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Acute Toxicity of Chromate, DDT, PCP, TPBS, and Zinc to *Daphnia magna* Cultured in Hard and Soft Water

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The pioneer work with Daphnia magna in toxicological research was done in the 1930:ies and 1940:ies by Nauman (1933 a, b), Anderson (1944) and Holm-Jensen (1944, 1948). Today Daphnia has been the most common invertebrate genus in studies of acute and chronic toxicity. Standardized test procedures have reduced the variations of toxicity values caused by ambient conditions like alkalinity, hardness, oxygen, pH and temperature. Specification on the use of species, usually D. magna or D. pulex, and age (< 24 h) has also reduced the variability. The quotient of the 48-h LC50's for D. magna and D. pulex was below 3 for 13 of 15 tested chemicals and below 6 for all 15 (Canton and Adema, 1977). The coefficient of variation of repeatability and reproducibility of the 24-h EC50 for potassium bichromate in D. magna were 14 and 39 % respectively, when determined at 46 laboratories in a ring-test of the ISO test procedure (ISO, 1982).

The advantages of using Daphnia in acute and chronic toxicity tests are its convenient size, short generation-time and easinesss of culture (Buikema et al, 1980). However, there is so far no generally accepted standard procedure for laboratory culture of - or for chronic tests with Daphnia. Most scientists use their own procedures developed at their home laboratory, as regards type of food and water, variables which may influence the susceptibility to chemicals, e.g. copper (Winner et al, 1977).

The aim of this study was to compare the toxicity of five chemicals to water fleas (Daphnia magna) cultured in either hard or soft water. The toxicity tests were made in reconstituted waters and the five chemicals to be tested were, p,p'-DDT, pentachlorophenol (PCP), tetrapropylenbenzyl sulfonate (TPBS), potassium bichromate ($K_2Cr_2O_7$) and zincsulfate ($ZnSO_4 \times 7H_2O$).

MATERIALS AND METHODS

For the purpose of this study Daphnia magna was cultured for several generations in both soft water and hard water. The soft water was aged local tapwater with a hardness of 50 mg/L as $CaCO_3$. Hard water for the cultures was prepared by addition of the chemicals in Table 1 to tapwater (300 mg/L as $CaCO_3$). Fifty percent of the water was renewed once a week. All cultures were fed Monday to Friday and with

no more than was consumed over night. The food suspension was a homogenate of tropical fish food (Tetra SM 80, Tetra Werke, W. Germany, and Wardleys basic food flakes, Wardley Prod., USA; 25 g of each/1 L deionized water). The cultures were aerated, kept at room temperature and illuminated by diffuse light from daylight-tubes 12 h a day. Juveniles (6-24 h old) for experiments were obtained from cultures containing 3-4 week old females.

Hard Reconstituted Water, HRW (Table 1), and hard and soft reconstituted water according to The Committee on Methods for Toxicity Tests with Aquatic Organisms (1975), was used as a dilution water in the toxicity tests. Stock solutions of PCP, $K_2Cr_2O_7$, TPBS and $ZnSO_4 \times 7H_2O$ were prepared in deionized water and for p,p'-DDT in acetone. All chemicals were of analytical grade. Test solutions were made in glass beakers. Concentrations were selected by logaritmic bisectioning. Test solutions with DDT were prepared by addition of acetone stock, evaporation to dryness, addition of water and mixing (Berglind and Dave, 1981). All test solutions were equilibrated at the regulated test temperature (20.5°C) for 2 h. The exposure was started (time 0) by addition of about 20 juveniles in 10 mL dilution water up to a final volume of 200 mL.

Water fleas which not were able to swim within 15 s after stimulation by water from the handling pipett, were considered immobilized. Median effective concentrations for immobilization (EC50's) after 24 and 48 h and 95 % confidence limits (CLs) were determined by log-probit analysis according to Davis (1971). Oxygen and pH was recorded prior to start and at termination. The oxygen concentration was between 92 and 100 percent of air saturation in all concentrations. In tests with HRW pH ranged 7.8 - 8.2 and in tests with hard and soft water 8.4 - 8.5 and 7.8 - 7.9, respectively.

Two experiments were made. In the first experiment all five chemicals were tested simultaneously in HRW with water fleas cultured in either hard or soft water. In the second experiment the toxicity of DDT was simultaneously tested in both hard and soft water with water fleas cultured in either hard or soft water.

Table 1. Quantities of reagent-grade chemicals required to prepare Hard Reconstituted water (HRW).

mg/L				Hardness as mg $CaCO_3$/L
$CaCl_2 \times 2H_2O$	$MgSO_4 \times 7H_2O$	$NaHCO_3$	$KHCO_3$	
240	215	100	20	250

Matrix: Milli Q Reagent Water (Millipore). pH adjusted to 7.8 by 1N HCl prior to use.

Table 2. Acute toxicity of five chemicals in hard reconstituted water to Daphnia magna cultured in either soft of hard water.

Culture water hardness	Time	EC50-values (95%-confidence limits)				
		$K_2Cr_2O_7$	Zn^{2+}	p,p'-DDT	PCP	TPBS
	h	mg/L	mg/L	µg/L	mg/L	mg/L
Soft	24	1.8 (1.6-2.0)	5.3 (3.9-7.0)	510 (230-1120)	0.50 (0.45-0.56)	8.5 (6.5-12)
Hard	24	1.7 (1.4-2.0)	3.0 (2.0-4.4)	71 (41 - 130)	0.51 (0.46-0.56)	8.7 (7.6-10)
Soft	48	0.77 (0.65-0.91)	1.7 (1.3-2.1)	1.1 (0.89-1.7)	0.37 (0.32-0.44)	4.0 (2.3-7.1)
Hard	48	0.90 (0.74-1.1)	1.1 (0.89-1.4)	0.68 (0.46-1.0)	0.44 (0.40-0.48)	7.1 (6.0-8.5)

Table 3. Acute toxicity of p,p'-DDT in hard and soft water to Daphnia magna cultured in either soft or hard water.

Culture water hardness	Time h	EC50-values (95% CL) µg/l	
		soft dilution water	hard dilution water
Soft	24	-	98 (75 - 127)
Hard	24	0.99 (0.66 - 1.49)	42 (32 - 56)
Soft	48	-	1.3 (1.1 - 1.5)
Hard	48	-	0.50 (0.41 - 0.61)

- Excessive mortality in the controls

RESULTS AND DISCUSSION

The 24- and 48-h EC50's from the first experiments are given in Table 2, and for the second experiment in Table 3. The hardness of the culture water did not affect the toxicity of PCP, $K_2Cr_2O_7$, TPBS or Zn^{2+}. However, DDT was more toxic to hard water cultured than to soft water cultured water fleas, especially after 24 h. In the second experiment all soft water cultured water fleas died within the first 24 h in the soft diultion water, but hard water cultured water fleas survived. DDT was about 40 times more toxic to the water fleas in the soft compared to the hard dilution water (Table 3).

The present study has shown that the hardness (mg $CaCO_3$/L)of the Daphnia culture medium affected the sensitivity to DDT but not to PCP, $K_2Cr_2O_7$, TPBS or Zn^{2+} in hard water. In a similar study by Maki and Bishop (1979) the sensitivity of D. magna to an anionic - but not a nonionic surfactant was affected by the culture water hardness. In both studies the acclimatization to soft water decreased the sensitivity to certain chemicals like DDT and LAS (linear alkyl benzene sulfonate), but not to others like PCP, $K_2Cr_2O_7$, TPBS, Zn^{2+} and NEODOL 45-7 (nonionic surfactant). The reason for this is probably a difference in mode of action.

DDT, which is mainly considered to alter the transport of sodium

and potassium ions across the membranes of nerve axons (Murphy, 1980), has also been shown to inactivate the osmoregulatory enzymes (Na, K-ATPases) in gills of fish (Leadman et al, 1974) and crustaceans (Neufeld and Pritchard, 1979). A possible explanation to the lower sensitivity of the soft water cultured water fleas may thus be that these had an overcapacity of osmoregulatory enzymes when transferred to hard water. Induction of these enzymes has been observed in blue crabs transferred from high to low saline water (Mantel and Olson, 1976; Towle et al, 1976).

This study and that by Maki and Bishop (1979) have both shown that the hardness of the culture medium can affect the toxicity of certain chemicals. Goulden et al. (1981) has shown that offsprings from water fleas fed on a trout-chow mixture were more sensitive to isophorone, polychlorinated biphenyl and copper, than offsprings from water fleas which were fed on algae. Thus, culture conditions like water hardness and type of food can affect the acute toxicity of certain chemicals even if the exposure is made under identical conditions. As a logical consequence the "normal" sensitivity to a reference toxicant, e.g. potassium bichromate, does not necessarily imply a "normal" sensitivity to all other toxicants. Nevertheless, the use of a reference toxicant like potassium bichromate is a necessary step towards harmonization of toxicity tests, because it can reflect other causes of variation related to the condition of the test organisms as well as to the composition of the dilution water (Müller, 1980). However, the results from this study together with the recent results by Maki and Bishop (1979) and by Goulden et al. (1981) suggest that the culture medium should be as similar to the test medium as possible and the kind of food should be described.

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