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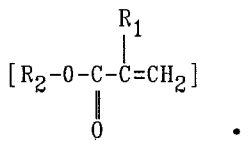
Acute Toxicity and Behavioral Effects of Acrylates and Methacrylates to Juvenile Fathead Minnows

C. L. Russom,* R. A. Drummond, and A. D. Hoffman

U.S. Environmental Protection Agency, Environmental Research Laboratory—Duluth, 6201 Congdon Boulevard, Duluth, Minnesota 55804

Acrylate and methacrylate esters are reactive monomers that are used primarily in the synthesis of acrylic plastics and polymers. The basic structure for this series of compounds is:



The R_1 functional group is hydrogen for acrylates and a methyl group for methacrylates.

Veith et al. (1985) proposed a Quantitative Structure-Activity Relationship (QSAR) for esters:

$$\log \text{Molar LC50} = -0.535 \log P - 2.75 \qquad \text{eq. 1}$$
$$SD \pm 0.34,\ n = 25,\ R^2 = 0.828 \bullet$$

Acrylate and methacrylate data were not used in the development of this QSAR. Veith found that more polar esters were more toxic than would be predicted using a baseline narcosis QSAR. A previous study by Dillingham et al. (1983) concluded that acrylates and methacrylates have a nonspecific, membrane-mediated, mode of action (MOA). Lawrence et al. (1972) reached a similar conclusion except they noted that increased toxicity was observed for compounds with an hydroxyl or amino group at the R_2 position.

The research presented here was initiated to better define QSARs for acrylates and methacrylates. Flow-through 96-h acute toxicity tests were conducted using juvenile fathead minnows, Pimephales promelas. The 96-h LC50 was then correlated against

*To whom correspondence should be sent.

log P. Log P was used as the independent variable in order to compare this study's findings with the QSAR of Veith et al. (1985). In coordination with the toxicity test, a behavioral screen was conducted. Previous work on classification of chemicals based on behavioral and morphological signs of stress, using the juvenile fathead minnow, has proved to be helpful in predicting MOA (Drummond et al. 1986; McKim et al. 1987).

MATERIALS AND METHODS

Ninety-six hour flow-through acute toxicity tests were conducted with fathead minnows (Pimephales promelas) on six acrylates and six methacrylates (Table 1). Two exposure systems were used. Isopropyl methacrylate was tested using an ABC (Analytical Bio-Chemistry Laboratories, Inc., Columbia, Missouri) solenoid operated electronic diluter system. All other exposures were conducted using a continuous-flow modified Benoit mini-diluter system (Benoit et al. 1982). A detailed description of these exposure systems can be found in Geiger et al. (1985). A 16-h light, 8-h dark photoperiod was used during all tests.

In general, biological and chemical procedures followed American Society for Testing and Materials recommendations (ASTM, 1980). The fathead minnows used during the exposures ranged in age from 28 to 34 days, with an average wet weight and standard length of 0.134 $\pm$ 0.034 g and 20.9 $\pm$ 2.0 mm, respectively.

Lake Superior water, filtered through a sand/gravel filter, was used as dilution water throughout the exposures. The lake water entered the diluter through a headbox where it was aerated and heated. Dissolved oxygen, pH and temperature were measured each day of the 4-d exposure. The average pH, temperature and dissolved oxygen concentration were 7.62 $\pm$ 0.12, 24.6 $\pm$ 0.4°C and 6.71 $\pm$ 0.57 mg/L, respectively. Alkalinity and hardness were determined from water sampled from control chambers as well as from chambers of the low, middle and high toxicant concentrations. Average alkalinity and hardness were 47.0 $\pm$ 3.2 and 45.3 $\pm$ 1.0 mg/L as $CaCO_3$, respectively. Water chemistry methods were those recommended by the American Public Health Association (APHA et al. 1980).

During exposures, fish were observed daily at 8, 24, 48, 72 and 96 h. Abnormal behavioral and morphological changes were recorded using a previously developed checklist (Geiger et al. 1985). Each abnormality was assigned a predefined code number. These data were then evaluated using discriminant function analysis with BMDP-79 software (Dixon and Brown 1979). A detailed description of these procedures has been presented (Drummond et al. 1986).

The test compounds were obtained from Scientific Polymer Products, Inc. (Ontario, New York) and were more than 97%

Table 1. Observed and predicted acute toxicity (96-h LC50) of acrylates and methacrylates exposed to fathead minnows.

CHEMICAL	MOLECULAR WEIGHT	LOG P	OBSERVED LC50 (mg/L)	PREDICTED[a] LC50	TR[b]
1 Lauryl acrylate	240.0	6.57	c	0.13	-
2 Hexyl acrylate	156.2	3.39	1.09	4.27	3.9
			1.14[d]		3.7
3 Cyclohexyl acrylate	154.2	2.78	1.48	8.93	6.0
4 Isobutyl acrylate	128.2	2.22[e]	2.09	14.8	7.1
			2.11[d]		7.0
5 2-Hydroxypropyl acrylate	130.1	0.35[e]	3.61	150	42.0
			3.10[d]		48.0
6 2-Hydroxyethyl acrylate	116.1	-0.21[e]	4.80	267	56.0
7 Allyl methacrylate	126.0	1.57	0.99	32.4	33.0
8 Benzyl methacrylate	176.2	2.82	4.67	9.71	2.1
9 2-Ethoxyethyl methacrylate	158.2	1.40	27.7	50.1	1.8
10 Tetrahydrofufuryl methacrylate	170.0	1.30	34.7	60.9	1.8
11 Isopropyl methacrylate	128.2	2.25[e]	38.0	14.3	0.38
12 2-Hydroxyethyl methacrylate	130.1	0.47[e]	227	130	0.57

[a] Calculated using eq 1 (log Molar LC50 = -0.535log P - 2.75).

[b] Calculated using eq 2: $TR = \frac{\text{LC50 pred}}{\text{LC50 obs}}$

[c] Not toxic at 4.33 mg/L

[d] Second test

[e] Measured Log P value from STARLIST (Leo et al. 1985).

pure. The chemicals contained small amounts (50-600 mg/L) of either hydroquinone or methyl ester hydroquinone to prevent polymerization. These inhibitors were removed prior to testing by slowly passing the acrylate through Resin HR4 inhibitor removing columns provided by Scientific Polymer Products. Once the inhibitors were removed, the chemicals were stored at 4°C to prevent breakdown of the parent compound.

Chemical stock solutions were prepared and renewed daily, except for 2-hydroxyethyl acrylate and 2-hydroxypropyl acrylate in which one stock solution was used for the entire test. Chemical analysis established that these stock solutions remained stable throughout the 4-d exposure. All stock concentrations were below toxicant solubility except for lauryl acrylate which was an emulsion.

Chemical analysis was performed on a Hewlett-Packard Model 5730 GC equipped with dual flame-ionization detectors and 120 cm x 2 mm I.D. glass columns. One column was packed with 60-80 mesh Tenax GC and the other was packed with 10% Carbowax 20 M on 80-100 mesh Gas Chrom Q. Aqueous samples from the second acute exposures of hexyl acrylate and isobutyl acrylate were analyzed using a 15 m x 0.53 mm Megabore FSOT, coated with a bonded

polyethylene glycol phase of 1.0 μm thickness. With capillary GC, better sensitivity and peak symmetry was achieved. Nitrogen (25-30 mL/min) was used as the carrier gas, and hydrogen (20-25 mL/min) and air (240 mL/min) were used for flame operation. Most of the compounds were analyzed on the Tenax column, which was run isothermally (165-210°C), using on-column aqueous injections. Lauryl acrylate and cyclohexyl acrylate were analyzed on the 10% Carbowax column at temperatures of 200 and 130°C, respectively, also with on-column aqueous injections. Hexyl acrylate and isobutyl acrylate analyses on capillary GC were run at 88 and 55°C, respectively. Injector and detector temperatures were 250° and 300° C, respectively, for all analyses. Peak area calculations were performed with a Hewlett-Packard Lab Automation Data System (Model 3356). Percent spike recovery ranged between 88.7-109.8% and percent agreement of duplicates ranged between 93.0-99.5%. Toxicant water concentrations were corrected for recovery.

Calculated and measured log P values were taken from the MedChem CLOG P (version 3.33/3) and STARLIST programs of the Medicinal Chemistry Project at Pomona College, Claremont, California (Leo et al. 1985). All LC50s were calculated using the average tank concentrations and a computerized Trimmed Spearman-Karber Method (Hamilton et al. 1977).

RESULTS AND DISCUSSION

The observed 96-h LC50s as well as calculated or measured log P values for the tested compounds, are presented in Table 1. The most toxic acrylate tested was allyl methacrylate (#7) with a 96-h LC50 of 0.99 mg/L. The least toxic compound tested was 2-hydroxyethyl methacrylate (#12) with a 96-h LC50 of 227.0 mg/L. 2-Hydroxyethyl acrylate (#6) was 47 times more toxic than 2-hydroxyethyl methacrylate, with a 96-h LC50 of 4.80 mg/L. Lauryl acrylate (#1) did not elicit mortality or behavioral signs of intoxification at 4.33 mg/L. This concentration was as close to water solubility as could be obtained using our technique. Also presented in Table 1 are predicted LC50 values calculated using eq. 1. A toxicity ratio (TR) of the predicted and observed 96-h LC50 values was calculated using eq. 2.

$$TR = \frac{\text{LC50 pred}}{\text{LC50 obs}} \qquad \text{eq. 2}$$

The ratio provides a means of assessing the agreement of the observed LC50 to that predicted by eq. 1. Plotted in Figure 1 are the log molar LC50s of the methacrylates and acrylate vs. log P, as well as a line representing eq. 1. Figure 2 presents the three MOA groups (categories) that acrylates and methacrylates were placed into based on an assessment of behavioral and morphological signs of intoxification.

Figure 1. A plot of log P vs. the log of the 96-h LC50 in Mol/L for six acrylates and six methacrylates. The numbers correspond to Table 1 with "a" and "b" referring to first and second test, respectively. NT means not toxic.

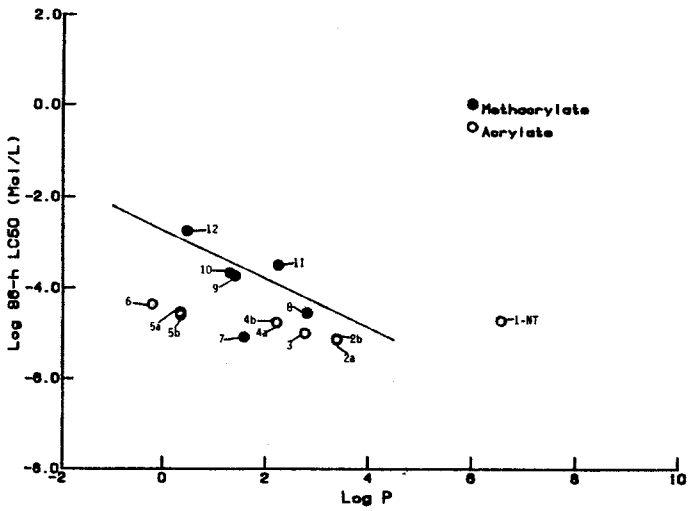


Figure 2. Classification of acrylate[a] and methacrylate chemicals based on fish behavioral and morphological indices. Chemical number (Table 1) shown in parenthesis.

Hypoactive and Underreactive	Hyperactive and Overreactive	
	Without scoliosis/lordosis	With scoliosis/lordosis
- Allyl methacrylate (7)	Without tetany	-2-Hydroxypropyl acrylate (5)
- Benzyl methacrylate (8)	- Hexyl acrylate (2)	-2-Hydroxethyl acrylate (6)
- 2-Ethoxyethyl methacrylate (9)	- Cyclohexyl acrylate (3)	
- Tetrahydrofurfuryl methacrylate (10)	-2-Hydroxyethyl methacrylate (12)	
- Isopropyl methacrylate (11)	With tetany	
	- Isobutyl acrylate (4)	
MOA: Narcosis	MOA: Inhibitor of respiratory or metabolic function (e.g., uncouplers)	MOA: Acetylcholinesterase inhibition/neurotoxic

[a] Lauryl acrylate (#1) - no behavioral stress observed.

In general, the observed LC50s for acrylates largely deviated from the predicted LC50 value, with toxicity being approximately 4 to 56 times greater than predicted. Deviations between predicted and observed LC50 values were smallest for hexyl acrylate (#2), cyclohexyl acrylate (#3), and isobutyl acrylate (#4) (TRs of 4-6). Compounds #2, #3 and #4 elicited behavioral

and morphological signs indicative of an inhibitor of respiratory or metabolic function (Drummond et al. 1986) (Figure 2). Hyperactive locomotor activity and overreactivity to outside stimuli were observed toxicant-induced stresses, with no signs of permanent deformities (lordosis/scoliosis). Fish exposed to isobutyl acrylate (#4) showed tetany. The remaining acrylates, 2-hydroxypropyl acrylate (#5) and 2-hydroxyethyl acrylate (#6), were 42 to 56 times more toxic, respectively, than predicted. These two compounds showed behavioral and morphological signs in the fish indicative of a neurotoxicant (Drummond et al. 1986). These compounds caused fish to become hyperactive, overreactive to outside stimuli, and exhibit scoliosis and lordosis deformities.

Lawrence (1972) suggested that either nucleophilic attack or electrostatic binding were important in the increased toxicity of acrylates. Autian (1975) proposed that acrylates undergo hydrolysis to acrylic acids, which are more toxic than the parent acrylate. Previous work has also concluded that the addition of the hydroxy group at the R_2 position increases the toxic effect of acrylates (Lawrence et al. 1972; Autian 1975).

The observed LC50s for methacrylates were in close agreement with predicted values, with the exception of allyl methacrylate which was 33 times more toxic than predicted (eq. 1). All the methacrylates, with one exception, were associated with a narcosis MOA (Figure 2) (Drummond et al. 1986). Fish exposed to these methacrylates were hypoactive in terms of spontaneous locomotor activity and did not respond to outside stimuli. The only methacrylate not classified as a narcotic was 2-hydroxyethyl methacrylate (#12). Signs of toxicant induced stress for 2-hydroxyethyl methacrylate (#12) included hyperactive locomotor activity and overreactivity to outside stimuli, which are associated with response elicited by inhibitors of respiratory or metabolic function (Drummond et al. 1986). None of the exposed fish showed signs of permanent deformities. It should be noted that the addition of an hydroxy group to the R_2 position of this methacrylate did not elicit increased toxicity as was observed with similar additions to acrylates, but could be associated with the observed behavioral aberrations. Allyl methacrylate (#7) also was unique in that it elicited very few signs of toxicity. Based on available behavioral data it was classified as a narcotic.

Autian (1975) and Lawrence (1972) concluded that a methyl group in the R_1 position interfered with hydrolysis to acrylic acid and therefore decreased the toxicity of methacrylates in comparison to acrylates. Our study concurs with the findings of these workers with the exception of allyl methacrylate. Previous work by Busfield et al. (1985) demonstrated that hydrogen abstraction from the two allylic positions of allyl methacrylate (#7) using t-butoxy radicals was a major reaction leading to

increased reactivity of the compound. The methyl group at the R_1 site may hinder hydrogen abstraction at that allylic position, but the terminal double bonded carbons at the R_2 position are not hindered in respect to hydrogen abstraction. The difunctional property of this compound may account for its high TR value.

In conclusion, the results of the present study demonstrate that acrylates and methacrylates, in general, exhibit separate MOAs, with acrylates being more toxic. Methacrylate toxicity can be predicted, within a factor of two by using eq. 1, except for compounds with an additional reactive site such as allyl methacrylate. Equation 1 does not provide adequate LC50 estimations for acrylates. A QSAR for acrylates based on this study is defined by eq. 3.

$$\log \text{Molar LC50} = -0.194 \text{ Log P} - 4.45$$
$$R^2 = 0.957, \text{ SD} = \pm 0.074, \text{ N} = 5 \qquad \text{eq. 3}$$

This equation is based on five acrylate values (#2-#6), with replicates averaged. Lawrence et al. (1972) developed a QSAR based on acute LD50 values of mice using a model developed by Hansch (Hansch et al. 1964) with log P and the net charge on the carbonyl carbon as independent variables. These researchers concluded that both independent variables contributed significantly to acrylate toxicity. Regarding methacrylates, Lawrence et al. (1972) stated that log P contributed significantly to toxicity but that the charge on the carbonyl carbon did not. In order to improve the QSAR for acrylates, another independent variable representing the charge on the carbonyl carbon should be included in the regression equation.

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