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Influence of a Co-Solvent (Acetone) and Ultrasonication on the Acute Toxicity of a Quaternary Amine (Aliquat 336) and an Organophosphorus Compound (HDEHP) to *Daphnia magna*

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In acute toxicity tests with chemicals on aquatic animals, some lipophilic chemicals may not produce sufficient mortality for determinations of LC50s. One way to overcome this problem is to increase the solubility of the toxicant by an additive (co-solvent). According to THE COMMITTEE ON METHODS FOR TOXICITY TESTS WITH AQUATIC ORGANISMS (1975), the final concentration of the co-solvent must not exceed 0.5 mL/L in static tests and 0.1 mL/L in flow-through tests.

There is a scarcity of information on the interaction between co-solvents and toxicants in aquatic organisms. When atrazine was tested for long-term toxicity to Daphnia pulex, the toxicity was enhanced on addition of ethanol at 0.5 mL/L (SCHOBER & LAMPERT 1977). Although several investigations have shown that surfactants can interact with organic chemicals (DUGAN 1967; SOLON et al. 1969; SOLON & NAIR 1970) as well as with metals (BROWN et al. 1968; CALMARI & MARCHETTI 1973; TSAI & MCKEE 1980), studies on influence of commonly used co-solvents on toxicity are lacking. The complex nature of toxicity interaction on addition of surfactants has been discussed by FLORENCE & GILLAN (1975) and FLORENCE (1977). Thus, in experiments on goldfish (Carassius auratus) the reciprocal death time in 0.04% thiroidazine hydrochloride was increased in the presence of low concentrations of some non-ionic detergents and decreased in high concentrations (FLORENCE 1977). In the presence of Polysorbate 80, the magnitude of the interaction was dependent on surfactant concentration, ambient pH and in addition, on molecular weight of the toxicant (FLORENCE & GILLAN 1975).

In the present study the acute toxicities of two solvent extraction chemicals (Aliquat 336 and HDEHP) were determined in Daphnia magna, and the influence of addition of

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acetone and ultrasonication of stock-solutions were investigated.

MATERIALS AND METHODS

The two solvent extraction chemicals tested were Aliquat 336, which is a quaternary amine (methyltricaprylyl ammonium chloride) manufactured by General Mills, USA, and HDEHP (di-2-(ethylhexyl)phosphoric acid) manufactured by Bayer, Germany. Both chemicals were of technical grade. Approximate solubilities in water are 10,000 and 100 mg/L for Aliquat 336 and HDEHP, respectively, and tested concentrations were kept below these values.

Three different techniques were used in preparing the final tests concentrations. Stock solutions were made either in acetone or in water. For stock solutions in water appropriate amounts of Aliquat 336 or HDEHP and 5 mL deionized water were sonicated (Sonifer B-12, Branson Sonic Power Co. USA, equipped with a converter and a microtip) in a 10-mL vial for 30 s, diluted by Standard Reference Water (SRW, Table 1) and allowed to equilibrate for one h. Final test concentrations, which were selected by progressive bisectioning on logaritmic scale, were prepared by diluting appropriate volumes of stock solutions in SRW up to 190 mL, and left for equilibration for 2 h. The final 10 mL SRW was then added together with the animals (time 0). When stock solutions in acetone were used, further predilutions in acetone (analytical grade) were made to produce desired concentrations on addition of 50 µL acetone solution to 200 mL dilution water. The acetone was either evaporated just to dryness before addition of SRW or not. Furthermore, in two experiments (Tables 2 and 3) acetone was added to the test solutions prepared from stock solutions in water. In this experiment exposures were initiated after equilibration for 3 h on addition of animals (time 0).

Table 1. Chemical composition of reconstituted Standard Reference Water (SRW) for toxicity tests on water fleas.

Ingredient[a]	Final concentration, mg/L
$NaHCO_3$	200
K_2SO_4	26
K_2HPO_4	6.8
$CaCl_2 \times H_2O$	297

a All ingredients were of analytical grade and dissolved in carbon-filtered deionized water. Prior to use, a sufficient volume was prepared and aerated for one day.

Young water fleas, D. magna, (less than 66 h old) were used in all tests. Culturing methods and test procedures were as described by DAVE et al. (1981). Water temperature was 20±0.5°C and pH was 8.3 - 8.4 in controls. Recorded pH at start and termination ranged 8.0 - 8.1 in the highest and 8.3 - 8.4 in the lowest toxicant concentrations. All tests were performed in duplicates or triplicates using 9 - 14 animals per concentration. The animals were classified as dead when their second antenna did not move within 15 s after irrigation by a water stream from the handling pipet. Dead and live animals were recorded after 3, 24, 48, 72, and 96 h. LC50 values and 95% confidence limits were calculated by the aid of microcomputer using probit analysis according to DAVIS (1971). For test series with insufficient partial mortality, LC50s were estimated on log-probit paper. These values are presented together with the highest concentration causing 0% mortality and the lowest concentration causing 100% mortality. All values are based on nominal concentrations calculated from added amounts of the toxicants.

The experiments were designed to reveal possible influence of two alternate procedures for preparation of test solutions in each experiment. The alternatives examined were (1) absence or presence of acetone at 0.5 mL/L (Aliquat 336 and HDEHP tested), (2) addition of toxicant dissolved in 50 µL acetone or in SRW (Aliquat 336 tested), (3) addition of toxicant dissolved in 50 µL acetone followed by evaporation just to dryness or in SRW (Aliquat 336 tested). The experimental design allows direct comparisons to be made only when two alternatives were tested simultaneously, using animals from the same culture produced at the same time and when all other procedures were identical as the exposures were performed simultaneously. Mortality data from the replicates (duplicates adn triplicates) were not pooled but analysed separately by probit analyses or graphic estimation on log-probit paper.

RESULTS

In most experiments LC50 values from replicates agreed reasonably. Over-all means and S.D.s of LC50s obtained (independent of procedure) were for HDEHP: 72-h LC50: 36.5±10.0 mg/L (n=6), 96-h LC50: 16.5±9.8 mg/L (n=6), and for Aliquat 336 24-h LC50: 37.1±9.7 µg/L (n=16), 48-h LC50: 18.1±10.1 µg/L (n=16).

(1) Influence of acetone. Additions of acetone to a final concentration of 0.5 mL/L were not found to influence LC50 values for either HDEHP (Table 2) or Aliquat 336 (Table 3). In tests with HDEHP, 24 h and in some tests also 48 h exposures did not produce sufficient mortality for calculations of LC50s. This is consistent with previous findings

Table 2. Acute toxicity of HDEHP to water fleas, in tests with and without acetone.

Final acetone conc. µL/L	Replicate no.	LC50 mg/L			72-/96-h LC50
		48 h	72 h	96 h	
500	1	60.7 (46.7-78.8)[a]	30.2 (23.9-38.0)	18.4 (14.4-23.4)[e]	1.6[b]
500	2	75 -	29.0 (18.1-46.6)	12.1 (7.28-20.0)[e]	2.4
500	3	75 -	47.9 (35.9-63.9)	28.7 (22.5-36.7)[e]	1.7
	Mean	-	35.7	19.7	1.9
0	1	76.9 (42.7-137)	24.5 (19.1-31.3)	2.82[c] (0.213-37.3)[e]	8.7[d]
0	2	75	47.4 (24.2-93.0)	11.1 (3.39-36.4)[f]	4.3
0	3	83.7 (59.4-117)	40.2 (31.5-51.3)	26.0 (21.2-31.9)[g]	1.6
	Mean	-	37.4	18.6	3.0

a 95% confidence limits
b Ratio between 72- and 96-h LC50
c Exceptionally low value and wide confidence limits make the result from this replicate unreliable
d Caused by exceptionally low 96-h LC50
e Mortality in control at 96-h 0%
f Mortality in control at 96-h 12.5%
g Mortality in control at 96-h 8.3%

Table 3. Acute toxicity of Aliquat 336 to water fleas, in tests with and without acetone.

Final acetone conc. µL/L	Replicate no.	LC50 µg/L		24-/48-h LC50
		24 h	48 h	
500	1	41.9 (32.3-54.3)[a]	15.7 (11.9-20.8)	2.8[b]
500	2	35.2 (18 - 56)[c]	12.9 (8.67-19.1)	2.7
	Mean	38.6	14.3	2.7
0	1	29.3 (22.3-37.4)	9.47 (7.12-12.6)	3.1
0	2	33.8 (26.5-43.0)	10.0 (6.44-15.6)	3.4
	Mean	31.6	9.7	3.3

a.b. Symbols as in Table 2.
c No confidence limit due to insufficient partial mortality. Range mortality 0 - 100%.

Table 4. Acute toxicity of Aliquat 336 to water fleas, in tests with stock solutions prepared either in acetone or in water.

Toxicant addition	Replicate no.	LC50 µg/L		24-/48-h LC50
		24 h	48 h	
From acetone stock solutions (50 µL/200 mL) to a final acetone conc. of 0.25 mL/L	1	43.1 (31.6-59.0)[a]	41.3 (27.4-62.3)	1.0[b]
	2	59.6 (47.2-75.4)	37.2 (30.4-45.4)	1.6
	3	46.4 (38.9-55.4)	32.6 (27.1-39.1)	1.4
	Mean	49.7	37.0	1.3
From ultrasonicated stock solutions in water	1	22.5 (10 - 32)[c]	12.0 (3.2 - 18)[c]	1.9
	2	21.4 (17.7-25.9)	12.0 (5.6 - 18)[c]	1.8
	3	28.5 (20.6-39.4)	8.49 (6.54-11.0)	3.4
	Mean	24.1	10.8	2.4

a. b. Symbols as in Table 2.
c No confidence limit due to insufficient partial mortality. Range mortality 0 - 100%.

Table 5. Acute toxicity of Aliquat 336 to water fleas, in tests with toxicant added either in acetone followed by evaporation or in water solution.

Toxicant addition	Replicate no.	LC50 µg/L		24-/48-h LC50
		24 h	48 h	
From acetone stock solutions (50 µL) followed by evaporation before addition of water and test organisms	1	44.3 (36.9-53.2)[a]	19.4 (17.0-22.1)	2.3[b]
	2	31.1 (24.8-39.1)	13.0 (11.3-14.9)	2.4
	3	40.8 (35.0-47.4)	22.3 (18.1-27.4)	1.8
	Mean	38.7	18.2	2.2
From ultrasonicated stock solutions in water	1	41.2 (33.6-50.5)	14.4 (10 - 18)[c]	2.9
	2	33.0 (27.1-40.2)	15.1 (12.4-18.5)	2.2
	3	42.0 (33.8-52.2)	14.5 (12.4-16.9)	2.9
	Mean	38.7	14.7	2.7

a. b. Symbols as in Table 2.
c No confidence limit due to insufficient mortality. Range mortality 0 - 100%.

(DAVE et al. 1981). Tests with Aliquat 336 were terminated after 48 h, the most commonly used period in acute tests on water fleas. In tests of longer duration with no feeding, mortality in controls often increases. Therefore, 72- and 96-h values were only recorded in tests with HDEHP. In the latter tests control mortality was always below 12.5% (0% mortality in all except two).

Although there is no significant (95% confidence limits overlap) difference in LC50 values for Aliquat 336 with and without acetone (Table 3), LC50 values from tests with acetone added are consistently higher than those without acetone added.

(2) Stock solutions prepared in acetone versus in water. The results obtained in a series when Aliquat 336 was added either in acetone (0.05 mL/200 mL; final concentration of acetone 0.25 mL/L) or in SRW (sonicated stock solution) are presented in Table 4. These results show that the toxicity was lower (LC50 higher) when Aliquat 336 was added from an acetone stock solution compared to an ultrasonicated stock solution. Furthermore, the ratio between 24-h and 48-h LC50s was lower when the toxicant was added from the acetone stock solution.

From the results presented in Tables 3 and 4, an increased resistance to Aliquat 336 in the presence of acetone at 0.25 and 0.5 mL/L was suggested. Therefore, another experiment similar to that presented in Table 4 was performed - the only difference being that the 50 µL of acetone was evaporated just to dryness before addition of water. In Table 5 it can be seen that the difference disappeared when the acetone was no longer present, as did the difference in ratio between 24- and 48-h LC50s.

All together, the results presented in Tables 3, 4, and 5 suggest that the presence of acetone caused an increased resistance to Aliquat 336 in the water flea.

DISCUSSION

The influence of co-solvents, carriers, surfactants or other vehicles on toxicity of pesticides have been reviewed by BROWN (1980). The findings cited revealed that route of administration as well as species and even sex can in unpredictable manners interact and alter the influence caused by the vehicle on the acute toxicity of chemicals. Although such complex interactions are so far unknown in aquatic animals, the existence of them is probable. Therefore, it is premature to extrapolate the findings in the present study, that acetone decreased the acute toxicity of Aliquat 336 in D. magna, to other species

of aquatic organisms and certainly to other toxicants.

In all studies on interactions caused by abiotic as well as biotic factors on toxicity of chemicals to aquatic organisms the magnitude of the interaction should be considered. There is an apparent tendency in many reports to overemphasize the importance of - although statistically significant - comparatively small variations in toxicity. In the present study 95% confidence limits in some replicates of tests with and without acetone overlapped. The calculated difference in mean values from tests with and without acetone in comparable tests with Aliquat 336 (data from Tables 3 and 4 considered separately) was 1.2- and 2.0-fold for 24-h LC50s and 1.5- and 3.4-fold for 48-h LC50s. Thus the interaction of acetone in these tests with Aliquat 336 in D. magna was somewhat greater for 48-h values compared to 24-h values.

From existing limited knowledge on the influence of common co-solvents in toxicity tests on aquatic organisms it can just be concluded that this possibility can not be neglected. Furthermore, interactions between co-solvents and toxicants can obviously occur even if the concentration of the co-solvent is far below those producing toxicity per se. Thus, the LC50 for acetone in D. magna is 12,600 - 12,700 mg/L (CANTON & ADEMA 1978) or about 25 or 50 times greater than the concentrations used in the present study. If one regards acetone as a toxicant then the present study would reveal antagonism between Aliquat 336 and acetone. That conclusion seems illogical and a more probable explanation is that the presence of small amounts of acetone have delayed the uptake and/or lethal action of Aliquat 336 in D. magna.

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