Some Aspects of the Chemistry and Acute Toxicity of the Iron Ore Flotation Agent Dimethyl Ammonium Alkyl Hydroxamate and Some Related Compounds to Brook Trout

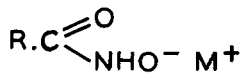
by G. L. FLETCHER* and R. F. ADDISON
Fisheries Research Board of Canada
Marine Ecology Laboratory
Bedford Institute
Dartmouth, Nova Scotia

SUMMARY

Dimethyl ammonium alkyl hydroxamate (DMAH) was lethal to brook trout (*Salvelinus fontinalis*) at concentrations as low as 4.5 mg/l. Since DMAH consisted of a number of molecular species in equilibrium, experiments were conducted with several of them in an attempt to evaluate their contribution to its toxicity. Of the compounds tested dimethylamine proved to be non-toxic, hydroxylamine was as lethal as DMAH (on a molar basis) and NaC_{10} hydroxamate was 5-10 times more toxic than hydroxylamine or DMAH. These results suggested that although hydroxylamine may have played a small part in the toxicity of DMAH, the hydroxamate group appeared to be the major lethal component.

INTRODUCTION

Flotation agents are extensively used in the mining industries for separation of ores from unwanted materials such as silica. These compounds are amphiphilic substances, such as straight chain fatty acid salts (which are widely used in North America) alkyl aryl sulphonates, fatty amines, and so on, depending on the requirements of the particular process involved (1). Recently, a number of reports have appeared in the Russian literature, describing the use of alkyl hydroxamic salts for this purpose (2 - 5). These compounds have the structure



* Present address: Marine Sciences Research Laboratory, Memorial University of Newfoundland, St. John's, Newfoundland.

Bulletin of Environmental Contamination & Toxicology, Vol. 7, No. 2/3, 1972 © Springer-Verlag New York Inc.

where R is an alkyl group, generally in the C_7-C_9 range, and M is a cation such as Na or K. They are usually prepared by reacting hydroxylamine with the appropriate fatty acid methyl ester.

One Canadian company is currently evaluating the use of a hydroxamate mixture for iron ore flotation. The dimethyl ammonium alkyl hydroxamate (DMAH) preparation is a mixture of hydroxamic acids prepared from a distillation cut of coconut oil methyl esters, partially neutralised with dimethylamine, and suspended in technical grade isopropanol to a concentration of 75%. Since some of this material may eventually be released into local rivers and lakes, we were invited to assess its toxicity to fish. So far, no information on this subject is available in the literature other than the fact that subcutaneous injections of acetohydroxamic acid are lethal to rabbits (6).

EXPERIMENTAL

Chemical Studies

1. Fatty acid composition of DMAH

A sample of the material was saponified, and the fatty acids produced by acidification of the soaps were esterified with boron trifluoride-methanol. The methyl esters were analysed by gas liquid chromatography (GLC) on a 6' x 1/8" glass column packed with 3% EGSS-X on 100-120 mesh Gas Chrom Q in a Packard 7400 instrument fitted with a flame ionisation detector (FID). Operating conditions were: column temperature 100°C; inlet, 240°C, and detector, 200°C. Carrier gas was He at 40 ml/min.

2. Amine composition of DMAH

Standard methylamine ($MeNH_2$), dimethylamine (Me_2NH) and trimethylamine (Me_3N) in aqueous solution were separated by GLC on a 6' x 1/8" stainless steel column, packed with Chromosorb 103, in an Aerograph 500D instrument fitted with FID. Conditions were column, 130°C, inlet, 210°C, and carrier gas, He at 30 ml/min. A qualitative and semi-quantitative estimate of the amine content of DMAH under various conditions of pH and air blowing was obtained by direct injection of the DMAH sample, diluted (where necessary) with isopropanol.

3. Synthesis of hydroxamates

The preparation of sodium caprohydroxamate (C_{10}) was based on the method described by Blatt (7) for potassium salts of

aromatic hydroxamic acids. Methyl caprate (Eastman Kodak) was the starting material. C and N analyses, performed on a Perkin Elmer C,H and N analyser gave the following results: C, 57% (calc. for $C_{10}H_{20}O_2NNa$, 57.4%), N, 6.0% (calc. 6.7%). Acetohydroxamic acid (C_2) was prepared by the method of Fishbein *et al* (7). C & N analyses, performed on a F and M instrument, gave C, 33.9% (calc. for $C_2H_5O_2N$, 32.0%), N, 18.7% (calc. 18.7%).

4. Estimation of hydroxamates

Hydroxamates were determined spectrophotometrically by reading the absorbance of their complex with $FeCl_3$ at 540 nm in a Unicam SP 500 (8). The NaC_{10} hydroxamate was used as standard and it and the DMAH were made up in 25% aqueous isopropanol. (The effects of varying amounts of isopropanol and dimethylamine on the method were tested by replacing water with isopropanol and a 1.5 x 10^{-3}M solution of dimethylamine, both separately and together).

Toxicity Studies

1. Fish

Brook trout (*Salvelinus fontinalis*) were obtained from Cold Spring Fish Hatchery, Nova Scotia and maintained in charcoal filtered water (16-18C) for at least two weeks prior to experimentation. A group of fingerlings was gradually adapted (7 days) to 3.5°C and held at this temperature two to four weeks before use. All fish were fed Purina Trout Chow every two to three days.

2. Bioassays

Bioassays were conducted using five fish per test. Each fish, when dead, was removed from its tank and Lt50's (lethal time for 50% of the individuals) were determined by probit analysis (9) using the IBM 360 computer at Dalhousie University, Halifax. Static bioassays were conducted using 2.5 to 130 liters of water, depending on the size of the fish. The test fish were fed every 24 hours followed, within a few fours, by a complete renewal of the test mixture. Continuous flow tests were performed using tanks containing four liters of water flowing at 600 ml/min. The toxicant was pumped into the tank at the water inlet. These fish were also fed daily. Further details of the bioassays are presented with the Results.

RESULTS AND DISCUSSION

DMAH was supplied by the Ashland Chemical Company as a "75% active" suspension in technical isopropanol. Approximate acyl radical distribution in the mixture was: 6:0*, trace; 80, 59%; 10:0, 33%, 12:0, 2%, 14:0, 3%, 16:0, 3%, 18:1, trace. Analysis of the amine content of the mixture showed that Me_2NH was the only detectable amine present.

Spectrophotometric assay of the hydroxamate content of the mixture (carried out several months after receipt of sample) showed a hydroxamate content of 50% (read against Na C_{10} hydroxamate). Since some obvious changes (such as precipitation) had taken place during storage of the sample, it seems possible that the "75% active" claim of the original sample was probably fairly accurate. (The presence of Me_2NH and isopropanol did not affect the absorption spectrum of the Fe^{+++} hydroxamate complex, provided concentration of isopropanol did not exceed about 25% by volume).

Toxicity of freshwater preparations of DMAH

The initial experiments were conducted using trout fingerlings (0.3-1.0g) in glass tanks containing 2.5 liters of freshwater (16-17C). No aeration was used and the test mixture was renewed every 24 hours. At DMAH concentrations above 5 µl/l, a white precipitate was evident and the pH was reduced below control values (pH of control, 6.6; pH of 500 µl/l DMAH, 6.0). DMAH was lethal at all concentrations tested (10-1,000 µl/l (Table 1). Unexposed control fingerlings and those tested in 100 µl/l isopropyl alcohol had no mortalities in 200 hours.

Two additional tests indicated that continuous aeration of the bioassay water did not modify the results significantly. The Lt50's for the continuously aerated tank containing 20 and 50 µl/l DMAH were 20.7 $\pm$ 2.98 (SE) hr. and 7.32 $\pm$ 0.90 hr. respectively.

Analysis of amine content of the mixture during air blowing indicated that at pH's normally encountered during bioassays (6-8) or under projected plant conditions (8.3) no change in the Me_2NH content of the sample occurred. Only at high pH's (approximately 12) could any reduction in Me_2NH content during air blowing be detected.

---

*Shorthand notation for no. of C atoms: no. of double bonds

TABLE 1

Toxicity of dimethyl ammonium alkyl hydroxamate (DMAH) to brook trout fingerlings

DMAH (µl/l)	Lt50[1] (hrs)
Static[2] (16-17°C)	
1000	0.0224 ± 0.001
500	0.138 ± 0.019
100	0.583 ± 0.040
75	1.06 ± 0.011
50	4.51 ± 0.433
35	9.17 ± 0.534
20	30.1 ± 6.23
10	133.0 ± 12.5
10	80.2 ± 7.04
Static[2] (3.5°C)	
300	0.798 ± 0.030
100	2.79 ± 0.112
50	13.8 ± 1.19
25	112.0 ± 5.54
Continuous flow[3] (16-18°C)	
150	0.252 ± 0.013
60.0	0.899 ± 0.082
15.0	15.6 ± 0.500
6.0	142.0 ± 26.8

[1]Lt50's expressed as mean ± standard error.

[2]Static tests were conducted using 2.5 liters of water.

[3]Continuous flow test conducted in 4 liters flowing at 600 ml/min.

Several investigators (9, 10, 11) have emphasized that static testing conditions can produce inaccurate results. Therefore, to validate the data from the static tests, four continuous flow experiments were carried out. No aeration was used and the water temperature was 16-18°C. The results obtained confirmed those observed in the static tests and indicated that the DMAH preparation was lethal at concentrations at least as low as 6 µl/l (4.5 mg/l) (Table 1, Figure 1).

The temperature of the rivers and lakes expected to receive the DMAH is generally lower than 16-18°C. Therefore, several static bioassays were conducted at 3.5°C to check the effects of temperature on the toxicity of DMAH. Although it was evident from the results that the lower temperature increased the time taken for the fish to die, DMAH remained lethal at the lowest concentration tested (25 µl/l) (Table 1).

One question of interest when assessing the toxicity of a compound is: can fish survive exposures which are not immediately lethal, or will death result at some later time? For example, trout can be exposed to potassium cyanide almost to the point of death and if they are transferred to clean water, they will recover and survive indefinitely (12, 13). In contrast, similar experiments exposing salmon to yellow phosphorus for a few hours resulted in mortalities which were delayed up to two weeks following exposure (14). DMAH appears to be similar to cyanide in this respect. Four experiments were performed in which trout fingerlings were exposed to 50 and 100 µl/l DMAH until they lay inverted on the bottom of the tank. When they were transferred to clean water they recovered within 20 minutes and remained normal during a 6 month observation period.

The toxicity of DMAH to yearling brook trout (70 to 120 g) was determined using static conditions. The tank contained 130 l of water maintained at 16-17°C. The water and toxicant were renewed daily and no aeration was used. The results obtained were as follows:

DMAH (µl/l)	Lt50 (hr)
100	1.34 ± 0.16 (SE)
50	6.99 ± 1.41
25	55.0 ± 7.12

A comparison of these values with those obtained for the fingerlings (Table 1, Figure 1) indicated that yearling trout were as susceptible to DMAH as were the fingerlings.

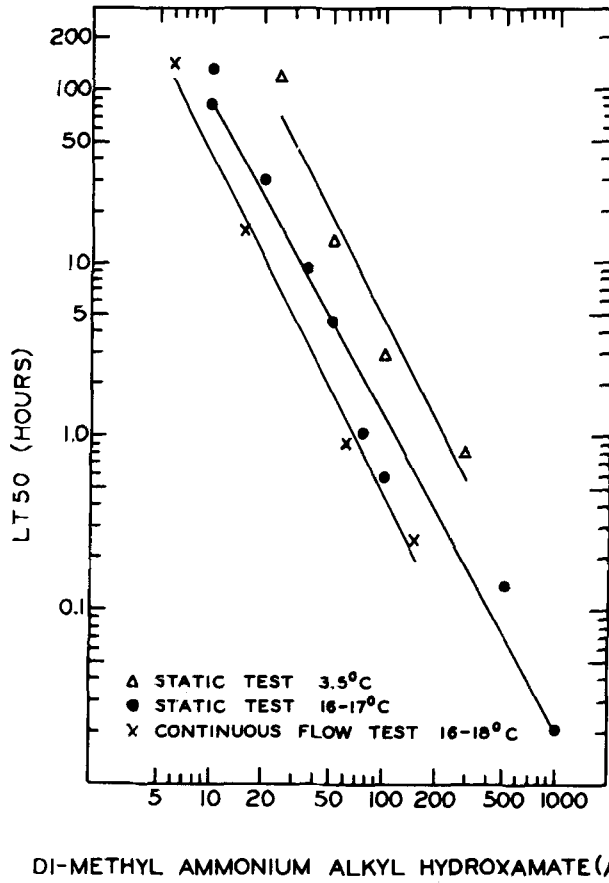


Figure 1. The acute toxicity of dimethyl ammonium alkyl hydroxamate (DMAH) to brook trout.

Static test (3.5°C)	$Y = 39{,}800X^{-1.96}$
	$r = -0.974$
Static test (16-17°C)	$Y = 5{,}240X^{-1.80}$
	$r = -0.986$
Continuous Flow test	$Y = 4{,}000X^{-1.98}$
	$r = -0.995$

A preliminary series of experiments was conducted to determine whether the toxicity of an aqueous preparation of DMAH would change as a function of time. A 15 liter aqueous solution of DMAH (50 µl/l) was prepared and the toxicity of 2.5 liter aliquots was tested over a period of three weeks. The DMAH preparation and the bioassays were held at 16-17° and no aeration was used. The results obtained were as follows:

Hours after preparation of 50 µl/l DMAH	Lt_{50} (hr)
0	4.04 ± 0.31)SE)
24	5.38 ± 0.36
72	8.20 ± 0.42
356	12.4 ± 2.70
504	13.4 ± 1.00

These results indicated that the toxicity of the DMAH preparation decreased to one half within three days. However, it did not continue to decline at this rate but slowed considerably after 356 hours. This slowing may reflect some limiting component in the DMAH stock solution.

The toxicity of buffered (pH 7.5) preparations of DMAH, methylamine, dimethylamine, trimethylamine, hydroxylamine hydrochloride and sodium hydroxamate

The experiments reported in the previous section clearly indicated that DMAH was lethal to brook trout at concentrations at least as low as 6 µl/l (4.5 mg/l). This concentration of DMAH was considerably lower than that proposed for the effluent of the iron ore company. Therefore, further studies were necessary in order to evaluate which component of the DMAH preparation was responsible for the lethal effects.

The major constituents of the DMAH preparation were believed to be dimethylamine, hydroxylamine and hydroxamic acid. Hydroxylamine was tested as hydroxylamine hydrochloride and hydroxamic acid as a sodium salt of a C_{10} hydroxamate. All of these experiments were conducted under static conditions using 8 liters of continuously aerated water (16-18°C). All other conditions were as previously described. Stock solutions of methylamine, dimethylamine, trimethylamine and hydroxylamine hydrochloride were aqueous while those of sodium hydroxamate and sodium caprate were in 70% isopropanol. Test concentrations of the compounds varied considerably in pH. Therefore, in order to allow a direct comparison of the results, all experiments were conducted in Tris-buffer (5 gm/l at pH 7.5).

Tris-buffer (Tris hydroxymethyl aminomethane, Sigma Chemical Co.) was chosen because it appears to be non-toxic and has been used in the transportation of fish (15).

The results from the toxicity experiments with DMAH in Tris-buffer differed somewhat from those obtained in the initial series of tests (compare Tables 1 and 2). Although the

TABLE 2

Toxicity of dimethyl ammonium alkyl hydroxamate (DMAH) in Tris-buffer (pH7.5) to brook trout fingerlings

DMAH (µl/l)	Lt50[1] (hrs)
200	1.24 ± 0.02
125	4.73 ± 0.38
100	13.5 ± 0.81
100	12.4 ± 1.60
50	16.0 ± 2.41
30	14.7 ± 1.43
20	25.9 ± 4.86
15	50.5 ± 7.43

[1] Lt50 values expressed as mean ± standard error

toxicities of 15, 20 and 30 µl/l were essentially identical in both series of tests (Fig. 2), buffered concentrations greater than 30 µl/l were considerably less toxic than those not buffered. The reason for the reduced toxicity of the higher concentrations of buffered DMAH is not clear. In the several months which had lapsed since the initial tests the DMAH preparation supplied to us had become considerably more acid. Thus, whether the reduction in toxicity was due to the buffered conditions or to some chemical change in the DMAH is not known.

Dimethylamine was tested at 500 mg/l and was not toxic in 106 hrs. Two related compounds were also tested: methylamine and trimethylamine. The Lt50's observed for 90 and 150 mg/l methylamine were 21.7 ± 1.22 (SE) hr and 62.2 ± 2.03 hr respectively. Trimethylamine (1,000 mg/l) was not toxic in 106 hrs.

The results from the bioassays of hydroxylamine hydrochloride and NaC_{10} hydroxamate are presented in Tables 3 and 4. Two

TABLE 3

Toxicity of hydroxylamine hydrochloride (NH_2OH HCl) in Tris-buffer (pH 7.5) to brook trout fingerlings

NH_2OH HCl (mg/l)	Lt50[1] (hr)
50.0	1.05 ± 0.04
25.0	2.04 ± 0.27
25.0	2.31 ± 0.24
15.0	4.66 ± 1.14
10.0	12.4 ± 1.71
7.5	14.1 ± 2.93
5.0	45.1 + 16.1
5.0	29.7 ± 0.94
4.0	80.4 ± 26.2
2.5	160.0 ± 11.0

[1] Lt50 values expressed as mean ± standard error.

TABLE 4

Toxicity of Na-C_{10} - Hydroxamate in Tris-buffer (pH 7.5) to brook trout fingerlings

Na-C_{10}-Hydroxamate (mg/l)	Lt50[1] (hr)
25.0	4.30 ± 0.55
12.5	3.97 ± 0.03
6.25	5.18 ± 0.37
3.13	14.0 ± 1.38
2.5	20.2 ± 2.18
1.56	43.4 ± 4.40
0.78	81.8 ± 4.19

[1] Lt50 values expressed as mean ± standard error.

additional bioassays were conducted to test the toxicity of NaC_{10} hydroxamate in the absence of Tris-buffer. The Lt_{50}'s for 1 and 2 mg/l were 84.0 ± 0.3 (SE) hr and 31.7 ± 1.74 hr respectively. These results were not significantly different from those obtained using Tris-buffer.

Three sets of control experiments were run: 5 g/l Tris-buffer (pH 7.5); 1 ml/l isopropanol in 5 g/l Tris-buffer (pH 7.5); 25 mg/l NaC_{10} caprate in 1 ml/l isopropanol plus 5 g/l Tris-buffer (pH 7.5). No mortalities were observed in any of these tests in 150 hrs.

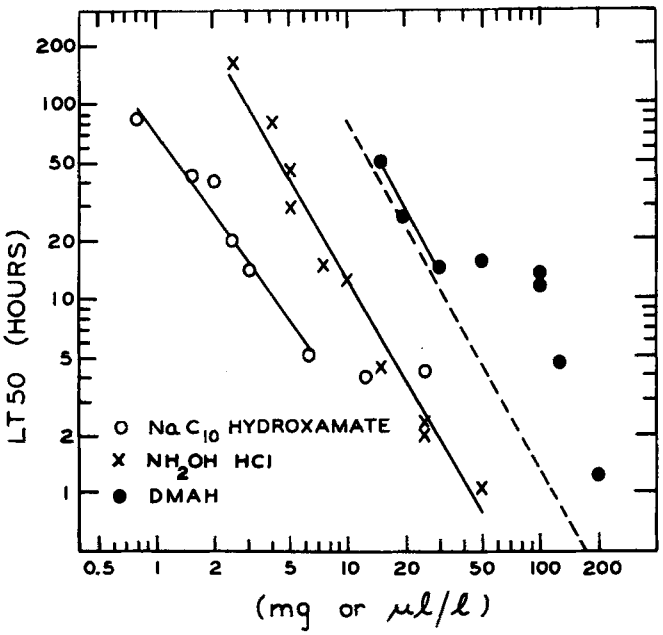


Figure 2. The acute toxicity of di-methyl ammonium alkyl hydroxamate (DMAH), hydroxylamine hydrochloride and NaC_{10} hydroxamate to brook trout.

DMAH. $Y = 5{,}520X^{-1.76}$, $r = -0.990$. Regression computed from the three lowest concentrations tested.

Hydroxylamine hydrochloride (NH_4OH HCl) $Y = 658X^{-1.74}$, $r = -0.989$

NaC_{10} Hydroxamate $Y = 72.6X^{-1.37}$, $r = 0.978$. Regression does not include 12.5 and 25.0 mg/l.

The broken line represents the regression for DMAH as presented in Figure 1.

Calculated on the basis of weight both hydroxylamine hydrochloride and NaC_{10} hydroxamate were considerably more toxic than DMAH (Figure 2). However, since the molecular weights of these compounds differ it is more appropriate to compare their toxicities when they are expressed on a molecular basis. Since most of the DMAH consisted of an 8 carbon hydroxamate, the molecular weight was approximated as 200. Comparing the compounds at an arbitrary Lt50 of 50 hours the respective concentrations required to produce this Lt50 were: DMAH, 54.8 µM/l; hydroxylamine, 63.1 µM/l; NaC_{10} hydroxamate, 6.27 µM/l. Expressed in this fashion, hydroxylamine appears to be no more toxic than DMAH. Since free hydroxylamine was unlikely to be present in the DMAH preparation (as hydroxamates dissociate to carboxylates and hydroxylamine only at extremes of pH), it is unlikely that it contributed significantly to the toxicity of the DMAH. The fact that sodium hydroxamate was considerably more toxic than hydroxylamine emphasizes this conclusion and indicates that hydroxamate is the major molecule responsible for the toxicity of the DMAH preparation.

References

1. Frommer, D. W. J. Amer. Oil Chem. Soc. 47, 189, (1970).

2. Strel'tsin, V. G., Khobotova, N. P. Chem. Abst. 70, 117275e.

3. Gorlorskii, S. I., Khobotova, N. P., Shchukina, N. E. Chem. Abst. 69, 98604x, (1968).

4. Gorlorskii, S. I., Khobotova, N. P., Shchukina, N. E. Chem. Abst. 66, 4983g, (1967).

5. Gorlorskii, S. I., Khobotova, N. P., Shchukina, N. E. Chem. Abst. 65, 363b, (1966).

6. Acosta, C. O. and Pallares, E. S. Rev. col. farm nacl (Argentine), 10, 87-88, (1943).

7. Blatt, A. H. Organic Syntheses, Collective Vol. 2, New York, Wiley & Sons Inc. p. 67-68, (1943).

8. Fishbein, W. N., Daly, J., and Streeter, C. L. Anal. Biochem. 28, 13-24, (1969).

9. Finney, D. J. Probit Analysis, Second Edition, Cambridge University Press (1952).

10. Mount, D. I. and Warner, R. E. U. S. Public Health Serv. Pub. No. 999-WP-23, 16 p., (1965).

11. Burke, W. D. and Ferguson, D. E. Trans. Am. Fisheries Soc. 97, 498, (1968).

12. Herbert, D. W. M. and Downing, K. M. Ann. Appl. Biol. 43, 237, (1955).

13. Neil, J. H. "Some effects of potassium cyanide on speckled trout (*Salvelinus fontinalis*)". Fourth Ontario Industrial Waste Conference, Ontario Water Resources Commission, (1957).

14. Fletcher, G. L. and Hoyle, R. J. J. Fish. Biol. In press. (1971).

15. McFarland, W. M. Calif. Fish and Game. 44, 291, (1958).