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Comparison of Short-Term Aquatic Toxicity: Formulation vs Active Ingredients of Pesticides

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Abstract. EC_{50} values for algae and water fleas and LC_{50} values for fish of formulated pesticide products from standardized short term toxicity tests have been compared with the corresponding values of the respective active ingredients (AI). The results suggest that toxicity tests conducted with the technical material will provide adequate information for estimating toxicological hazard of formulations to aquatic organisms. A preliminary hazard assessment performed by using the toxicity of the technical grade material divided by a species and formulation related factor of extrapolation uncertainty would allow a hazard assessment for the formulation with a high degree of reliability. Based on the presented data, this factor could be as low as 5 for green algae, 10 for water fleas, and 5 for fish without an unreasonable risk (2% or less) of substantially increased, environmentally relevant formulation toxicity. Such an approach would save considerable numbers of test animals and also allow improvement of formulations without unnecessary or even economically prohibitive replications of numerous tests.

Since most pesticides are poorly soluble in water, they are formulated with solvent aids which produce very fine emulsions when added to water (Rand and Petrocelli 1985). These adjuvants in a formulated product, however, may alter the toxicity of the active ingredient (Solon *et al.* 1969; Folmar *et al.* 1979; Beusen and Tucker 1989). One of the most comprehensive comparisons of technical active ingredient vs formulation toxicity to date consists of 161 acute aquatic toxicity studies performed by the U.S. Fish and Wildlife Service (Mayer and Ellersieck 1986). In this study, as well as in smaller studies (Murty 1986), high variations of the ratios between AI and formulations occurred, giving the impression that the formulation toxicity cannot reliably be predicted from that of the active ingredient. Differences in those studies, however, may be due to differences in test methods, chosen test species and strains, or the overall performance of individual laboratories. They do not necessarily or unequivocally reflect the real differences in the toxicity between AI and formulations. To overcome this problem, recent results from highly standardized tests following OECD (Organization for Economic Cooperation and Development), EEC (European Economic Community), or EPA (Environmental Protection Agency) / ASTM (American Society for Testing of Material) guidelines have been compiled by nine manufacturers of agricultural chemicals. Due to the standardized quality of these tests, differences in results are attributed to intrinsic differences in the formulations and not to methods or test system performance.

If in these highly standardized tests, formulations appeared to be substantially more toxic than the AI, an attempt was made to relate this increased toxicity to criteria such as the chemical class of compound, the type of formulation, or toxic adjuvants. This analysis was conducted in order to identify critical formulation types which may require higher factors of extrapolation uncertainty. Since formulations typically persist only for a limited time after entering the aquatic environment, and since soil passage separates the adjuvants from the AI, only the short term standard tests were considered: algae EC_{50} (72 h), water flea EC_{50} (48 h), and fish LC_{50} (96 h).

Material and Methods

Test Procedures

The tested green algae (predominantly *Selenastrum capricornutum* or *Scenedesmus subspicatus*), water fleas *(Daphnia magna)* and fish *(Oncorhynchus mykiss, Cyprinus carpio, Lepomis macrochirus, Leuciscus idus melanotus)* were exposed to various concentrations of the test material for a total of 72 h for green algae, 48 h for water fleas, and 96 h for fish. Details of the test conditions and procedures are outlined in the respective international testing guidelines (for green algae: OECD 1984a; EPA 1986; for water fleas: OECD 1984b; EPA 1985; for fish: OECD 1984c; EEC 1984; EPA 1982) For green algae, the effect on growth of several generations was measured every 24 h. The testing endpoints for water fleas were behavioral impairments and mortality.

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Table 1. Test organisms, test parameter (ratio) and size of the data base

Test organism	Evaluated ratio	Number of data pairs
Green algae	$EC_{50\ AI}/EC_{50\ formulation}$	49
Water fleas	$EC_{50\ AI}/EC_{50\ formulation}$	69
	$NOEC(48\ h)_{AI}/NOEC(48\ h)_{formulation}$	29
Fish	$LC_{50\ AI}/LC_{50\ formulation}$	145

Lethality was the endpoint of the fish testing. The results obtained at the various test concentrations in comparison with an untreated control were used to calculate the EC_{50}/LC_{50}, *e*ffective/*l*ethal *c*oncentration at which the growth rate is inhibited by *50%* (green algae) or *50%* of the test animals are sublethally (water fleas) or lethally (fish) affected. The second important parameter was the NOEC (*no o*bserved *e*ffect *c*oncentration), the highest tested concentration of a test substance at which no statistically significant lethal or sublethal or other effect is observed.

Data Selection Criteria

The data base comprises mostly unpublished data of several chemical companies (American Cynamid, Bayer, BASF, Ciba-Geigy, Dow Elanco, Hoechst, Sandoz, Schering, Shell Agrar). The studies were conducted following standard test protocols, appeared valid (*e.g.*, no increased control toxicity, experienced laboratory, feasible concentration/response relationship) and used the standard test organisms as outlined in the preceeding chapter. If possible, data pairs from the same laboratory were used. Every data set that could be obtained from the nine chemical companies and fulfilled the above described criteria were included in the presented data base, and no data pairs were advertently omitted due to unfitting results. Table 1 summarizes the tested organisms, the evaluated ratio indices, and the number of considered data pairs.

Mathematical Transformation of the Data

The ratios of EC_{50} or LC_{50} of technical grade AI to those of formulations, based on active ingredient (AI), were compared. The following sample calculation illustrates the transformation of the actual data on formulations: The EC_{50} of active ingredient "x" for algal growth inhibition is 100 mg AI/L. A formulated product containing 10% AI also has an EC_{50} of 100 mg Formulation/L. Based on AI, the EC_{50} in the formulation test would be 10 mg AI/L. The ratio of the EC_{50} of technical grade pesticide to that of the formulation is then 100/10 = 10, *i.e.*, the formulation is ten times more toxic as the pure active ingredient.

In some cases, the toxicity was not given as a finite number, but in the form of "greater than" or "smaller than." To avoid uninterpretable ratios, no calculations were performed with values of "smaller than" for the denominator (= formulation) or "greater than" for the nominator (= AI). For formulations which contained more than a single AI, the effect of the combined AI was assumed to be additive (Anonymous 1988) and was calculated from Finney's harmonic-mean formula:

$$C_A/T_A + C_B/T_B + \cdots + C_Z/T_Z = 100/T_M \qquad \text{(WHO 1986)}$$

where

C = % concentration of active ingredients A, B, ..., Z
T = the toxicity (EC_{50}, LC_{50} values) of A, B, ..., Z
TM = the resulting toxicity value of the mixture

Evaluation of the Data Pairs

The EC_{50}/LC_{50} value of the active ingredient was divided by the corresponding toxicity value of the formulated product to achieve the ratio of AI vs formulation toxicity. A ratio greater than 1.0 indicates an increased toxicity of the formulation or synergism, if more than one AI is present and a ratio less than 1.0 a decreased toxicity of the formulation. However, ratios between 0.5 and 2.0 are considered as being within the range of normal experimental variation. Therefore, the toxicity of the technical compound and the formulation are considered to be equal if the ratio is between 0.5 and 2.0.

Results and Discussion

Green Algae

The comparison of the EC_{50} of technical AI vs formulation toxicity consisted of 95 studies conducted for 44 AIs (including combined AIs) and 51 end-use products (Table 2). As the No Observed Effect Concentrations (NOEC) were not routinely measured, a comparison of the NOEC (EC_0) data for AIs with such for formulations was not performed due to the low number of available data. The ratios of the EC_{50} of technical AI vs formulation toxicity are categorized according to the types of formulation in Table 3 and ranked into different toxicity classes as illustrated in Figure 1.

Based on the active ingredient, the toxicity of the formulation was equal to or less toxic than the AI in 75% of the cases. All but two out of the 12 more toxic formulations were less than 5 times more toxic than the technical compound. In no case, were the formulated products more toxic than by a factor of 50 when compared with the corresponding AI. The highest factor was 43. Due to the limited number of data, the type of formulation with a higher probability of an increased toxicity cannot conclusively be identified. It is, however, notable that most of the formulations which were more toxic than the AI were emulsifiable concentrates (EC). EC formulations may be more effective because the pesticide molecules are more evenly distributed in the test medium or because they contain adjuvants which themselves may have certain toxic properties against algae. Particularly the last mentioned point becomes more commanding the less toxic the AI is. It is, therefore, important to note that a significant increase in toxicity of the formulation (by a factor of 43) was only observed for a substance with a very low AI toxicity of 380 mg/L, a concentration which could not be reached by contamination from ground application in good agricultural practice. The second formulation with an increased toxicity (more than 5 times) had a factor of 7.3 (Figure 1).

Water Fleas

A total of 69 pesticidal formulations (62 AIs including combinations) consisting of 20 fungicides, 33 herbicides, and 16 insecticides were evaluated. The EC_{50} and the NOEC (48 h) values are listed in Table 4. Of the 69 pesticides, 68 (61 AIs) could be evaluated with respect to EC_{50} values. The EC_{50} toxicity ratios of AI vs formulated products are listed in Table 5 according to types of formulation. Ranking of the EC_{50} ratios according to toxicity classes are illustrated in Figure 2.

Overall, the formulations were equally or less toxic than the AI in nearly 65% of the cases. The formulation was less toxic or

Table 2. Toxicity of selected agrochemicals to green algae (in mg/L). The toxicity of formulations is based on the active ingredient (AI) content. For formulations which contained more than a single AI, the effect of the combined AIs was assumed to be additive and was calculated from Finney's harmonic-mean formula. The full names of formulation type acronyms are outlined in Table 3

Compound	Technical AI EC_{50}	Type of formulation	Formulation EC_{50}	Factor AI/formulation
		(A) Acaricides/Insecticides		
Organotin Compounds	0.16	WP	0.91	≤2
Carbamate	7.0	SC	2.03	>2–5
Organophosphorus	8.04	VL	13.27	≤2
Carbamates	7.4	EW	6.88	≤2
Organophosphorus	0.55	EC	0.74	≤2
Chloronicotinyl compound	>10.0	WE	142.0	≤2
Organophosphorus	86.0	SL	63.64	≤2
Organophosphorus	3.0	WP	2.49	≤2
Organophosphorus	167.5	SL	646.55	≤2
Organophosphorus	0.5	SL	0.47	≤2
Organophosphorus	0.5	EC	0.49	≤2
Organophosphorus	5.3	EC	2.6	>2–5
Organophosphorus	1.2	EC	3.6	≤2
		(B) Herbicides		
Azole	2.3	WG	5.7	≤2
Urea	0.079	WP	0.03	>2–5
Urea	0.13	WP	0.17	≤2
Triazinone	0.22	WG	0.92	≤2
Urea	0.042	WP	0.106	≤2
Triazinone	0.0069	WG	0.016	≤2
Urea	0.002	WP	0.01	≤2
Dinitrophenol derivative	0.62	EC	9.7	≤2
Urea	0.016	WP	0.024	≤2
Phenoxy-propionic acid	1.5	EC	0.3	>2–5
Phenoxy-propionic acid	1.5	EC	0.76	≤2
Phenoxy-propionic acid	0.52	EC	0.45	≤2
Sulfonyl urea	47.0	WG	20.0	>2–5
Biscarbamate	0.13	OF	52.67	≤2
Biscarbamate	0.13	EC	>1.6	≤2
Biscarbamate/benzofurane	<1.1	EC	0.826	≤2
Biscarbamate/benzofurane	<0.67	WP	70.92	≤2
Biscarbamate/benzofurane	<0.4	WP	16.36	≤2
Benzofurane	<0.31	EC	4.85	≤2
Cyclohexandione	16.0	EC	4.73	>2–5
Cyclohexandione	16.0	WP	33.75	≤2
		(C) Fungicides		
Morpholine	0.013	EC	0.0043	>2–5
Chinoxaline	0.068	WP	0.0558	≤2
Urea	0.56	FS	18.98	≤2
Carbamate	0.12	WG	0.49	≤2
Azole	1.64	EW	2.36	≤2
Sulfonyl amide	1.45	WG	5.00	≤2
Azole	0.9	WG	0.41	>2–5
Azole	3.7	WG	1.48	>2–5
Azole	0.073	EC	0.01	5–10
Not identified	380.0	EC	8.94	10–50
Not identified	380.0	EC	577.0	≤2
		(D) Others or Not Specified		
Pyridine	7.0	AS	11.0	≤2
Organophosphorus	0.66	EC	0.18	>2–5
Phenoxy-propionic acid	1.80	EC	4.40	≤2
Not identified	12.50	SC	14.0	≤2

less than 5 times more toxic than the technical grade compound in more than 80% of the cases. All formulations except 3 (95%) were at most 10 times more toxic than the technical AI. Similar results are obtained when the acute NOEC of AI is compared to that of the corresponding formulations (not presented in Table 5): In more than 85% of the cases, the formulation was less toxic or less than 5 times more toxic than the technical grade compound. The toxicity ratio of 4 compounds exceeded the factor 10 compared to the AI toxicity.

Three herbicides were more than ten times as toxic as the corresponding active ingredient. The comparison of herbicidal formulations with the corresponding AI revealed ratios of 10,

Table 3. EC_{50} ratios of active ingredient (AI)/formulation, classified according to the type of formulation: green algae

Type of formulation	Ratio of $EC_{50\ AI}/EC_{50\ formulation}$						
	>100	50–100	10–50	5–10	>2–5	≤2	Total
AS (aqueous solution)	—	—	—	—	—	1	1
EC (emulsifiable concentrate)	—	—	1	1	5	11	18
EW (emulsion, oil in water)	—	—	—	—	—	2	2
FS (flowable concentr., seed treatment)	—	—	—	—	—	1	1
OF (oil misc. flowable concentr.)	—	—	—	—	—	1	1
SC (suspension concentrate)	—	—	—	—	1	1	2
SL (soluble concentrate)	—	—	—	—	—	3	3
VL (vapor releasing liquid)	—	—	—	—	—	1	1
WP (wettable powder)	—	—	—	—	1	10	11
WG (water dispersible granules)	—	—	—	—	3	6	9
Total	—	—	1	1	10	37	49

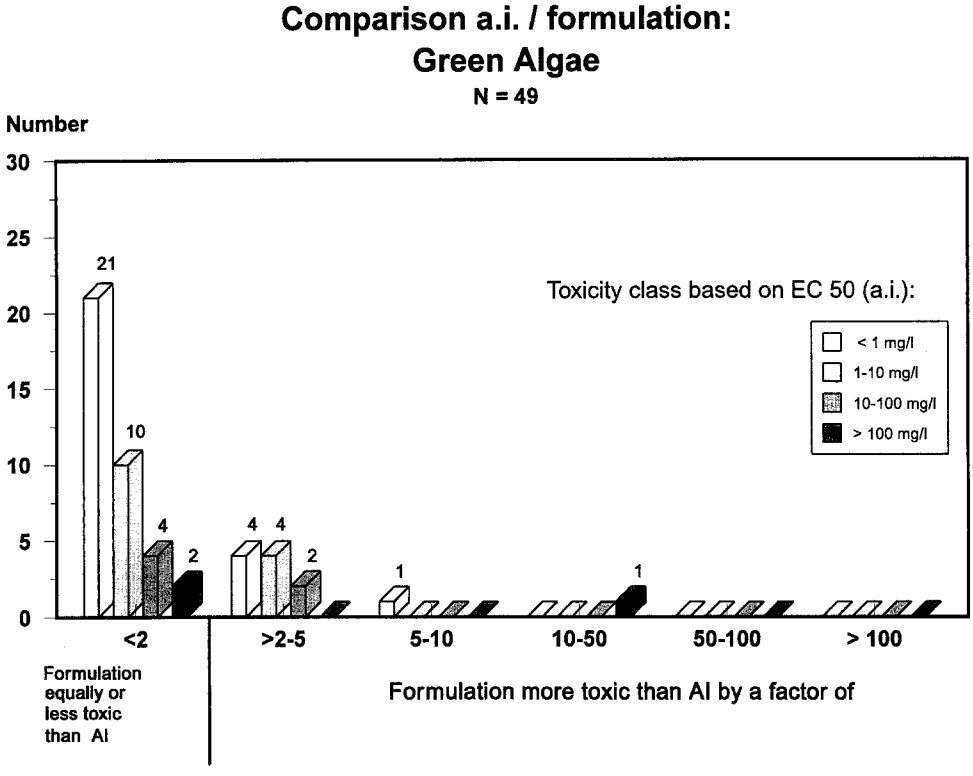

Fig. 1. Ratios of the short-term toxicity of pesticidal formulations vs the active ingredients to algae, ranked into different toxicity classes of the active ingredient (AI).

40, and 248, respectively. The considered formulated herbicides, however, are characterized by a low acute toxicity of the AI to water fleas, which is positively correlated with the ratio indices (EC_{50} of 6, 120, and 670 mg/L, respectively). Such formulations usually do not pose a specific hazard to aquatic invertebrates since under actual farming conditions, the *E*stimated (*P*redicted) maximum *E*nvironmental *C*oncentrations (EEC or PEC) would be well below the concentration at which toxic effects would be expected.

Fish

The comparison of technical vs formulation toxicity consisted of 273 acute studies conducted according to standard test protocols (OECD, EPA, EEC, or comparable). The LC_{50} values of the AIs and the formulations on different fish species are listed in Table 6. The LC_{50} ratios of AI vs formulated product are listed in Table 7 according to types of formulation. Ranking of the LC_{50} ratios according to toxicity classes are illustrated in Figure 3. Based on these comparisons, more than 75% of the formulated products did not show a higher toxicity than the AI. In more than 95% of the cases, the formulation was maximally 5 times more toxic than the AI. A factor of 10 would encompass 98% of the products. Only three formulations, all EC formulations, were more toxic by a factor of or exceeding 10, and no formulation was more toxic than 100 times the AI toxicity. The higher factors were 63, 14, and 12, respectively. Two out of the three formulations that exhibited an increased toxicity by factors more than 10 showed again a very low acute toxicity of the AI (LC_{50} = 447 mg/L). Therefore, the maximum contaminations expected under actual farming conditions are well below

Table 4. Acute toxicity of selected agrochemicals to water fleas (in mg/L). The toxicity of formulations is based on the active ingredient (AI) content. For formulations which contained more than a single AI, the effect of the combined AIs was assumed to be additive and was calculated from Finney's harmonic-mean formula. The full names of formulation type acronyms are outlined in Table 5

Compound	Type of formulation	Technical AI EC_{50}	Formulation EC_{50}	Factor AI/formulation	Technical AI $NOEC_{48\,h}$	Formulation $NOEC_{48\,h}$	Factor AI/formulation
			(A) Acaricides/Insecticides				
Organophosphorus	WP	0.0011	0.0022	≤2	—	—	—
Pyrethroid	SC	0.00029	0.00025	≤2	—	—	—
Pyrethroid	EC	0.00014	0.000025	5–10	—	—	—
Organophosphorus	VL	0.0230	0.022	≤2	<0.01	—	—
Organophosphorus	EC	0.0017	0.0013	≤2	—	—	—
Chloro-nicotinyl deriv.	WG	85.0	169.15	≤2	—	—	—
Chloro-nicotinyl deriv.	WP	85.0	65.45	≤2	—	—	—
Organophosphorus	DS	0.0073	0.00088	5–10	—	—	—
Organophosphorus	VL	0.19	0.13	≤2	0.0630	<0.1	—
Triazapentadiene	EC	0.035	0.014	>2–5	0.01	0.008	≤2
Organophosphorus	EC	0.0017	0.0003	5–10	—	0.00015	—
Organophosphorus	EC	0.0170	0.80	≤2	—	—	—
Organophosphorus	EC	0.0030	0.0005	5–10	—	0.00024	—
Organophosphorus	EC	0.0220	0.03	≤2	0.0086	0.0040	>2–5
Organophosphorus	WP	0.0070	0.0060	≤2	0.0020	0.0015	≤2
Organophosphorus	EC	0.0173	0.002	5–10	0.02	0.0005	10–50
			(B) Herbicides				
Azole	WP	1.54	1.77	≤2	—	—	—
Organophosphorus	EC	0.12	0.041	>2–5	—	—	—
Triazinone	WG	63.0	28.98	>2–5	—	—	—
Triazinone	WG	101.70	145.43	≤2	—	—	—
Dinitroaniline deriv.	SC	0.28	18.72	≤2	—	—	—
Dinitroaniline deriv.	SC	0.28	20.30	≤2	—	—	—
Biscarbamate	EC	6.0	0.581	10–50	—	—	—
Biscarbamate/benzofurane	EC	35.23	20.8	≤2	—	—	—
Biscarbamate	OF	6.0	8.389	≤2	—	—	—
Biscarbamate/benzofurane	WP	30.72	9.061	>2–5	—	—	—
Biscarbamate/benzofurane	WP	41.97	45.1	≤2	—	—	—
Benzofurane	EC	13.52	46.60	≤2	8.55	—	—
Cyclohexanedione	EC	120.0	2.975	10–50	—	—	—
Cyclohexanedione	EC	6.20	1.4	>2–5	—	—	—
Pyridine	AS	225.0	103.0	>2–5	100.0	50.0	≤2
Pyridine	EC	0.56	0.29	≤2	0.56	0.0470	10–50
Phenoxy-propionic ac.	EC	6.10	0.70	5–10	1.10	0.24	>2–5
Azole	SL	125.0	>500.0	≤2	62.50	240.0	≤2
Pyridazinone	WP	164.0	258.0	≤2	62.50	81.30	≤2
Urea	WP	33.0	3.90	5–10	18.0	0.16	>100
Urea	EC	0.75	0.46	≤2	0.32	—	—
Phenoxy-propionic ac.	EC	0.23	0.39	≤2	0.08	0.16	≤2
Phenoxy-propionic ac.	EC	3.20	2.70	≤2	0.32	1.0	≤2
Not identified	SL	670.0	2.70	>100	420.0	1.80	>100
Phenoxy-propionic ac.	EW	3.0	0.42	5–10	1.0	0.20	>2–5
Urea	SC	507.0	>450.0	≤2	—	56.0	—
Urea	SC	67.0	124.0	≤2	18.0	43.30	≤2
Urea	WG	67.0	110.0	≤2	18.0	55.50	≤2
Triazine	SC	21.20	>500.0	≤2	8.0	500.0	≤2
Triazine	WP	87.0	>50.0	≤2	18.0	29.0	≤2
Triazine	SC	87.0	230.0	≤2	18.0	75.0	≤2
Triazine	SC	>100.0	191.0	—	58.0	100.0	≤2
Benzoic acid derivate	SL	111.0	768.0	≤2	56.0	268.8	≤2
Benzoic acid derivate/phenoxy carboxylic acid	SL	189.5	153.2	≤2	51.5	66.6	≤2
			(C) Fungicides				
Morpholine	EC	0.66	0.62	≤2	—	—	—
Azole	WP	2.79	4.21	≤2	—	—	—
Urea	EC	0.27	131.76	≤2	—	—	—
Urea	DS	0.27	>12.69	≤2	—	—	—
Azole	EC	4.20	5.59	≤2	0.74	—	—
Azole	EW	4.20	2.44	≤2	0.74	—	—
Sulfonyl amide	WP	0.57	0.36	≤2	—	—	—
Azole	WP	11.30	8.0	≤2	5.6	2.80	≤2
Azole	WG	51.0	20.9	>2–5	—	—	—

Table 4. Acute toxicity of selected agrochemicals to water fleas (in mg/L). The toxicity of formulations is based on the active ingredient (AI) content. For formulations which contained more than a single AI, the effect of the combined AIs was assumed to be additive and was calculated from Finney's harmonic-mean formula. The full names of formulation type acronyms are outlined in Table 5 (*continued*)

Compound	Type of formulation	Technical AI EC_{50}	Formulation EC_{50}	Factor AI/formulation	Technical AI $NOEC_{48\ h}$	Formulation $NOEC_{48\ h}$	Factor AI/formulation
Azole	EC	51.0	60.18	≤2	—	—	—
Azole	EC	2.60	0.48	5–10	—	0.1280	—
Morpholine	EC	2.4	3.9	≤2	1.0	1.35	≤2
Carbamate	SC	12.7	5.5	>2–5	3.2	3.2	≤2
Organophosphorus	EC	0.0002	0.0007	≤2	—	0.0003	—
Carbamate	WP	0.13	0.0960	≤2	0.01	0.03	≤2
Azole	EC	4.80	0.56	5–10	1.70	0.80	>2–5
Azole	EC	0.77	0.83	≤2	<0.10	0.50	≤2
Not identified	SC	2.43	0.6	>2–5	0.31	0.1	>2–5
Azole	EC	26.0	5.9	>2–5	4.6	—	—
Azole/chlorobenzene derivative	EC	0.19	0.058	>2–5	—	0.042	—

Table 5. EC_{50} ratios of active ingredient (AI)/formulation, classified according to the type of formulation: water fleas

Type of formulation	Ratio of $EC_{50\ AI}/EC_{50\ formulation}$						
	>100	50–100	10–50	5–10	>2–5	≤2	Total
AS (aqueous solution)	—	—	—	—	1	—	1
DS (powder for dry seed treatment)	—	—	—	1	—	1	2
EC (emulsifiable concentrate)	—	—	2	7	5	16	30
EW (emulsion, oil in water)	—	—	—	1	—	1	2
OF (oil misc. flowable concentr.)	—	—	—	—	—	1	1
SC (suspension concentrate)	—	—	—	—	2	7	9
SL (soluble concentrate)	1	—	—	—	—	3	4
VL (vapor releasing liquid)	—	—	—	—	—	2	2
WP (wettable powder)	—	—	—	1	1	11	13
WG (water dispersible granules)	—	—	—	—	2	3	5
Total	1	—	2	10	11	45	69

the effect level. The third formulation, which exhibited an increased toxicity by more than 10-fold, had a moderate acute toxicity (LC_{50} = 30 mg/L), and was 12 times more toxic than the technical grade compound.

Discussion

Previous reviews of technical vs formulation toxicity showed that approximately one-third of the considered formulated products were more toxic than the technical grade material (Murty 1986; Mayer and Ellersieck 1986). This result would appear to give a basis to require toxicity testing for formulated as well as technical grade material. However, these compilations included published data from different sources which may have used different test mediums, test methods, and test species/strains. Water quality parameters (temperature, hardness, pH, suspended solids) are known to affect the toxicity of organic compounds (Fry 1971; Rand and Petrocelli 1985; Doe *et al.* 1988; Fisher 1991). If the active ingredient or an adjuvant causes a shift in the pH of the low volume test medium by ionization, the observed effects may not be representative of the formulation toxicity in the field. The above cited data compilation do, therefore, not necessarily or unequivocally reflect the real differences in the toxicity between AI and formulations. To overcome this problem, recent results from highly standardized tests following OECD (Organization for Economic Cooperation and Development), EEC (European Economic Community), or EPA (Environmental Protection Agency) / ASTM (American Society for Testing of Materials) guidelines have been compiled by nine manufacturers of agricultural chemicals. Due to the standardized quality of these tests, differences in results are assumed to be attributed to intrinsic differences in the formulations and not to methods or test system performance.

In the present paper, a total of 265 pesticides consisting of 75 insecticides, 99 herbicides, 69 fungicides, and 22 not specified pesticides were evaluated. The calculated AI/formulation toxicity ratios showed that before testing of formulated products is considered, a preliminary hazard evaluation could be performed based solely on the toxicity of the technical grade compound. The degree of extrapolation reliability is related to the organism and the type of formulation. The highest AI/formulation toxicity ratios found were 43, 248, and 63 for green algae, water fleas, and fish, respectively. A higher AI/formulation toxicity ratio than 10 was recorded in seven of 263 cases (3%) with one of 49 (2%), three of 69 (4%), and three of 145 (2%) for green algae, water fleas, and fish, respectively. Six of these higher ratios were found for EC formulations (emulsifiable concentrates). The increase in toxicity of EC formulations may be due to toxicity of an adjuvant and/or enhanced uptake of the AI due to increased availability. Beusen

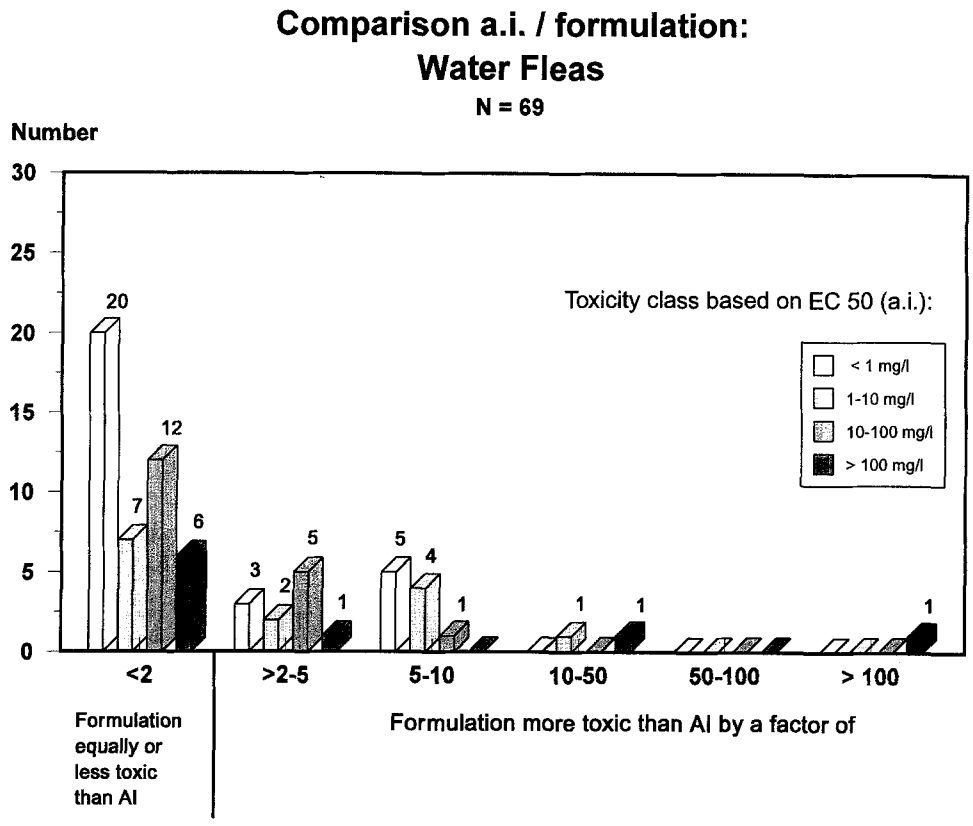

Fig. 2. Ratios of the short-term toxicity of pesticidal formulations vs the active ingredients to water fleas, ranked into different toxicity classes of the active ingredient (AI).

Table 6. Toxicity of selected agrochemicals to fish (in mg/L). The toxicity of formulations is based on the active ingredient (AI) content. For formulations which contained more than a single AI, the effect of the combined AIs was assumed to be additive and was calculated from Finney's harmonic-mean formula. The full names of formulation type acronyms are outlined in Table 7

Compound	Technical LC_{50}	Type of formulation	Formulation LC_{50}	Factor AI/ formulation
	(A) Acaricides/Insecticides			
Organotin compound	0.0040	WP	0.0021	≤2
Pyrethroid	89×10^{-6}	EC	65×10^{-6}	≤2
Pyrethroid	33×10^{-6}	EC	0.0294	≤2
Carbamate	12.80	WG	10.10	≤2
Carbamate	12.80	SL	5.80	>2–5
Carbamate	12.80	EC	6.40	≤2
Carbamate	12.80	EW	166.0	≤2
Carbamate	61.80	WG	50.70	≤2
Carbamate	61.80	SL	6.80	5–10
Carbamate	61.80	EW	8.01	5–10
Carbamate	1.0	SC	1.0	≤2
Carbamate	0.89	SC	1.69	≤2
Organophosphorous	1.50	EC	1.40	≤2
Organophosphorous	1.50	EC	1.02	≤2
Organophosphorous	0.57	EC	1.25	≤2
Organophosphorous	0.57	EC	1.05	≤2
Organophosphorous	9.10	SL	4.55	≤2
Organophosphorous	30.0	SL	37.90	≤2
Organophosphorous	0.87	EC	1.15	≤2
Organophosphorous	2.70	EC	2.15	≤2
Organophosphorous	2.70	WP	2.86	≤2
Organophosphorous	6.90	WP	8.99	≤2
Carbamate	4.70	FS	5.05	≤2
Carbamate	3.80	FS	7.50	≤2
Organophosphorous	17.0	EC	8.25	>2–5
Organophosphorous	17.0	EC	9.0	≤2

Table 6. *Continued*

Compound	Technical LC_{50}	Type of formulation	Formulation LC_{50}	Factor AI/ formulation
Organophosphorous	17.0	EC	7.25	>2–5
Organophosphorous	17.0	EC	12.24	≤2
Organophosphorous	447.30	EC	7.05	50–100
Organophosphorous	447.30	EC	32.60	10–50
Organophosphorous	40.0	SL	77.30	≤2
Carbamate	13.60	SP	24.50	≤2
Carbamate	12.40	EC	3.22	>2–5
Carbamate	12.40	SP	13.60	≤2
Pyrethroid/organoph.	0.01	SL	0.009	≤2
Organoph./organoph.	2.08	EC	2.6	≤2
Organoph./thiurame derivative	2.71	DS	2.3	≤2
Organoph./thiurame derivative	1.18	DS	0.97	≤2
Organoph./organoph.	1.57	EC	0.88	≤2
Pyrethroid/organoph.	0.15	SL	0.025	5–10
Organophosphorous	30.0	EC	31.0	≤2
Organophosphorous	0.70	EC	0.70	≤2
Triazapentadiene	0.74	EC	0.35	>2–5
Triazapentadiene	0.45	EC	3.14	≤2
Triazapentadiene	1.17	EC	0.29	>2–5
Organophosphorous	0.14	EC	0.17	≤2
	(B) Herbicides			
Urea	5.60	WP	66.40	≤2
Triazinone	326.0	WG	100.80	>2–5
Triazinone	443.0	WG	187.60	>2–5
Triazinone	64.0	WG	44.80	≤2
Triazinone	151.50	WG	59.50	>2–5
Urea	15.90	WP	14.0	≤2
Urea	578.0	WP	247.5	>2–5
Phenoxy carboxylic acid/phenoxy carboxylic acid	4.29	SL	166.0	≤2
Triazole/urea	15.7	—	88.0	≤2
Phenoxy carboxylic acid/trichlorophenoxy acid	141.1	EC	446.0	≤2
Urea/aminophosphorous phenoxy carboxylic acid	25.9	WG	70.7	≤2
Urea/aminophosphorous phenoxy carboxylic acid	21.8	WG	57.4	≤2
Azole/uracile deriv./phenoxy carboxylic acid/urea	23.8	WP	59.0	≤2
Phenoxy carboxylic acid/trichlorophenoxy acid	1.63	EC	2.7	≤2
Triazinone/biscarbamate/benzofurane deriv.	40.1	WP	56.6	≤2
Azole/urea	13.3	—	18.2	≤2
Azole/urea/phenoxy carboxylic acid	597.5	SP	800.0	≤2
Urea/phenoxy carboxylic acid	69.1	WP	87.0	≤2
Azole/urea/phenoxy carboxylic acid	665.7	SP	698.0	≤2
Urea/phenoxy carboxylic acid	41.6	WP	39.0	≤2
Triazinone/biscarbamate	27.1	WG	14.1	≤2
Trichlorophenoxy acid/phenoxy carboxylic acid	4.9	SL	1.6	>2–5
Dinitroaniline	0.2	SC	1.0	≤2
Dinitroaniline	2.75	SC	1.0	>2–5
Urea	60.0	WP	50.0	≤2
Urea	74.0	WP	68.0	≤2
Urea	3.2	EC	3.4	≤2
Phenoxy propionic acid	0.20	EC	0.53	≤2
Phenoxy propionic acid	0.40	EC	0.95	≤2
Phenoxy propionic acid	0.30	EC	0.26	≤2
Phenoxy propionic acid	0.50	EC	0.67	≤2
Phenoxy propionic acid	0.58	EW	0.33	≤2
Phenoxy propionic acid	0.57	EW	0.17	>2–5
Biscarbamate	1.41	OF	6.92	≤2
Biscarbamates	8.97	EC	7.8	≤2
Biscarbamates/benzofurane	16.7	EC	19.2	≤2
Biscarbamates	8.98	WP	32.45	≤2
Biscarbamate/benzofurane/triazinone	21.8	WP	43.25	≤2
Benzofurane	10.92	EC	32.05	≤2
Cyclohexandione	30.0	EC	2.53	10–50
Cyclohexandione	5.40	EC	1.30	>2–5
Cyclohexandione	>2600.0	WP	210.0	—
Phenoxyquinoxaline	0.72	EC	0.76	≤2

Table 6. Toxicity of selected agrochemicals to fish (in mg/L). The toxicity of formulations is based on the active ingredient (AI) content. For formulations which contained more than a single AI, the effect of the combined AIs was assumed to be additive and was calculated from Finney's harmonic-mean formula. The full names of formulation type acronyms are outlined in Table 7 (*continued*)

Compound	Technical LC_{50}	Type of formulation	Formulation LC_{50}	Factor AI/ formulation
Phenoxyquinoxaline	0.72	EC	0.29	>2–5
	(C) Fungicides			
Chinoxaline	0.24	WP	0.25	≤2
Carbamate	1.90	WG	4.69	≤2
Carbamate	133.0	WG	60.2	>2–5
Azole	23.50	WG	11.79	≤2
Azole	17.4	WG	10.3	≤2
Azole	13.6	WG	5.55	>2–5
Sulphamide	0.05	WP	0.08	≤2
Sulphamide	0.061	WP	0.085	≤2
Sulphamide	0.05	WG	0.08	≤2
Sulphamide	0.12	WP	0.12	≤2
Azole	6.40	EC	1.68	>2–5
Thiurame derivative	0.16	WP	0.22	≤2
Thiurame derivative	0.46	WP	1.28	≤2
Azole/phtalimide	0.03	WP	0.13	≤2
Azole/azole	24.9	DS	36.0	≤2
Azole	61.7	DS	78.0	≤2
Azole	38.4	FS	42.0	≤2
Azole	51.8	FS	53.7	≤2
Azole/benzotriazine/azole	58.9	FS	59.2	≤2
Azole	44.2	DS	41.0	≤2
Azole/benzotriazine/azole	80.7	FS	66.7	≤2
Azole/azole	23.9	EC	15.1	≤2
Azole/azole	23.7	EC	14.3	≤2
Azole/azole	29.2	EC	17.2	≤2
Azole/azole	29.2	EC	14.2	>2–5
Azole/morpholine der.	8.46	EC	3.53	>2–5
Azole/sulfamide	0.16	WG	0.054	>2–5
Azole/sulfamide	0.29	WG	0.099	>2–5
Azole/sulfamide	0.12	WG	0.035	>2–5
Azole/sulfamide	0.13	WG	0.035	>2–5
Azole/morpholine	22.01	EC	5.71	>2–5
Organophosphorus	6.1	EC	1.3	>2–5
Organophosphorus	1.1	EC	0.69	≤2
Carbamate	0.83	WP	0.17	>2–5
Azole	1.0	EC	1.17	≤2
Azole	19.0	EC	14.1	≤2
Azole	18.9	EC	8.84	≤2
Azole/chlorobenzene derivative	0.13	EC	0.075	≤2
	(D) Others, Not Specified			
Azole	37.0	WG	140.5	≤2
Azole	35.70	WG	39.55	≤2
Organophosphorus	2.60	WP	1.84	≤2
Organophosphorus	2.70	WP	1.90	≤2
Azole	4.50	EC	1.40	>2–5
Urea	20.0	WP	9.20	>2–5
Urea	20.0	WG	15.30	≤2
Urea	20.0	SC	22.60	≤2
Urea	50.0	WP	42.40	≤2
Organophosphorus	13.90	EC	10.0	≤2
Nitrile	0.80	FS	0.75	≤2
Triazine	3.80	SC	3.50	≤2
Triazine	11.0	SC	11.0	≤2
Triazine	11.0	WG	11.0	≤2
Triazine	19.0	SC	28.50	≤2
Triazine	70.50	SC	71.50	≤2
Triazine	3.0	SC	3.10	≤2
Urea	43.0	WP	29.0	≤2

Table 7. EC_{50} ratios of active ingredient (AI) formulation, classified according to the type of formulation: fish

Type of formulation	Ratio of $LC_{50\ AI}/LC_{50\ formulation}$						
	>100	50–100	10–50	5–10	>2–5	≤2	Total
DS (powder for dry seed treatment)	—	—	—	—	—	5	5
EC (emulsifiable concentrate)	—	1	2	—	13	37	53
EW (emulsion, oil in water)	—	—	—	1	1	2	4
FS (flowable concentr., seed treat.)	—	—	—	—	—	7	7
OF (oil misc. flowable concentr.)	—	—	—	—	—	1	1
SC (suspension concentrate)	—	—	—	—	1	9	10
SL (soluble concentrate)	—	—	—	2	2	5	9
SP (water soluble powder)	—	—	—	—	—	4	4
WP (wettable powder)	—	—	—	—	3	24	27
WG (water dispersible granules)	—	—	—	—	9	14	23
Not specified	—	—	—	—	—	2	2
Total	—	1	2	3	29	110	145

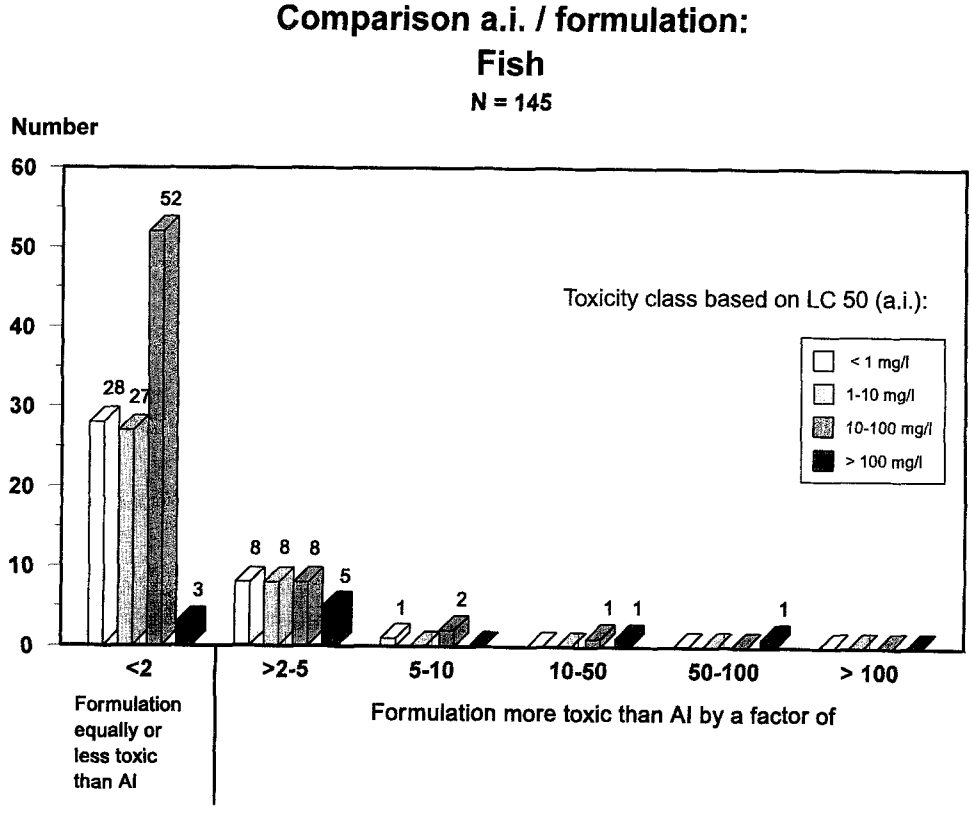

Fig. 3. Ratios of the short-term toxicity of pesticidal formulations vs the active ingredients to fish, ranked into different toxicity classes of the active ingredient (AI).

and Neven (1989) suggested that the greater toxicity of dimethoate emulsifiable concentrate to *Daphnia magna* was due to the additive toxicity of the xylene solvent in the formulation. In other cases, a facilitated transport of the AI across the gill membrane can be assumed to cause an increased formulation toxicity. Surfactants may induce micelle or mixed micelle formation in cell membranes by solubilizing membrane components which leads to increased membrane permeability (Florence 1977). However, Bradbury *et al.* (1985) found a decrease in the toxicity and uptake of the pyrethroid, fenvalerate, when formulated as emulsifiable concentrate. The authors speculated that either interactions of the emulsifiers with the gill membranes or the formation of a microemulsion may have reduced pesticide uptake. These results clearly indicate that alterations of the toxicity by formulation cannot be explained by only one factor. Nevertheless, the application of an organism- and/or formulation-type-specific safety factor which accounts for at least 95% of the ratios derived from the presented comparison of AI vs end-use products, would allow an estimation of the maximum expected increase of toxicity resulting from formulation of the AI at least 95% of the time. If the comparison of the maximum toxicity value (EC_{50}) of the AI divided by a factor of species-specific extrapolation uncertainty and the PEC or EEC indicates a high margin of safety for the considered organism, additional testing of the formulated product would not lead to new findings that predict unreasonable hazards. Additional testing of the formulated products appears justified only for those compounds which may pose a signifi-

cant hazard on the test organisms according to this preliminary hazard assessment.

For algae, a factor of 5 covers all but one case (98%) in the hazard-relevant range ($\leq$ 10 mg AI/L). For water fleas, with the factor of 10 on the AI, we would have failed to cover the toxicity of the formulation only in one case (98%) within the hazard-relevant range. If a factor of 5 is applied to the toxicity of the AI on fish, all but one case (99%) in the hazard-relevant range of toxicity ($LC_{50} \leq$ 10 mg/L) would have been covered.

In summary, the results suggest that toxicity tests conducted with the technical material will provide adequate information for identifying formulations which may pose a risk to aquatic organisms. These formulations can then be further examined. A preliminary hazard assessment performed by using the toxicity of the technical grade material divided by a factor of extrapolation uncertainty would allow a hazard assessment for the formulation with a high degree of reliability. Based on the presented data, this factor could be as low as 5 for green algae, 10 for water flea, and 5 for fish species without an unreasonable chance of substantially increased, environmentally relevant formulation toxicity. In fact, formulation toxicity may be far less relevant for the aquatic environment, since formulated pesticides are not completely transported to a body of water, except by spray drift or deliberate application to water bodies for weed or insect control. Adjuvants and active ingredients usually differ regarding their adsorptive properties and biotic/abiotic degradation rates, which cause them to separate before they reach an aquatic environment. The proposed preliminary risk assessment would, therefore, be of required reliability and environmental relevance at concurrently saving considerable numbers of test animals and economic resources.

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References

Anonymous (1988). Position paper on toxicology of crop protection products in combination. Groupement International des Associations Nationales des Fabricants de Pesticides (GIFAP). Brussels, Belgium

Bradbury SP, Coats JR, McKim JM (1985). Differential toxicity and uptake of two fenvalerate formulations in fathead minnows *(Pimephales promelas)*. Environ Toxic Chem 4:533–541

Beusen JM, Neven B (1989). Toxicity of dimethoate to *Daphnia magna* and freshwater fish. Bulletin Environ Contam Toxicol 23:126–133

Doe KG, Ernst WR, Parker WR, Julien GJR, Hennigar PA (1988). Influence of pH on the acute lethality of Fenitrothion, 2,4-D, and aminocarb and some pH-altered sublethal effects of aminocarb on rainbow trout *(Salmo gairdneri)*. Can J Fisheries Aquat Sci 45: 287–293

EPA (U.S. Environmental Protection Agency) (1982). Hazard Evaluation Division, Standard Evaluation Procedure—Acute toxicity for freshwater fish. U.S. Department of Commerce, National Technical Information Service, Springfield, IL

———(1985). Hazard Evaluation Division, Standard Evaluation Procedure—Acute toxicity for freshwater invertebrates. U.S. Department of Commerce, National Technical Information Service, Springfield, IL

———(1986). Hazard Evaluation Division, Standard Evaluation Procedure—Non-target plants: Growth and reproduction of aquatic plants—tiers 1 and 2. U.S. Department of Commerce, National Technical Information Service, Springfield, IL

Fisher SW (1991). Changes in the toxicty of three pesticides as a function of environmental pH and temperature. Bull Environ Contam Toxicol 46:197–202

Florence AT (1977). Biological implications of micelle formation. In: Mittal KL (ed) Micellization, solubilization, and microemulsions. Proceedings of the international symposium, 7th Northeast Regional Meeting, Am Chem Soc, Albany, NY, Aug 1976. Plenum, NY

Folmar LC, HO Sanders and AM Julin (1979). Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. Arch Environ Contam Toxicol 8:269–278

Fry FE (1971). The effects of environmental factors on the physiology of fish. In: WS Hoar and DJ Randall (ed) Fish physiology, Vol VI. Academic Press, NY

Mayer FL, Ellersieck MR (1986) Manual of acute toxicity: Interpretation and data base for 410 chemicals and 66 species of freshwater animals. U.S. Fish & Wildlife Service Resource Publication 160. U.S. Department of the Interior, Washington, DC, 579 pp

Murty AS (1986). Toxicity of pesticides to fish, Vol II. CRC Press, Boca Raton, FL, 143 pp

Rand GM, Petrocelli SR (1985). Fundamentals of aquatic toxicology. Hemisphere Publishing Corp, NY, 666 pp

OECD (Organization for Economic Cooperation and Development) (1984a). OECD guidelines for testing of chemicals: Alga, growth inhibition test, Guideline 210. OECD Publications Service, Paris

———(1984b). OECD guidelines for testing of chemicals: Daphnia sp., Acute immobilization test and reproduction test, Guideline 202. OECD Publications Service, Paris

———(1984c). OECD guidelines for testing of chemicals: Fish, acute toxicity test, Guideline 203. OECD Publications Service, Paris

Solon JM, Lincer JL, McNair JH III (1969). The effect of sublethal concentrations of LAS on the acute toxicity of various insecticides to the fathead minnow (*Pimophales promelas* Rafinesque). Water Res 3:767–775

WHO World Health Organization (1986). The WHO recommended classification of pesticides by hazard and guidelines to classification 1986–87. WHO-VBC/86.1, p 5