Toxicity of Alkyldinitrophenols to Some Aquatic Organisms

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Data on the acute toxicity of eighteen 2-alkyl-4,6-dinitrophenols to juvenile Atlantic salmon (*Salmo salar*), crayfish (*Orconectes limosus*), and lobster (*Homarus americanus*) are presented. The toxicity of six compounds was determined and correlated with their respective octanol-water partition coefficients. The resulting structure-toxicity relationship (see for example ZITKO 1975) was used to predict the toxicity of the remaining compounds.

Alkyldinitrophenols are highly toxic to salmon and lobster and their levels in the runoff from agricultural areas should be surveyed to assess the impact of the current usage patterns. Dinoseb at 4.81 mg/ℓ was detected in a stream in New Brunswick, Canada.

Alkyldinitrophenols are used as fungicides, acaricides, and herbicides. The latter use includes potato-vine killing (see for example MURPHY and GOVEN 1975). In spite of the relatively high dosage rates of some of these compounds (dinoseb, 3.6-6.8 kg/ha for potato-vine killing), little attention has been paid to their effects on the aquatic environment and to safe practices in operating the spray equipment, avoiding a direct contamination of surface waters.

The relationship between chemical structure and pesticidal activity of alkyldinitrophenols has been reviewed (BRONISZ et al. 1974; PIANKA 1967; PIANKA and EDWARDS 1968). The mammalian toxicity of dinitrocresol and dinoseb was studied in detail (see for example FROESLIE 1973, 1974; GIBSON 1973; GIBSON and RAO 1973). The metabolism of alkyldinitrophenols in mammals involves the reduction of nitro groups (ERNST 1969), hydrolysis of ester groups, oxidation of alkyl groups, and conjugation. Some of these transformations may be also induced by sunlight (BANDAL and CASIDA 1972). The persistence of alkyldinitrophenols in soil is about one month (KUTHAN and JAVORSKA 1975).

Acute toxicities of only dinitrocresol, dinoseb,

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and dinocap are given in the reviews by PIMENTEL (1971) and BATTELLE'S COLUMBUS LABORATORIES (1971). The LC50 values range from 0.13 to 9 mg/ℓ. CHRISTIE and PENNEY (1972) reported 24- and 96-h LC50, 0.18 and 0.11 mg/ℓ, respectively, of dinoseb to speckled trout (*Salvelinus fontinalis*). According to GROBA (1972), the 14-day maximum tolerable levels of dinitrocresol and 2-isopropyl-4,6-dinitrophenol to rainbow trout (*Salmo gairdneri*) are 0.10 and 0.56 mg/ℓ, respectively.

The acute toxicity of alkyldinitrophenols to marine fauna has not been reported. The toxicity of 2,4-dinitrophenol to herring (*Clupea harengus*) eggs, expressed as the 48-h LC50, is 0.92 mg/ℓ (ROSENTHAL and STELZER 1970). A similar study with rainbow trout eggs indicates that the toxic concentration of 2,4-dinitrophenol is approximately 18 mg/ℓ (DEVILLERS and CHANCONIE 1972).

EXPERIMENTAL

Alkyldinitrophenols. 2,4-dinitrophenol was obtained from Fisher Scientific Company, dinoseb from Anachemia, and the remaining tested alkyldinitrophenols from the Pesticide Kit (Chem Service, Media, Pa.). Dinosam was prepared by saponification of dinocap.

Toxicity tests with juvenile Atlantic salmon. Static tests were conducted in 4-ℓ Erlenmeyer flasks, containing 3 ℓ of gently aerated test solution, and 3 fish, average length 6.59 cm, weight 3.30 g. The flasks were kept in a water bath at 9.0°C. The test solutions were changed at 48 hours and the test was terminated at 96 hours. The time-to-median mortality was plotted against concentration of the tested compound in log-log coordinates. Lethal threshold was determined by calculating the geometric mean of the lowest concentration at which median mortality was observed, and the highest concentration at which no mortality occurred. The former concentration was not less than 50% of the latter. No mortality occurred in control tests, run under identical conditions.

Toxicity tests with lobster and crayfish. Tests with recently hatched lobster larvae were conducted with 1 larva in a glass jar with 500 mℓ of test solution, a group of 5 larvae being tested at each concentration. Temperature was maintained at 20°C, solutions were changed and the larvae were fed live *Artemia* daily. In tests with adults, 2 lobsters, each 450 g, were exposed in 6 ℓ of gently aerated solution at 6° and 10°C in fiberglas tanks, and the solutions were changed every two days. Tests with crayfish were conducted in 4-ℓ flasks containing 1 ℓ of solution and 5 small crayfish, average weight 1.0 g, or

containing 2 ℓ of solution and 5 large ones, average weight 7.2 g. The flasks were maintained at 8° and 12°C, the solutions were gently aerated and were renewed every two days. Lethal threshold was calculated as described for the salmon tests.

Analytical methods. Alkyldinitrophenols in concentrations above 1 mg/ℓ were determined spectrophotometrically at 370 nm, directly in the test solutions. In the case of lower alkyldinitrophenol concentrations, an aliquot of the test solution (25-200 mℓ) was extracted with 6 mℓ of chloroform and the concentration of alkyldinitrophenol was determined in the chloroform solution spectrophotometrically at 267 nm.

The rate of hydrolysis of binapacryl and dinocap was determined spectrophotometrically at 370 nm. The concentration of the compounds was 5 mg/ℓ, and the hydrolysis rate was measured at pH = 13.

In a water sample from Godin Brook on September 11, 1975, near Grand Falls, New Brunswick, dinoseb was determined spectrophotometrically without extraction, as described above. To confirm its identification, an aliquot was acidified and extracted with ether. The ether extract was concentrated in a rotatory evaporator, treated with diazomethane, concentrated further, and analyzed by gas chromatography-mass spectrometry (a Finnigan GC/MS system, with a 4-ft 1/4-inch column of 3% OV-1 HP Chromosorb W 80/100, operated at 150°C).

Octanol-water partition coefficients. The octanol-water partition coefficient of 2,4-dinitrophenol was obtained from the review of LEO et al. (1971); partition coefficients of alkyldinitrophenols were calculated by using aromatic substituent (HANSCH et al. 1973) and fragment constants (LEO 1975).

RESULTS AND DISCUSSION

Toxicity. The toxicity of alkyldinitrophenols to juvenile Atlantic salmon, expressed as the lethal threshold, ranges from 0.70 mg/ℓ for 2,4-dinitrophenol to 0.02 mg/ℓ for dinocap. The logarithm of the lethal threshold (log c, c in mmole/ℓ) is a linear function of the logarithm of the octanol-water partition coefficient (log P):

$$\log c = -0.309 \log P - 2.31 \qquad (1)$$

The correlation coefficient is 0.991 and this high correlation indicates that the rate of tissue penetration is the primary factor determining the relative toxicity

of alkyldinitrophenols. The tested compounds included both phenols and their esters and the data indicate that the ester group increases the transport rate of the compound to the active site but has no effect on the toxic mechanism. 2,4-dinitrophenol is a well-known uncoupler of oxidative phosphorylation. It is likely that alkyldinitrophenols act by the same mechanism. The presence of positional isomers in dinocap and dinosam (2,6-dinitro-4-octyl and 2,4-dinitro-6-octyl) and the isomeric alkyl chains (octyl = 1-methylheptyl, 1-ethylhexyl, 1-propylpentyl) may have little effect on the toxicity.

In view of the high correlation between log c and log P and the factors discussed above, equation (1) may be used to predict the lethal thresholds of related alkyldinitrophenols to juvenile salmon (Table 1).

The octanol-water partition coefficient increases with increasing chain length of the alkyl substituent R_2, and consequently, octyldinitrophenols are the most toxic pesticidal alkyldinitrophenols. The effect of substituents R_1 on the toxicity is relatively less pronounced. Carboxylic esters are more toxic than the corresponding carbonates.

The predicted toxicities of dinofenate and etinofen are less certain than those of the remaining compounds since the substituents are significantly different from those used as the basis for deriving equation (1).

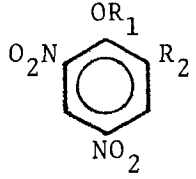
The salmon toxicity curves of the tested alkyldinitrophenols are, with the exception of that of dinosam, practically parallel (Table 2).

Dinoseb is extremely toxic to lobster larvae and has a lethal threshold of 0.0075 mg/ℓ. Adult lobsters are more resistant, those tested at 1 mg/ℓ having a median mortality time of 3 hours compared with 0.3 hour for larvae. The threshold for adults, based on 96-h exposure tests, is estimated to be 0.3 mg/ℓ or lower. Crayfish are considerably more resistant since they survived exposure to 1 mg/ℓ for 215 h and to 10 mg/ℓ for 144 h with no apparent effect.

The toxicity of additional alkyldinitrophenols to lobster larvae should be determined to establish a structure-toxicity relation analogous to equation (1).

Stability of alkyldinitrophenols in water. Concentrations of the tested compounds, measured after 48 hours, were no less than 80% of the nominal ones.

TABLE 1. Lethal thresholds of alkyldinitrophenols



Compound	R_1	R_2	Mol. wt.	log P	Lethal threshold, mg/ℓ Eq.(1)	Detd.
2,4-dinitrophenol	H	H	184	1.53	0.30	0.70
dinitrocresol	H	methyl	198	2.39	0.18	0.20
2-isopropyl-4,6-dinitrophenol	H	isopropyl	226	3.06	0.13	
dinoseb	H	1-methylpropyl	240	3.69	0.085	0.070
dinoseb acetate	acetyl	"	282	3.72	0.098	
binapacryl	3-methyl-2-butenoyl	"	322	4.52	0.063	0.060
dinofenate	2,4-dinitrophenyl-carbonyl	"	450	2.86	0.29	
dinobuton	isopropyloxycarbonyl	"	326	3.80	0.11	
dinoterb	H	*tert*-butyl	240	3.51	0.097	
dinoterb acetate	acetyl	" "	282	3.54	0.11	
dinoterbon	ethoxycarbonyl	" "	312	3.10	0.17	
dinopenton	isopropyloxycarbonyl	1-methylbutyl	340	4.34	0.076	
dinitrocyclohexylphenol	H	cyclohexyl	266	4.89	0.040	
dinosam	H	octyl[1]	296	5.43	0.030	0.030
dinocap	2-butenoyl	"	364	6.22	0.021	0.020
dinocton	methoxycarbonyl	"	354	4.62	0.065	
dinosulfon	methylthiocarbonyl	"	370	5.65	0.032	
etinofen	H	ethoxymethylenyl	242	1.77	0.34	

[1] a mixture of isomers: 1 methylheptyl, 1-ethylhexyl, 1-propylpentyl

TABLE 2. Toxicity curves log(LT50) = -A log c - B (LT50 hours, c, mmole/ℓ)

Compound		A	B
dinitrophenol		2.09	2.92
dinitrocresol		2.21	4.49
dinoseb	salmon	2.80	7.93
	lobster larvae	1.19	3.67
binapacryl		2.32	6.72
dinosam		1.41	3.69
dinocap		1.93	6.30

Accordingly, the losses of alkyldinitrophenols by evaporation, adsorption, and uptake by the test animals were practically negligible. The hydrolysis rate of the esterified alkyldinitrophenols, binapacryl and dinocap, is quite slow. At pH = 13 the respective half-lives were 24 and 40 min, and no hydrolysis was observed during 96 h in the toxicity tests. It is likely that in the environment the esters will be eventually hydrolyzed, primarily by micro-organisms.

Levels of alkyldinitrophenols in the environment. Levels of these compounds in surface waters have not been monitored systematically. During 1975, 33 streams in western New Brunswick were surveyed for other purposes. At Godin Brook, near Grand Falls, New Brunswick, the water was a conspicious yellow color about 300 m downstream from where a sprayer was being filled or rinsed. A sample was taken and the presence of dinoseb was confirmed later by gas chromatography-mass spectrometry (dinoseb methyl ether, molecular ion [M], m/e = 254, relative abundance 12%, additional characteristic ions: m/e = 225 [M - 29], 100%, m/e = 195 [M - 59], 40%). The concentration, determined spectrophotometrically, was 4.81 mg/ℓ. Dinoseb was not detectable in two other samples taken at the same time in the general area for other purposes.

Godin Brook had no fish in a 100-m section electrofished, compared with an average of 172 trout and 178 sculpin in the other streams. Also virtually no insects were present, except for chironomidae, but their number was only 14% of the number in the other streams. Some other streams in the Grand Falls area also had low fish and benthos populations, attributed mainly to chemical contamination. For example, one stream had 166 trout per 100 m upstream, and only one trout per 100 m downstream from an area where sprayers were rinsed and filled.

The evidence suggests that there is a severe local aquatic contamination from agricultural chemicals. Because of a sporadic nature of such contamination,

routine water quality monitoring is not likely to delineate the problem.

CONCLUSIONS

The toxicity of alkyldinitrophenols to juvenile Atlantic salmon increases with increasing octanol-water partition coefficient of these compounds. Some alkyldinitrophenols are extremely toxic to juvenile Atlantic salmon, larvae and adult lobsters, but comparatively nontoxic to crayfish. The unexpected low toxicity of dinoseb to crayfish is interesting also from the point of structure-activity relations and emphasizes the need of testing these in different species of aquatic fauna. No data are available on the levels of alkyldinitrophenols in fresh water, estuaries, and coastal areas. There is an indication that at least in certain streams the concentration may temporarily exceed many times the lethal threshold and have a severe impact on aquatic life. Alkyldinitrophenols are not likely to be bioaccumulated and biomagnified and their presence would not be detected by analyses of aquatic fauna. A detailed survey of their levels in surface waters, linked to their usage patterns, should be carried out.

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