The Short-Term Toxicity of Some Feed Additives to Different Freshwater Organisms

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Feed additives are compounds which are added to the feed of different live-stock animals for growth promotion and improvement of feed conversion. These substances are excreted in faeces and/or urine partly unchanged and partly as metabolites. It is clear that this may cause contamination of the environment, including water. The excrements are also used as fertilizers, so that crops cultured on fertilized field may contain residues of these substances.

Because of the lack of data about the toxicity of these feed additives to water organisms, the present study was initiated to obtain information on the short-term toxicity of these compounds to fresh water organisms.

Materials and methods

The following is a list of the chemicals tested, along with their source of supply and purity. In some cases this purity was not known.

Amprolium : 1-[(4-amino-2-propyl-5 pyrimidinyl)-methyl]-2 picolinium-chloride hydrochloride
Merck Sharp and Dohme

Buquinolate : Ethyl-4-hydroxy-6,7-diisobutoxy-3-quinoline-carboxylate
The Norwich Pharmacal Company; purity $\geq$ 90%

Carbadox : 3-2(quinoxalinyl methylene) carbazic acid, methylester, N,N'-dioxide
Pfizer Inc.; purity 96%

Clopidol (meticlorpindol) : 3,5-dichloro-2,6-dimethyl-4 pyridinol
Dow Chemical Company

Decoquinate : ethyl-6-decyloxy-7-ethoxy-4 hydroxy quinoline-3-carboxylate
Rhone-Poulenc; purity > 99%

Ethopabate : 4-acetamido-2-ethoxybenzoic acid methylester
Merck Sharp and Dohme

Furazolidone : 3-(5-nitrofurfurylideneamino)-2-oxazolidone
Norwich Pharmacal Company

Grofas® : quinoxaline 1,4-di-N-oxide
I.C.I.; purity > 95%

Pyrimethamine : 2,4-diamino-5-(p-chlorophenyl)-6-ethyl-pyrimidine
Merck Sharp and Dohme

Bulletin of Environmental Contamination & Toxicology,
Vol. 15, No. 6 © 1976 by Springer-Verlag New York Inc.

Robenidine : 1,3-bis(p-chlorobenzylidene amino) -guadinine hydrochloride
American Cyanamid; purity > 96%

Stenorol : dl-trans-7-bromo-6-chloro-3-(-3-(3-hydroxy-2-piperidyl)acetonyl)-4 (3H)-quinazolinone hydrobromide
Roussel Uclaf; purity ≥ 97%

Sulfaquinoxaline : N'-(2-quinoxalinyl)-sulfamilamide
Merck Sharp and Dohme

Zoalene® : 2-methyl-3,5-dinitrobenzamide
Dow Chemical Company

The short-term toxicity of these compounds was determined using Chlorella pyrenoidosa, a unicellular alga, Daphnia magna, a crustacean and two fish species Lebistes reticulatus and Salmo gairdneri (CANTON et al., 1975). After a single administration of different amounts of each of the test compounds to the water, the organisms were placed into the medium for 2 or 4 days and a LC_{50} or EC_{50} was determined. A 4 day exposure period was used only if no LC_{50} or EC_{50} values could be estimated after 2 days. It was impossible to determine the exact concentrations of each compound in the water during the tests due to the lack of suitable analytical methods. Table 1 outlines the experimental conditions.

TABLE 1
Experimental conditions

Species	Age	Number of organisms per group	Test vol. per group in litres	Temp in °C ± 1	Medium
Chlorella pyrenoidosa	taken from a culture in log-phase	ca. 10^7	0.4	24	according to WANKA (1965)
Daphnia magna	< 1 day	20	1	20	according to FREEMAN and FOWLER (1953)
Lebistes reticulatus	ca.3 weeks	10	1	24	according to ALABASTER and ABRAM (1965)
Salmo gairdneri	ca.3 months	10	10	15	Tap-water

During the experiment the test solutions were not aerated except in the algae test (test procedure according to KOCH, 1953).

TABLE 2

EC_{50} and LC_{50} values (in mg/l) of some feed additives to 4 freshwater organisms after 2 days

	Chlorella	Daphnia		Lebistes	Salmo
	EC_{50} a)	EC_{50} b)	LC_{50}	LC_{50}	LC_{50}
Amprolium	160	230 (197-269)	610 (554-671)	270 (210-340)	1550 (670-3600)
Ethopabate	no EC_{50}; up to 30% growth inhibition after 2 and 4 days	170 (157-184)	200 (179-224)	no LC_{50}; EC_{50} c): 105 (90-125)	23 (17-32)
Furazolidone	1.3	- d)	- d)	no LC_{50} 2 days LC_{50} 4 days:25	- d)
Pyrimethamine	20	4.8 (4.1-5.6)	5.8 (5.0-6.7)	7.5	5.9 (5.0-7.0)
Robenidine	0.56	0.056 (0.049-0.064)	0.075 (0.066-0.086)	0.2	0.075
Stenorol	46	-	0.018 (0.015-0.021)	1.6 (1.3-1.9)	2.9 (2.1-3.8)
Zoalene	120	155 (146-164)	165 (157-173)	- e)	- e)

Between brackets the 95% confidence limits are given

a) E = growth inhibition
b) E = mortality + immobilization
c) E = immobilization

- : In 2 days no effects could be noticed within the solubility area
d) tested up to 30 mg/l
e) tested up to 200 mg/l

TABLE 3

Effects of some feed additives on 4 freshwater organisms when added to the medium up-to the highest soluble concentration

	Tested max. concentration in mg/l	Testorganisms			
		Chlorella	Daphn.	Leb.	Salmo
Buquinolate	ca. 0.75	-	-	-	-
Carbadox	ca.30	ca. 25% growth stimulation after 2 and 4 days	-	-	-
Clopidol	ca.10	-	-	-	-
Decoquinate	ca.10	-	-	-	-
Grofas	ca.25	ca. 25% growth inhibition after 2 days	-	-	-
Sulfaquinoxaline	ca. 7.5	ca. 25% growth inhibition after 2 and 4 days	-	-	-

- : in 2 days no effects could be noticed within the tested concentrations

TABLE 4

Short-term toxicity classification

	Degree of short-term toxicity to water organisms	Feed additive
< 1 mg/l	very toxic	robenidine (to all tested org.) stenorol (to Daphnia)
1-10 mg/l	moderate toxic	pyrimethamine
> 10 mg/l	little toxic	amprolium ethopabate furazolidone zoalene
none, within area of water solubility	not toxic	buquinolate carbadox* clopidol decoquinate grofas* sulfaquinoxaline*

* these chemicals influence only the growth of Chlorella

Results and discussion

Table 2 summarizes the results on compounds which gave a LC_{50} and EC_{50} after a 2 or 4 day exposure. Where possible, the 95% confidence limits (LITCHFIELD AND WILCOXON, 1949) are reported. Due to a poor water solubility, it was impossible to estimate an EC_{50} or LC_{50} (2 or 4 days) for several compounds under investigation. The results of the experiments with these compounds are given in table 3.

In table 4 the test compounds are classified according to the degree of short-term toxicity to the most sensitive tested freshwater organism - the classification is based on the results, mentioned in table 2 and 3 -. This has to be preferred above the classification of NISHIUCHI (1974), who distinguishes different toxicity classes for different organisms.

Due to the high toxicity to the tested organisms, further investigations should be carried out with robenidine, stenorol and pyrimethamine. To get more information about the influence of these compounds on the ecosystem, long-term toxicity studies should be done as well as accumulation/elimination and persistence experiments.

Summary

The short-term toxicity (EC_{50} respectively LC_{50} after 2 or 4 days) of 13 feed additives was determined to 4 freshwater organisms of different trophical levels: Chlorella pyrenoidosa, Daphnia magna, Lebistes reticulatus and Salmo gairdneri.

The most toxic ($LC(EC)_{50} < 1$ mg/l) were robenidine (to all tested organisms) and stenorol (to Daphnia); moderately toxic ($1 < LC(EC)_{50} < 10$ mg/l) was pyrimethamine. Amprolium, ethopabate, furazolidone and zoalene proved to be little toxic ($LC(EC)_{50} > 10$ mg/l); whereas buquinolate, carbadox, clopidol, decoquinate, grofas and sulfaquinoxaline were under the experimental conditions not toxic for the tested organisms.

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