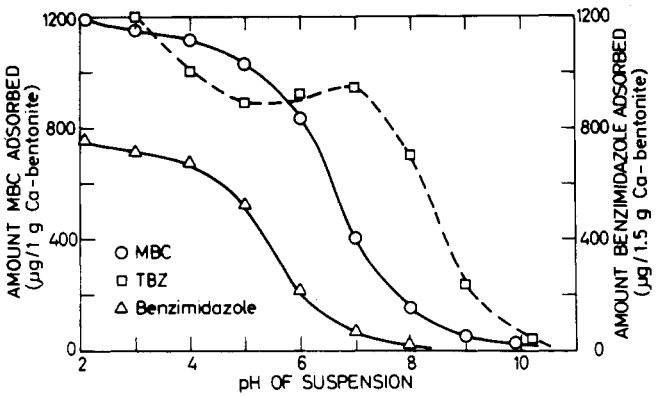

Figure 4. Adsorption of benzimidazole and MBC on Ca-bentonite as a function of the pH of the suspension (ionic strength of the suspension 0.01 *M* $CaCl_2$); details for TBZ are as in Figure 3.

Table III. Adsorption of TBZ and MBC on Al_2O_3 and MgO in Aqueous Suspensions

Fungicide	Adsorbent, mg/150 ml	Amount of fungicide added, μg	Amount of fungicide adsorbed, μg	pH of suspension[a]
TBZ	Al_2O_3, 100	247	0	3.0
TBZ	Al_2O_3, 100	247	0	8.2
TBZ	Al_2O_3, 100	247	0	10.8
MBC	Al_2O_3, 100	220	0	3.0
MBC	Al_2O_3, 100	220	0	8.0
TBZ	MgO, 200	245	242	10.6
TBZ	MgO, 200	245	221	11.1
TBZ	MgO, 200	245	162	12.2
MBC	MgO, 100	156	136	10.6
MBC	MgO, 100	156	123	11.2

[a] The pH of the suspension was changed by the addition of either HCl or NaOH.

other than coulombic ones are operating in the adsorption of the benzimidazole derivatives, and to understand the nature of these forces, further studies are required.

Adsorption of TBZ and MBC on MgO and the effect of pH on this process are shown in Table III. The pH was changed by using either NaOH or HCl. The two fungicides were not adsorbed on alumina oxide. The adsorption of TBZ and MBC on MgO may suggest another specific site of adsorption on montmorillonite clays. It has been shown (Barshad, 1960) that decreasing the pH of a montmorillonite suspension increases the amount of adsorbed magnesium on the clay surfaces.

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Dinitramine: Residues in and Toxicity to Freshwater Fish

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Dinitramine, a herbicide registered for use on cotton and soybeans, is toxic to fish and accumulates in fish tissue after brief exposures to low concentrations. The 96-hr LC_{50} values for several species of coldwater and warmwater fish ranged from 0.590 to 1.52 mg/l. at 12°. Muscle, plasma, and gallbladder bile of carp (*Cyprinus carpio*) and channel catfish (*Ictalurus punctatus*) were examined for uptake and retention of dinitramine after a 12-hr exposure to a 1 mg/l. concentration. Accumulation in tissues analyzed exceeded the exposure concentration in all instances, and the herbicide was not completely eliminated after the fish had been held in fresh water for 24 hr.

Dinitramine (N^3,N^3-diethyl-2,4-dinitro-6-trifluoromethyl-*m*-phenylenediamine) is a preplant, incorporated herbicide (U.S. Borax and Chemical Corporation) registered for use on cotton and soybeans for control of many annual grasses and broadleaf weeds (Berg, 1974). Other names include cobex, USB-3584,and Cobeko.

Newsom and Mitchell (1972) reported on the assay of dinitramine residues in soil and plant tissue. Widespread use of a terrestrial herbicide may lead to the introduction of the compound into surface waters because of runoff, direct application over water, or possible misuse. Therefore, we determined the uptake and retention of dinitramine in carp (*Cyprinus carpio*) and channel catfish (*Ictalurus punctatus*) and the toxicity to several species of fish.

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Table I. Toxicity of Dinitramine (99+%) to Nine Species of Freshwater Fish in Soft Water[a] at 12°

Species	Av wt, g	LC_{50} and 95% confidence interval (mg/l.) at 24 hr	LC_{50} and 95% confidence interval (mg/l.) at 96 hr
Coho salmon (*Oncorhynchus kisutch*)	0.8	>1.51	0.600
			[b]
Steelhead trout (*Salmo gairdneri*)	0.3	1.20	0.590
		1.06–1.36	0.510–0.682
Brown trout (*Salvelinus trutta*)	0.7	1.27	0.590
		1.05–1.53	0.510–0.682
Lake trout (*Salvelinus namaycush*)	0.6	1.15	0.920
		0.986–1.34	0.776–1.09
Carp (*Cyprinus carpio*)	1.0	>2.00	1.18
			1.02–1.36
Fathead minnow (*Pimephales promelas*)	0.8	2.63	1.44
		[b]	1.07–1.93
Channel catfish (*Ictalurus punctatus*)	0.8	2.99	1.37
		2.20–4.06	1.04–1.81
Bluegill (*Lepomis macrochirus*)	1.4	2.88	1.52
		1.96–4.24	1.14–2.02
Yellow perch (*Perca flavescens*)	0.8	1.00	1.00
		0.870–1.15	0.870–1.15

[a] pH 7.2–7.6; total hardness = 40–44 mg/l. as $CaCO_3$. [b] Insufficient data for computation of confidence intervals.

EXPERIMENTAL SECTION

Apparatus and Reagents. A Tracor MT220 gas chromatograph equipped with a ^{63}Ni electron capture detector and a 1-mV recorder was operated at an electrometer sensitivity of 1.6×10^{-9} A full scale deflection. A 180 cm × 4 mm i.d. glass column packed with 3% OV-101 on Chromosorb W/HP (80–100 mesh) was used for the residue determinations. Residue determinations were based only on retention time because this was a controlled study utilizing fish with no background levels of dinitramine. Operation conditions were: column temperature, 180°; inlet temperature, 217°; detector temperature, 360°; nitrogen carrier flow, 65 cm^3/min; and nitrogen purge flow, 25 cm^3/min.

Dinitramine (99+%) was supplied by U.S. Borax Research Corporation, Anaheim, Calif. We used pesticide grade solvents for all residue work and commercial grade acetone to prepare stock solutions for toxicity tests. Florisil, heated at 130° in an oven for more than 16 hr, was used for muscle cleanup.

Toxicity Testing. Toxicity tests were done in facilities similar to those described by Lennon and Walker (1964). Stock solutions of dinitramine were prepared just prior to the tests, and portions were delivered by pipet to give the desired concentrations in test chambers. Test water was prepared by adding reagent grade chemicals to deionized water (Marking, 1969). Fish were obtained from Federal hatcheries and were maintained according to the procedures suggested by Hunn et al. (1968). We analyzed the data according to the method of Litchfield and Wilcoxon (1949) to determine LC_{50} values (concentration producing 50% mortality) and 95% confidence intervals for dinitramine.

Exposure of Fish and Sample Collection for Residue Analysis. Carp and channel catfish that had been fasted for at least 4 hr were exposed to 1.0 mg/l. of dinitramine in aerated well water (pH 7.7–7.9; total hardness = 220–230 mg/l. as $CaCO_3$) at 12° in polyethylene tanks. After 12 hr of exposure, the fish were transferred to screened cages in flowing well water at 12°. Samples of blood, gallbladder bile, and muscle were collected after selected time intervals (Hunn and Allen, 1975).

Table II. Dinitramine Residues in Fish Muscle, Plasma, and Gallbladder Bile after Exposure of Fish to 1 mg/l. for 12 hr and Withdrawal in Fresh Water

Species	Type of sample	hr of withdrawal	Concn[a] Mean	Concn[a] Range
Carp (200–500 g)	Muscle	0	3.96	1.50–6.73 (5)[b]
		4	4.00	2.45–5.90 (5)
		8	2.96	2.00–4.37 (5)
		12	5.15	4.19–6.93 (3)
		24	4.98	4.33–5.71 (5)
		Control	N.D.[a]	(5)
	Plasma	0	24.4	22.4–25.1 (5)
		4	11.4	7.3–14.7 (4)
		8	10.3	7.0–13.7 (5)
		12	9.3	(1)
		24	5.1	4.3–12.0 (5)
		Control	N.D.	(5)
	Bile	0	22.7	20.1–27.0 (5)
		4	18.9	15.3–22.7 (5)
		8	22.5	16.6–24.9 (4)
		12	31.0	30.0–31.9 (3)
		24	13.8	12.0–15.7 (4)
		Control	N.D.	(4)
Channel catfish (100–200 g)	Muscle	0	46.1	29.9–62.3 (2)
		24	20.0	19.0–21.0 (2)
		Control	N.D.	(1)
	Plasma	0	58.5	54.0–63.0 (2)
		24	19.6	10.8–28.4 (2)
		Control	N.D.	(1)
	Bile	0	13.4	(1)
		24	14.5	(1)
		Control	N.D.	(1)

[a] Concentrations in milligrams per kilogram for muscle and milligrams per liter for plasma and bile; N.D. = not detectable (<0.01 mg/l.). [b] Number of samples in parentheses.

Blood samples collected with heparinized syringes were centrifuged at 2000 rpm for 20 min to obtain the plasma. Bile samples were also collected with syringes, and muscle samples were collected by filleting. The fish were not fed during the experiment.

Extraction and Cleanup. Fish muscle was frozen, cubed, and blended with Dry Ice (Benville and Tindle, 1970). Samples were extracted with 180 ml of petroleum ether–ethyl ether (85:15) in a column described by Hesselberg and Johnson (1972). Cleanup was done on a glass chromatographic column (17 cm × 1 cm i.d.) packed with 7 g of preheated Florisil. A 1 to 1.5 g equivalent of sample extract quantitatively transferred to the column was eluted with 60 ml of petroleum ether–ethyl ether (85:15) solution. Recoveries of 70.4 and 73.1% were obtained for muscle samples fortified with 2.5 and 5.0 μg/g, respectively. The data reported are not corrected for recovery.

A measured volume (≤1.0 ml) of blood plasma or bile was placed in a centrifuge tube capped with a Teflon-lined screw cap. A volume of petroleum ether–ethyl ether (85:15) equal to twice the volume of the sample was added to the tube. The tube was tightly capped, and the sample was shaken on a mechanical mixer for 60 sec. If an emulsion occurred, the centrifuge tube was placed in a sonic cleaner for 2 min and then centrifuged at 1800 rpm for 15 min to separate the phases. Dinitramine partitioned into the organic phase and was injected on the GC with no further cleanup. Recoveries of 97.3 and 105% were obtained for plasma samples fortified with 5.0 and 10.0 μg/ml, respectively. Due to lack of sample and similarity to plasma analysis, recoveries were not run on bile.

RESULTS AND DISCUSSION

Dinitramine was toxic to coldwater and warmwater fishes at concentrations of 0.590–1.52 mg/l. in 96-hr static toxicity tests (Table I). Other terrestrial herbicides with similar uses have toxicities less and greater than dinitramine. Walker (1964) reported a 96-hr toxicity value for atrazine against sunfish species of approximately 5 mg/l. (active ingredient). Mullison (1970), in his review on herbicides, reported that treflan (trifluralin) was toxic to fish at concentrations of 11–210 μg/l.

Dinitramine residues in samples of carp and channel catfish muscle taken immediately after exposure (0 hr) and after 24 hr of withdrawal exceeded the exposure concentration (Table II). The mean concentration of dinitramine residue in carp muscle, ranging from 2.96 to 5.15 μg/g, did not show evidence of elimination during the postexposure period. Residues in channel catfish muscle had declined more than 50% 24 hr after withdrawal, but the concentration was still 20 times the exposure concentration. Persistence beyond 24 hr after withdrawal was not determined. However, dinitramine residues are much more persistent in fish than are residues of TFM (3-trifluoromethyl-4-nitrophenol), quinaldine, and MS-222 (tricaine methanesulfonate) (Hunn and Allen, 1974).

Carp and channel catfish blood plasma contained high concentrations of dinitramine immediately following exposure (Table II). After 24 hr in fresh water, the concentration in plasma decreased from 24.4 to 5.1 mg/l. in carp and from 58.5 to 19.6 mg/l. in channel catfish. The high concentration of the herbicide in plasma as compared with the concentration in water after a relatively short exposure indicates that partitioning of dinitramine across the gill is favored over retention in the water. The partitioning is predictable because of the low solubility of dinitramine in water (1 mg/l.) and its high solubility in organic solvents (57% in acetone) as given in the U.S. Borax Research Technical Data Sheet. Hunn and Allen (1974) stated that solubility in lipids and low solubility in water resulted in rapid uptake of drugs across the gills of fishes.

The concentration of dinitramine in gallbladder bile of the fish after exposure was much higher than the exposure concentration (Table II). However, the magnification was not as great as reported by researchers for other compounds; magnification factors of 124 to 1061 were reported by Lech et al. (1973) for DDT, 2,4-dichlorophenoxyacetic acid, and carbaryl and by Hunn and Allen (1974) for TFM and MS-222. Dinitramine residue concentrations in carp bile are similar in magnitude to those in plasma and may result from equilibrium between plasma and bile.

This preliminary study does not answer all questions regarding what would happen to dinitramine in the aquatic environment. The lack of available information concerning the dynamics of the herbicide in the aquatic ecosystem was a major consideration for doing the study. More work needs to be done using other species and longer withdrawal periods before the dynamics will be understood. However, the data from this study do support two conclusions.

CONCLUSIONS

(1) Residue concentrations of dinitramine in fish muscle, plasma, and bile exceeded the bath exposure concentration and persisted longer than 24-hr postexposure. (2) In several instances, the toxicity of dinitramine to fish was near the limit of solubility of the herbicide in water under our experimental conditions.

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Effect of Sodium Chloride Concentration on the Nitrosation of Proline at Different pH Levels

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Acidity had a determining influence in regard to activation and inhibitory effects of sodium chloride on the rate of nitrosation of proline. In 1 *M* sodium chloride the rate of nitrosation was increased by 325% at pH 0.5 and decreased by 36 and 50% at pH 4.0 and 5.5, respectively. At pH 2.5 a slight inhibiting effect was noticed. Multiple regression analysis showed the best fitted model was of the form, ln (initial rate of nitrosation) = $a + b[\mathrm{NaCl}] + c[\mathrm{NaCl}]^2$.

The carcinogenicity of *N*-nitrosamines has been recognized for some time (Druckrey et al., 1967; Magee and Barnes, 1956). The discovery of volatile nitrosamines in several types of foods treated with nitrite has initiated investigations into the conditions which may promote their formation in food processing and also during digestion (Fan and Tannenbaum, 1973; Bills et al., 1973; Pensabene et al., 1974; Sen et al., 1974; Sander and Burkle, 1969).

It has been demonstrated that several anions exert an accelerating effect on the nitrosation of amines in acidic media (Ridd, 1961; Boyland et al., 1971; Boyland, 1971). The order of the accelerating effect was $I^- > SCN^- >$ acetate $> Br^- > Cl^-$ with the chloride ion exhibiting a very

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