ACUTE TOXICITY OF SODIUM ISOPROPYLXANTHATE TO THE FATHEAD MINNOW (Pimephales promelas) AND (Daphnia pulicaria)

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ACUTE TOXICITY OF SODIUM ISOPROPYLXANTHATE TO THE FATHEAD MINNOW (*Pimephales promelas*) AND *Daphnia pulicaria*)

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ABSTRACT

INTRODUCTION TO THE REGIONAL COPPER-NICKEL STUDY

The Regional Copper-Nickel Environmental Impact Study is a comprehensive examination of the potential cumulative environmental, social, and economic impacts of copper-nickel mineral development in northeastern Minnesota. This study is being conducted for the Minnesota Legislature and state Executive Branch agencies, under the direction of the Minnesota Environmental Quality Board (MEQB) and with the funding, review, and concurrence of the Legislative Commission on Minnesota Resources.

A region along the surface contact of the Duluth Complex in St. Louis and Lake counties in northeastern Minnesota contains a major domestic resource of copper-nickel sulfide mineralization. This region has been explored by several mineral resource development companies for more than twenty years, and recently two firms, AMAX and International Nickel Company, have considered commercial operations. These exploration and mine planning activities indicate the potential establishment of a new mining and processing industry in Minnesota. In addition, these activities indicate the need for a comprehensive environmental, social, and economic analysis by the state in order to consider the cumulative regional implications of this new industry and to provide adequate information for future state policy review and development. In January, 1976, the MEQB organized and initiated the Regional Copper-Nickel Study.

The major objectives of the Regional Copper-Nickel Study are: 1) to characterize the region in its pre-copper-nickel development state; 2) to identify and describe the probable technologies which may be used to exploit the mineral resource and to convert it into salable commodities; 3) to identify and assess the impacts of primary copper-nickel development and secondary regional growth; 4) to conceptualize alternative degrees of regional copper-nickel development; and 5) to assess the cumulative environmental, social, and economic impacts of such hypothetical developments. The Regional Study is a scientific information gathering and analysis effort and will not present subjective social judgements on whether, where, when, or how copper-nickel development should or should not proceed. In addition, the Study will not make or propose state policy pertaining to copper-nickel development.

The Minnesota Environmental Quality Board is a state agency responsible for the implementation of the Minnesota Environmental Policy Act and promotes cooperation between state agencies on environmental matters. The Regional Copper-Nickel Study is an ad hoc effort of the MEQB and future regulatory and site specific environmental impact studies will most likely be the responsibility of the Minnesota Department of Natural Resources and the Minnesota Pollution Control Agency.

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INTRODUCTION

Xanthates are dithiocarbonates which are used in the flotation processes for most sulfide minerals, the metallic elements such as copper, nickel, lead, zinc, silver and gold, and a number of oxidized minerals of lead and copper. Flotation is a physiochemical method of concentrating finely ground ores. The process involves chemical treatment of an ore pulp to create conditions favorable for the attachment of certain mineral particles to air bubbles. The air bubbles carry the selected minerals to the surface of the pulp and form a stabilized froth which is skimmed off while the other minerals remain submerged in the pulp. Xanthates fit into this process by functioning as "collectors." Collectors aid in the attachment of a mineral particle to an air bubble. Sodium isopropylxanthate is one of the most commonly used xanthates (Dow Chemical Co., 1976).

Since xanthates may be discharged from tailing ponds into nearby water systems, toxicity information is important. Acute toxicity values found in the literature are summarized in Table 1.

Hawley (1972) and Hardie, *et al.*(1974) did not describe test conditions, so the data they obtained are hard to compare with the data from other investigations. Web, *et al.*(1975) obtained their data from tests in static (unrenewed) solutions with hardness of 118-125 mg/1. They also ran an eight-day, continuous-flow experiment in which 100% mortality occurred at a concentration of 9.3 mg/1 of sodium isopropylxanthate. Fuerstenau (1974), in static tests using rainbow

trout in water of 348 ppm hardness found that the 96-hr LC50 of sodium isopropyl-xanthate was 18-20 mg/1.

TABLE 1. Ninety-six hour LC50 values for sodium isopropylxanthate

Species	96-hr LC50 (mg/1)	Reference
Daphnia magna	0.1-1.0	Hawley (1972)
Notropis atherinoides	0.01-0.1	Hawley (1972)
Pimephales promelas	0.32-5.6	Hawley (1972)
Catfish	>10	Hardie, *et al.*(1974)
Snails	10-100	Hardie, *et al.*(1974)
Tadpoles	10-100	Hardie,*et al.* (1974)
Salmo gairdneri	100-180	Webb, *et al.* (1975)

Chemical degradation is also of concern in xanthate toxicity. Xanthate solutions are not stable in acid medium (Dyer and Phifer, 1969). However, literature reports indicate that alkaline solutions of xanthate are somewhat stable. Harris (1970), quoting other papers, reported that, in one study, 75% decomposition of a xanthate solution took place over an eight-day period at pH 6.5 but only 25% at pH 10.8. Other studies showed that decomposition of xanthate solutions was minimal at pH 10-13. Fuerstenau (1974), working with 52 ppm solutions of ethylxanthate and amylxanthate at 12°C and pH 8.6, observed no significant decomposition. Trofimovich, *et al.* (1976) reported that solutions of ethyl, isopropyl, isobutyl and isoamyl potassium xanthates are stable in water at neutral pH, at a temperature of 20°C and a concentration of 10 mg/1.

Joedodibroto (1963) showed that the decomposition rate decreased with increased molecular weight of the xanthate, and was slower in secondary alkyl xanthates than in primary alkyl xanthates.

The present study was undertaken to determine the acute toxicity of sodium isopropylxanthate and to determine whether the toxicity of sodium isopropylxanthate changes with time.

MATERIALS AND METHODS

Acute Toxicity - Fathead minnows and Daphnia pulicaria.

Acute toxicity of sodium isopropylxanthate to the fathead minnow was evaluated by 48-hr and 96-hr bioassays in static solutions. Dilution water for the 48-hr tests came from a well at the Department of Entomology, Fisheries and Wildlife, University of Minnesota, St. Paul, and from the South Kawishiwi River near Ely, Minnesota. Dilution water for the 96-hr tests was taken from Lake Superior at the U.S. EPA Environmental Research Laboratory, Duluth, Minnesota. Chemical characteristics of the waters are given in Table 2.

The fish used in the 48-hr tests were four-week-old fathead minnows, Pimephales promelas, reared at the St. Paul laboratory. The fish used in the 96-hr tests were eight-week-old fathead minnows reared at the Duluth laboratory. All fish were placed in testing chambers 24 hours before the addition of the toxicant. Ten fish were placed in each chamber. The fish were not fed during the experiments.

Twenty-liter glass test chambers (50 cm x 25 cm x 16 cm high) were used at the St. Paul laboratory and 6-liter, cylindrical polyethylene chambers were used at the Kawishiwi River and Duluth laboratories.

Sodium isopropylxanthate (supplied by the Dow Chemical Co. and manufactured under the Z-11 trademark) was introduced by siphoning approximately 75% of the water out of the test chamber into a glass jar. Toxicant concentrations were prepared by pipetting the proper amount of xanthate stock solution into the jar and then siphoning the solution back into the test chamber. Test

TABLE 2. Chemical characteristics of dilution water

Item	Concentration (mg/l) Laboratory Well	South Kawishiwi River	Lake Superior
Date sampled		L)?L@?&&	&?L#?&*
Total Hardness as $CaCO_3$	220	11	46
Calcium as $CaCO_3$	140	11	33
Magnesium as $CaCO_3$	76	8.3	12
Iron	2	0.6	0.06
Chloride	<1	2.0	1.4
Sulfate	<5	1.7	<5
Fluoride	0.22	<0.1	<0.1
Total Phosphates	0.03		
Sodium	6	1.1	1.1
Potassium	2	0.40	<0.5
Copper	0.0004	0.0019	0.0009
Manganese	0.0287	0.041	<3
Zinc	0.0044	<0.0015	1.1
Cobalt, Nickel	<0.0005	<0.0021	<.0002
Cadmium	<0.0001	0.0002	0.000021
Mercury	<0.0001	0.00024	0.00031
Total Organic Carbon		14	3
Color*		360	5
Turbidity*		1.9	7
Suspended Solids		1.6	5.6

* Standard units

solutions were not replaced during the experiment. Xanthate levels were checked by ultraviolet spectrophotometry (Dyer and Phifer, 1969). Temperature, pH and dissolved oxygen concentration in each chamber were recorded daily. Total alkalinity, hardness and specific conductance in controls were recorded at the termination of each experiment. These data are in Table 3.

TABLE 3. Test conditions for fathead minnow bioassays

	pH		Temperature °C		Dissolved Oxygen (mg/l)		Total Alkalinity	Hardness	Specific Conductance
	Mean	Range	Mean	Range	Mean	Range	Mean	Mean	Mean
Well water	8.45	8.31-8.5	24.6	23.9-25.6	6.9	6.8-7.1	227	220	-
River water	7.1	7.0-7.1	24.0	23.4-24.5	6.9	6.8-7.1	17	29	51
Lake water	7.6	7.5-7.7	24.5	24.0-25.0	6.7	5.5-7.9	41	49	95

The acute toxicity of sodium isopropylxanthate to Daphnia pulicaria was evaluated by 48-hour static bioassays. Dilution water was from Lake Superior. Test organisms were obtained from a laboratory culture of Daphnia pulicaria. Gravid adults were captured with a pipet and placed in a battery jar containing Lake Superior water. One ml of a food solution containing Cerophyl and trout pellets was added to this jar. Test solutions were mixed on the same day and allowed to stand in a water bath for 24 hours. Test chambers were 350 ml pyrex beakers containing 200 ml of test solution. Five treatment levels and a control were used in each experiment.

The following day, five young daphnids, 12$^{+}_{-}$ 12 hours old, were placed in each test chamber. The temperature in each chamber was recorded daily. The pH and toxicant level in each chamber, and the alkalinity and specific conductance in controls were measured at the end of each experiment. Test conditions are summarized in Table 4.

TABLE 4. Test conditions for Daphnia bioassays

Factor	Mean	Range
Temperature	18.1	17.5-18.5
pH	7.8	7.6-8.0
Total Alkalinity	39	
Hardness	46	
Specific Conductance	90	

The trimmed Spearman-Karber method described by Hamilton, et al. (1977) was used to estimate LC50's.

Degradation Tests

Two experiments were run with solutions of sodium isopropylxanthate to determine the degree of xanthate breakdown over time. Two bioassays were also run to determine whether toxicity changes as a xanthate solution ages.

In the first series of experiments, solutions of sodium isopropylxanthate were prepared and aged at room temperature in unstoppered glass Erlenmeyer flasks under a ventilation hood. The absorbance was read on a spectrophotometer at 302 nm every 24 hours.

One set of solutions was prepared with well water at xanthate concentrations

of 10 mg/1 and 50 mg/1. Another set of solutions was prepared with de-ionized water at a xanthate concentration of 5g/1.

In the second series of experiments, six test chambers were set up to determine the effects of pH, aeration and xanthate concentration on the breakdown rate of xanthates. Lake Superior water was used as the dilution water. The design is summarized in Table 5. Absorbance readings were taken at the beginning, after 24 hr, and after 96 hr.

TABLE 5. Degradation Test

Chamber	Xanthate Conc. (mg/1)	Factor	pH
1	75	Aerated with airstone	7.9
2	75	HCl added	1.3
3	75	NaOH added	12.3
4	75	-	7.9
5	150	-	7.9
6	0	-	7.9

Two series of 48-hr static bioassays were run to determine whether xanthate toxicity changes with time. These bioassays were conducted in the manner described in a previous section.

In the first series of bioassays, fathead minnows were used. One liter of stock solution with a concentration of 20g xanthate/1 was mixed in a volumetric flask. The flask was covered and stored in the testing room during the experiment. The first 48-hr static bioassay in this series was conducted using the fresh stock solution. The second bioassay used 24-h4-old stock.

The third used 48-hr old stock, and the fourth used 9-day-old stock. Well water was used for the dilution water.

Daphnia pulicaria were used in the sec ond series of 48-hr static bioassays. Lake Superior water was used as the dilution water. A stock solution of xanthate which had been allowed to stand uncovered for 72 hours was used. The stock was mixed at a nominal concentration of 250 mg xanthate/1. After 72 hours the stock solution was cloudy and the analyzed concentration was 256 mg xanthate/1.

Mixture Bioassay - Daphnia

A 48-hr static bioassay using Daphnia pulicaria in a mixture of copper and sodium isopropylxanthate was conducted using methods similar to the previous Daphnia bioassays. The LC50 value for copper, 9.29 µg CuH/1, was obtained from previous tests done in Lake Superior water by the Copper-Nickel Project. Xanthate LC50's were calculated from tests described elsewhere in this paper. The LC50 values were averaged using the LC50 divided by its 95% confidence interval as a weighting coefficient.

The test solutions were prepared with an attempt to keep the xanthate/copper ratio constant. The low treatment level contained about 37% of the LC50 value of each toxicant. The high treatment level contained about 80% of the LC50 value of each treatment. If the toxic interaction of copper and xanthate were strictly additive, the LC50 value for the mixture would be 50% of the LC50 value of each toxicant.

RESULTS

Spearman-Karber estimates of the all LC50 values are given in Tables 6 and 7.

Results of the degradation experiments are given in Tables 8 and 9.

Results of the copper-xanthate mixture bioassay are given in Table 10. The LC50 was calculated by expressing the level of toxicant in each chamber as the sum of the fractions of xanthate and copper LC50's present in the chamber. Thus an LC50 of 1 "toxic unit" would indicate a strictly additive effect; <1 would indicate more than additive effects; and >1, less than additive effects.

TABLE 6. Spearman-Karber estimates of LC50's
(mg sodium isopropyl/xanthate/liter)

Fathead Minnow Tests

Water	Duration	LC50	95% confidence limits
Well water	48 hr	31.11	26.24, 36.89
River water	48 hr	32.52	27.32, 38.72
River water	48 hr	35.65	29.63, 42.90
River water	48 hr	46.45	41.77, 50.56
L.Superior	96 hr	47.079	41.24, 53.75
L. Superior	96 hr	39.33	25.52, 60.60
L. Superior	96 hr	31.34	24.43, 40.19
L. Superior	96 hr	37.98	31.67, 45.54
L. Superior	96 hr	28.35	26.55, 30.26
L. Superior	96 hr	39.177	33,95, 45.21

Weighted mean of 96 hr LC50 = 37,72

Daphnia Tests

L. Superior	48 hr	21,74	20,00, 23.62
L. Superior	48 hr	21.72	19.49, 24.20
L. Superior	48 hr	22.76	19.46, 26.63

Weighted mean of 96 hr LC50 = 21.97

TABLE 7. Spearman-Karber estimates of the LC50's in bioassays using aged stock solutions

Fathead minnow tests

Age of Stock Solution	48 hr LC50 (mg/l)	95% Confidence limits
Fresh	24.86	21.74, 28.44
1-day-old	39.81	35.25, 44.95
2-day-old	25.14	20.00, 31.60
9-day-old	30.49	27.33, 34.02

Daphnia Test

72-hour-old	13.42	10.62-16.94

TABLE 8. Absorbance of Sodium Isopropyl-xanthate solutions over 96 hours

Age of Stock (hr)	Absorbance (302 nm)	% of Fresh Stock
Well Water 50 mg/1 (pH 8.60)		
Fresh	1.040	--
24	0,820	88.5
48	0.905	87.0
72	0.870	83.7
96	0.855	82.2
Well Water 10 mg/1 (pH 8.62)		
Fresh	0.970	--
24	0.920	94.8
48	0.880	90.7
72	0.820	84.5
96	0.780	80.4
Deionized Water 5 g/1 pH 10.22)		
Fresh	1.000	--
24	1.020	102.0
48	0.985	98.5
72	1.005	100.5
96	0.990	99.0

TABLE 9. Xanthate concentrations of solutions aged under different conditions

		Xanthate Concentration (mg/1)		
Chamber	Factor	0 hrs	24 hrs	96 hrs
1	aerated	81.6	82.5	85.5
2	acidic	0.0	0.0	0.0
3	basic	80.4	80.0	79.3
4	-	80.0	79.9	79.9
5	doubled concentration	157.1	155.7	158.6
6		0.0	0.0	0.0

TABLE 10. Copper-xanthate mixture bioassay.

Chamber	[Cu] (µg/1) / fraction of LC50	Xanthate (µg/1) / fraction of LC50	Percentage Survival
1	3.40 -- .37	5.82 -- .26	100
2	3.85 -- .41	8.05 -- .37	100
3	5.25 -- .57	10.87 -- .49	80
4	5.95 -- .64	19.82 -- .90	15
5	7.55 -- .81	19.01 -- .87	25

Spearman-Karber estimate of 48-hr LC50: 1.23

95% confidence limits: 1.11, 1.47

DISCUSSION

Acute Tests

The LC50 values reported in the literature are generally lower than the LC50 values found in the present study.

The course of toxic action of sodium isopropylxanthate appeared to be inconsistent. Mortality of fathead minnows would occur either in the first 24-hr period or after the second day. In some of the higher treatments, the solution would turn cloudy overnight. No fish survived in a cloudy solution. This cloudiness appeared with no apparent pattern. For example, in one case the second highest treatment level turned cloudy in 24 hours and the highest treatment level turned cloudy after 48 hours. If the high treatment did not turn cloudy the fish would still die, although at a slower rate. Toxicity was indicated by erratic swimming and loss of orientation, followed by lethargy and "gasping" on the bottom of the tank, and finally death. The type of dilution water did not appear to be a factor in the toxicity of xanthates.

Daphnids would occasionally get caught in the surface film of a xanthate solution, but this was never observed in a control chamber. If a daphnid became caught it usually would survive even if the other daphnids died.

Degradation Tests

The degradation experiments (Table 8) show that the lower concentrations of xanthates degrade to some extent with time. The higher concentrations did not degrade in 96 hours.

The data presented in Table 9 show that acidification was the only factor

which led to rapid xanthate breakdown. Cloudiness was observed in the stream from the pipette which was used to introduce the xanthate stock solution. This cloudiness disappeared after a few seconds. None of the solutions turned cloudy during the experiment.

In the bioassays conducted with fathead minnows using an aging stock solution, toxicity appeared to be lower after the first day, but there were no differences from the initial toxicity after 2 and 9 days of aging. No cloudy solutions were observed.

The 48-hour LC50 value for the Daphnia bioassay using 72-hour-old stock solution was 61% of the weighted mean LC50 value found using fresh stock. The stock solution used in this test was cloudy. The lower LC50 indicates that toxicity increased with degradation.

Copper-Xanthate Mixture Bioassay

The survival of daphnids in higher treatments in the mixture bioassay (Table 10) was the result of these daphnids becoming trapped in the surface film. One organism in chamber 4 was trapped in the surface film, as were all the survivors observed in chamber 5. If these daphnids were counted as mortalities, the Spearman-Karber estimate of the 48-hr LC50 is 1.22 "toxic units" with 95% confidence limits of 1.13-1.32 "toxic units".

Both of the LC50 estimates indicate that a copper-xanthate mixture is less toxic than either chemical alone.

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