 mg HA/L), $p < 0.05$.

Table 2. Influence of commercially available humic acid (HA) on the toxicity of selected carbamate insecticides

Insecticide	mg HA/L	Luminescence EC50 (mg/L) with 95% confidence limits		
		5 min	15 min	30 min
Carbaryl	0	4.00 (3.87 - 4.13)	4.28 (3.67 - 5.00)	3.89 (3.14 - 4.81)
	0.5	4.29 (3.90 - 4.72)	4.30 (3.92 - 4.71)	4.37 (3.96 - 4.83)
	5.0	4.06 (3.37 - 4.89)	4.05 (3.25 - 5.03)	4.54 (3.81 - 5.41)
	50	3.73 (3.45 - 4.02)	3.69 (3.39 - 4.01)	3.72 (3.38 - 4.09)
	100	3.16* (2.95 - 3.39)	3.31* (3.12 - 3.52)	3.25 (3.04 - 3.47)
Carbofuran	0	0.843 (0.692 - 1.03)	0.724 (0.547 - 0.959)	0.697 (0.512 - 0.949)
	0.5	1.13* (1.07 - 1.20)	1.02* (0.984 - 1.06)	1.01* (0.980 - 1.05)
	5.0	1.55* (1.28 - 1.88)	1.62* (1.36 - 1.92)	1.53* (1.27 - 1.83)
	50	1.14 (1.00 - 1.31)	0.944 (0.877 - 1.02)	0.721 (0.672 - 0.775)
	100	0.957 (0.906 - 1.01)	0.791 (0.722 - 0.867)	0.751 (0.648 - 0.871)

* Significantly different from control (0 mg HA/L), $p < 0.05$.

Table 3. Influence of commercially available humic acid (HA) on the toxicity of selected pyrethroid insecticides

Insecticide	mg HA/L	Luminescence EC50 (mg/L) with 95% confidence limits		
		5 min	15 min	30 min
Fenvalerate	0	0.494 (0.445 - 0.549)	0.516 (0.457 - 0.584)	0.580 (0.510 - 0.661)
	0.5	0.463 (0.401 - 0.535)	0.510 (0.447 - 0.582)	0.533 (0.440 - 0.646)
	5.0	0.578 (0.524 - 0.637)	0.607 (0.528 - 0.699)	0.667 (0.565 - 0.788)
	50	0.919* (0.774 - 1.09)	0.867* (0.811 - 0.928)	0.941* (0.866 - 1.02)
	100	1.28* (1.17 - 1.40)	1.45* (1.32 - 1.59)	1.73* (1.62 - 1.85)
Permethrin	0	0.980 (0.910 - 1.06)	1.07 (0.969 - 1.19)	1.24 (1.15 - 1.34)
	0.5	1.17 (1.04 - 1.32)	1.33* (1.21 - 1.46)	1.54* (1.42 - 1.66)
	5.0	1.19* (1.11 - 1.28)	1.32 (1.11 - 1.57)	1.44 (1.31 - 1.57)
	50	2.13* (1.99 - 2.27)	2.38* (2.26 - 2.51)	2.72* (2.56 - 2.86)
	100	3.91* (3.22 - 4.75)	4.29* (3.56 - 5.17)	4.72* (3.87 - 5.76)

* Significantly different from control (0 mg HA/L), $p < 0.05$.

Table 4. Influence of commercially available humic acid (HA) on the toxicity of selected triazine herbicides

Herbicide	mg HA/L	Luminescence EC50 (mg/L) with 95% confidence limits		
		5 min	15 min	30 min
Atrazine	0	85.5 (74.6 - 97.9)	73.9 (64.7 - 84.6)	82.4 (75.4 - 90.1)
	0.5	95.1 (80.2 - 113)	82.5 (69.5 - 97.9)	86.5 (74.9 - 100)
	5.0	103 (82.7 - 128)	107 (77.2 - 150)	107 (84.0 - 136)
	50	74.1 (60.4 - 90.8)	113* (89.7 - 142)	108 (85.1 - 138)
	100	232* (201 - 269)	234* (208 - 263)	280* (248 - 316)
Cyanazine	0	80.5 (73.5 - 88.1)	88.9 (81.9 - 96.7)	82.2 (74.6 - 90.6)
	0.5	81.2 (68.2 - 96.7)	86.0 (76.4 - 96.8)	81.9 (68.8 - 97.6)
	5.0	96.0 (85.7 - 107)	94.5 (80.3 - 111)	97.0 (89.5 - 105)
	50	146* (119 - 178)	128* (103 - 159)	135* (116 - 157)
	100	250* (181 - 344)	217* (166 - 283)	156* (114 - 213)
Simazine	0	211 (177 - 250)	181 (152 - 214)	242 (207 - 284)
	0.5	260 (213 - 318)	258 (210 - 317)	240 (184 - 313)
	5.0	261 (179 - 381)	259 (180 - 373)	258 (185 - 359)
	50	538* (372 - 778)	526* (384 - 720)	674* (473 - 959)
	100	838* (577 - 1217)	690* (485 - 981)	777* (517 - 1167)

* Significantly different from control (0 mg HA/L), $p < 0.05$.

Table 5. Influence of commercially available humic acid (HA) on the toxicity of selected herbicides

Herbicide	mg HA/L	Luminescence EC50 (mg/L) with 95% confidence limits		
		5 min	15 min	30 min
Glyphosate	0	26.8 (21.4 - 33.5)	26.5 (22.0 - 31.8)	25.5 (21.1 - 30.8)
	0.5	10.8* (9.07 - 12.7)	15.5* (12.1 - 19.5)	10.5* (8.86 - 12.4)
	5.0	24.0 (18.9 - 30.5)	21.8 (17.4 - 27.2)	21.2 (17.0 - 26.4)
	50	93.1* (56.0 - 155)	108* (67.9 - 171)	106* (63.0 - 178)
	100	65.5 (22.8 - 185)	74.3 (31.4 - 176)	112* (54.3 - 229)
MSMA	0	80.0 (68.7 - 92.4)	76.3 (66.9 - 87.0)	74.1 (64.5 - 85.1)
	0.5	65.8 (56.5 - 76.5)	64.4 (55.1 - 75.2)	62.6 (53.7 - 72.9)
	5.0	69.0 (63.5 - 75.0)	72.9 (68.9 - 77.2)	68.6 (64.1 - 73.4)
	50	82.3 (72.3 - 93.7)	81.4 (71.9 - 92.1)	82.4 (72.5 - 93.7)
	100	88.9 (79.1 - 99.8)	87.6 (78.0 - 98.4)	83.8 (69.7 - 101)
Pentachlorophenol	0	2.20 (1.97 - 2.47)	1.85 (1.65 - 2.07)	1.60 (1.43 - 1.79)
	0.5	1.74 (1.38 - 2.21)	1.66 (1.31 - 2.10)	1.69 (1.31 - 2.18)
	5.0	1.61 (1.27 - 2.05)	1.53 (1.18 - 1.98)	1.51 (1.16 - 1.97)
	50	1.78* (1.67 - 1.90)	1.63 (1.53 - 1.73)	1.59 (1.51 - 1.67)
	100	2.00 (1.89 - 2.10)	1.63 (1.48 - 1.79)	1.61 (1.47 - 1.76)

Table 5. Influence of commercially available humic acid (HA) on the toxicity of selected herbicides (Continued)

Herbicide	mg HA/L	Luminescence EC50 (mg/L) with 95% confidence limits		
		5 min	15 min	30 min
Trifluralin	0	3.56 (3.22 - 3.94)	3.65 (3.46 - 3.84)	3.33 (2.98 - 3.73)
	0.5	3.02* (2.92 - 3.12)	3.00* (2.91 - 3.09)	3.05 (2.98 - 3.13)
	5.0	3.14 (3.03 - 3.25)	3.05* (2.93 - 3.17)	3.12 (2.99 - 3.26)
	50	2.92* (2.76 - 3.10)	3.04* (2.86 - 3.23)	3.00 (2.84 - 3.17)
	100	3.08 (2.94 - 3.23)	3.02* (2.91 - 3.15)	3.14 (3.02 - 3.27)

* Significantly different from control (0 mg HA/L), $p < 0.05$.

Table 6. Influence of different sources of dissolved humic materials (DHM) on the toxicity of permethrin (Ambush)

DHM type	mg/L	Luminescence EC50 (mg/L) with 95% confidence limits		
		5 min	15 min	30 min
Commercial humic acid	0	0.980 (0.910 - 1.06)	1.07 (0.969 - 1.19)	1.24 (1.15 - 1.34)
	0.5	1.17 (1.04 - 1.32)	1.33* (1.21 - 1.46)	1.54* (1.42 - 1.67)
	5.0	1.19* (1.11 - 1.28)	1.32 (1.12 - 1.57)	1.44 (1.31 - 1.57)
	50	2.13* (1.99 - 2.27)	2.38* (2.26 - 2.51)	2.72* (2.59 - 2.86)
	100	3.91* (3.22 - 4.75)	4.29* (3.56 - 5.17)	4.72* (3.88 - 5.76)
Aquatic humic acid	0	0.980 (0.910 - 1.06)	1.07 (0.969 - 1.19)	1.24 (1.15 - 1.34)
	0.5	1.24* (1.16 - 1.32)	1.36* (1.29 - 1.32)	1.51* (1.43 - 1.60)
	5.0	1.27* (1.09 - 1.47)	1.48* (1.20 - 1.82)	1.60* (1.34 - 1.90)
	50	1.29* (1.14 - 1.46)	1.52* (1.28 - 1.81)	1.73* (1.51 - 1.98)
	100	1.68* (1.52 - 1.87)	1.96* (1.81 - 2.12)	2.35* (2.22 - 2.49)
Aquatic fulvic acid	0	0.980 (0.910 - 1.06)	1.07 (0.969 - 1.19)	1.24 (1.15 - 1.34)
	0.5	1.28* (1.22 - 1.34)	1.47* (1.39 - 1.56)	1.70* (1.56 - 1.85)
	5.0	1.21* (1.06 - 1.38)	1.37* (1.24 - 1.51)	1.65* (1.52 - 1.78)
	50	1.28* (1.15 - 1.42)	1.49* (1.28 - 1.73)	1.70* (1.40 - 2.06)
	100	1.36* (1.30 - 1.43)	1.50* (1.42 - 1.59)	1.63* (1.57 - 1.69)

* Significantly different from control (0 mg DHM/L), $p < 0.05$.

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