Bull. Environ. Contam. Toxicol. (1988) 40:204–211

Bulletin of Environmental Contamination and Toxicology

Toxicity of Sodium Chromate and 3,4-Dichloroaniline to Crustaceans

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Small adult and larval crustaceans are important components of a number of food webs. Therefore, it is important on the one hand to determine the sensitivity of these crustaceans to environmental contaminants and on the other hand to select the most sensitive species as test organisms to help establish the permissible contamination. Daphnia magna is by far the most investigated fresh water crustacean. Nevertheless the interpretation of the results obtained with this species is still open to discussion (see e.g. Müller 1980a; Müller 1980b; Parkhurst et al. 1981; Berglind and Dave 1984). In brackish water adults as well as larvae of the species Palaemonetes pugio and Uca pugilator have been most frequently investigated. These species can also be studied in sea water. The marine species Carcinus maenas, Crangon septemspinosa and Homarus americanus have also been studied relatively intensively.

The present paper describes the toxicity, at different salinities, of Na_2CrO_4 (chromate) and 3,4-dichloroaniline (3,4-DCA) to a number of relatively small adult and larval crustaceans. Chromate was chosen as a model of a heavy metal since, although its toxicity may be relatively low (Andersen 1950), it may under certain circumstances have considerable environmental effects (Trabalka and Gehrs 1977; Bookhout et al. 1984). Moreover, bichromate has been recommended as a standard in Daphnia tests. 3,4-dichloroaniline was chosen as a model of a chlorinated hydrocarbon, the handling of which does not need special precautions.

MATERIALS AND METHODS

Palaemonetes varians (Leach) was caught in brackish ditches in the province of Zeeland, The Netherlands. It was kept in 100% sea water (s.w.) at 15°C and fed with mashed shrimps and Enchytraeus albidus Henle. Young adults of about 2 cm were put separately in glass cylinders (outer diameter 3 cm, length 10 cm) with 12 slits (1.5 x 15 mm). Five cylinders were put in a jar of 500 mL. The animals were fed daily with mashed Enchytraeus.

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Control groups showed no mortality. Egg bearing females were separated. The morning after hatching, groups of 15 larvae were put in 500 mL and fed daily with Artemia salina L. nauplii. Control groups showed no mortality. Adults and larvae could be transferred from 100% s.w. (salinity 33 o/oo) to 10% s.w.(salinity 3.3 o/oo) without any ill effect.

Palaemon elegans Rathke was caught in the Oosterschelde, Zeeland, The Netherlands. Egg bearing females were put separately in 100% s.w. and fed with mashed shrimps and Enchytraeus. The morning after hatching, groups of 15 larvae were put in 500 mL. They were fed daily with Artemia nauplii. The control groups showed no mortality.

Neomysis integer (Leach) was caught in a lake near Landsmeer, North Holland, The Netherlands. It was kept in 10% s.w. at 20°C and fed with Artemia nauplii and mashed Enchytraeus. Adults were put in the cylinders described for P.varians (5 per 500 mL) and fed daily with Artemia nauplii and Scenedesmus sp.. Mortality in the control groups started at day 14, mortality was 20% at 15.0 days, 50% at 19.1 days and 80% at 24.3 days. Therefore, the experiments were restricted to 14 days.

Praunus flexuosus (Müller) was collected in the Oosterschelde, Zeeland, The Netherlands. It was kept in 100% s.w. at 20°C and fed with Artemia nauplii and mashed Enchytraeus. Six adults were put in 500 mL and fed daily with Artemia nauplii. Control mortality was negligible.

Daphnia magna Strauss, obtained from the Laboratory of Aquatic Ecology, University of Amsterdam, The Netherlands, was kept in 10% s.w. at 20°C and fed with Chlorella pyrenoidosa. Young specimens on the 1st or 2nd day after hatching were used (15 per 500 mL). They were fed daily with Chlorella. Mortality in the control groups was 2% on day 10 and 7% on day 26.

Sea water (s.w.) was made by dissolving Wiegandt (Krefeld, F.R.G.) sea salt plus bio-elements in distilled water to a salinity of 33 o/oo, pH 8.3. Seventy percent s.w. (salinity 23 o/oo) and 10% s.w. (salinity 3.3 o/oo) were obtained by dilution with distilled water. To the 10% s.w. ("fresh water") was added per L: 100 mg $NaHCO_3$, 20 mg $KHCO_3$, 200 mg $CaCl_2 . 2H_2O$ and 180 mg $MgSO_4$, pH 8.4, hardness 880 mg/L as $CaCO_3$.

Artemia nauplii, obtained from 50 mg eggs, were added to each experimental jar. Scenedesmus sp. was cultured in distilled water to which was added per L: 26 mg $KH_2PO_4 . 3H_2O$, 50 mg Na_2CO_3, 76 mg $NaSiO_3$, 33 mg $FeCl_3 .6 H_2O$, 104 mg $NaNO_3$ and 50 mL soil extract, at 15°C exposed to Philips 33 fluorescent light, day length 16h. Chlorella pyrenoidosa was cultured in a synthetic medium. (Macronutrients acording to Rodhe, micronutrients according to

Beyerinck, see Wayne Nichols, 1973). About 2 x 10^6 algal cells were added daily to the experimental jars, resulting in 4 x 10^3 cells per mL.

All tests were made at 20°C (unless otherwise stated) in cylindrical glass jars containing 500 mL of aerated water. The water and food in the jars was renewed daily. Animals, alive and dead (or motionless) were counted daily. Chromate was added daily in at most 10 mL aqueous solution, 3,4-dichloroaniline was dissolved in ethanol and added daily in a volume of 1 mL or less.

Usually the results of a number of experiments performed on different days were taken together. The minimum effective concentration (MEC) was determined by using the trend test (Peto et al. 1980) for "composite" contingency tables. In this method, the numbers of actual deaths in the control and the treatment group ("observed" =0) are determined on each day and the number of "expected" deaths (E) is calculated from the sum of the deaths in both groups in proportion to the numbers of animals alive in each group on the previous day. Dose rate times (O-E) gives the trend statistic T. The variance of T (V) is calculated for each day (Peto et al. 1980). The T's and V's obtained on each day are summed and $z = T/\sqrt{V}$ is calculated. A $z \geqq 1.96$ corresponds to a one-tailed P (P_1) $\leqq 0.025$ and is considered to indicate a significant increase in mortality. MEC is the lowest concentration at which $z \geqq 1.96$. For larval development the number of larvae reaching the 1st postlarval stage is taken instead of the number of deaths. In this case $z = \leqq -1.96$ corresponds to a one-tailed P (P_1) $\leqq 0.025$ and indicates a significant retardation of larval development. In some experiments a "no observed effect" concentration (NOEC), usually z = 0 (one-tailed P (P_1) = 0.50) could be determined. LC_{50}'s at 4 and 10 days were estimated by using the method of Litchfield and Wilcoxon (1949). For larval development the day at which 20%, 50% and 80% of the larvae reached the 1st postlarval stage were estimated from plots according to Litchfield and Wilcoxon (1949). In experiments with N.integer (used up to 14 days, when control mortality became considerable) and D.magna (slight mortality) the following correction was made

$t' = \frac{t-c}{100-c} \times 100$; t'= corrected % mortality (M) in the test group,

t= observed % M in the test group, c= % M in the corresponding controls. For larval development, the number of 1st postlarval animals on a certain day was calculated as % of all animals surviving at that day.

RESULTS AND DISCUSSION

A total of 255 young adults of Palaemonetes varians (including controls) was exposed for 26 to 40 days at 33 o/oo, 23 o/oo and 3.3 o/oo salinity to chromate from 10^{-4} to 2 x 10^{-3} mol/L. Average group size was 13. Table 1 shows that chromate is equally toxic at 33 and 23 o/oo salinity but about 3 times more toxic at 3.3 o/oo (10^{-4} mol/L corresponds to about 5 ppm chromium).
In all, 662 larvae of Palaemonetes varians, were exposed for 14 to 32 days at 33, 23 and 3.3 o/oo salinity to chromate from 10^{-5}

to 2 x 10^{-3} mol/L. Average group size was 29. Table 1 shows that the mortality at 33 and 23 o/oo is comparable, the larvae being as sensitive as the adults. According to the MEC (lowest concentration at which the one-tailed P (P_1) ≦ 0.025) chromate is about 30 times more toxic at 3.3 o/oo than at the higher salinities, while in this medium the larvae are about 10 times more sensitive than the adults. For retardation of larval development the MEC's are very similar to those for mortality. Only the results obtained at 3.3 o/oo salinity are in agreement with the general assumption that crustacean larvae are more sensitive than adults (see e.g. Greenwood and Fielder 1983).

Table 1. Chromate, added daily. Effect on mortality (M) and larval development (LD). MEC = minimum effective concentration (lowest concentration at which one-tailed P (P_1) ≦ 0.025), NOEC = no observed effect concentration (P_1=0.50 unless otherwise stated). In parentheses, for M: period of exposure (days), for LD: day at which all (remaining) larvae had reached the 1st postlarval stage. Sal.=Salinity, o/oo, P.var: <u>Palaemonetes varians</u>, P.el: <u>Palaemon elegans</u>, P.flex: <u>Praunus flexuosus</u>, N.int: <u>Neomysis integer</u>, D.mag: <u>Daphnia magna</u>. a: adults, l: larvae, y: young

Sal., o/oo	Species	M or LD	MEC,mol/L	NOEC,mol/L	Estimated LC50,mol/L 4d	10d
33	P.var,a	M	$2x10^{-4}$ (26)	-	$1.1x10^{-3}$	$6.9x10^{-4}$
	P.var,l	M	$2x10^{-4}$ (30)	10^{-4} (30)	$6.2x10^{-4}$	$3.9x10^{-4}$
	P.var,l	LD	10^{-4} (14)	-	-	-
	P.el,l	M	10^{-4} (23)	$3x10^{-5}$ (38)	$2.6x10^{-4}$	$1.4x10^{-4}$
	P.el,l	LD	$3x10^{-5}$ (38)	10^{-5} (36)**	-	-
	P.flex,a	M	10^{-4} (6)	$5x10^{-5}$ (6)	$1.3x10^{-4}$	$5.5x10^{-5}$
23	P.var,a	M	$2x10^{-4}$ (28)	10^{-4} (28)	$1.6x10^{-3}$	$6.3x10^{-4}$
	P.var,l	M	$2x10^{-4}$ (19)	10^{-4} (23)	$6.2x10^{-4}$	$3.9x10^{-4}$
	P.var,l	LD	10^{-4} (13)	-	-	-
	P.flex,a	M	$6x10^{-5}$ (23)	$2x10^{-5}$ (23)	$1.1x10^{-4}$	$3.9x10^{-5}$
3.3	P.var,a	M	$6x10^{-5}$ (40)	-	$2.6x10^{-4}$	$8.9x10^{-5}$
	P.var,l	M	$6x10^{-6}$ (30)	-	$7.4x10^{-5}$	$3.6x10^{-5}$
	P.var,l	LD	$6x10^{-6}$ (32)	-	-	-
	N.int,a	M	$6x10^{-6}$ (14)	$3x10^{-6}$ (14)***	-	$7.0x10^{-6}$*
	D.mag,y	M	$6x10^{-6}$ (30)	10^{-6} (16)	$3.0x10^{-5}$*	$1.8x10^{-5}$*

* corrected for mortality in the controls. **P_1=0.275.
***P_1 =0.316.

Table 2 shows that only a limited range of concentrations can be used to study the effect on larval development, while only at 33 o/oo a marked retardation of development was observed without accompanying mortality.

A total of 151 Palaemonetes varians larvae, with an average group size of 30, was exposed for 8 h, 1 day or 2 days to a number of concentrations of chromate. Table 3 shows that at 33 o/oo, exposure to 3-6 times the MEC during the first 2 days may cause a significant delay of the larval development (see P_1 values) even without mortality. At 3.3 o/oo exposure to 5 times the MEC during the first two days or to 10 times the MEC for 8h caused a significant delay in larval development with little mortality. Longer exposure to 10 times the MEC caused considerable mortality.

In all, 75 larvae of Palaemon elegans were exposed for 23 to 38 days to chromate from 10^{-5} to 3×10^{-4} mol/L at 33 o/oo salinity, with an average group size of 15. In these experiments the temperature was 17°C at night and 20°C during the day. Table 1 shows that the sensitivity to chromate of P.elegans larvae is comparable to that of P.varians larvae. Table 2 shows that the range of concentrations useful to study the effect on larval development is again very narrow. At a temperature of 20°C for 24 h, 20%, 50% and 80% of the control larvae reached the 1st postlarval stage after 16.6, 17.3 and 17.9 days, respectively.

Table 2. Chromate added daily at the indicated concentrations. Effect on larval development expressed as Et50 (number of days until 50% of the larvae had reached the 1st postlarval stage) and the period from Et20-Et80 (when 20%, respectively 80% had reached the 1st postlarval stage). Mortality (%) during Et20-Et80. MEC's (see Table 1) in parentheses.

P.varians			Chromate, mol/L			
Salinity, o/oo		Control	(10^{-4})	$2x10^{-4}$	$3x10^{-4}$	$6x10^{-4}$
33	Et50	9.0	11.7	12.1	17.6	no
	Et20-Et80	8.2-9.7	11.0-12.5	11.6-12.6	14.1-22.1	postl.
	Mortality	0-0	0-0	0-0	7-33	100
23	Et50	9.1	10.5	16.1	16.3	no
	Et20-Et80	8.3-9.9	10.0-11.2	13.1-19.6	13.6-19.7	postl.
	Mortality	0-0	3-3	0-60	20-80	100
		Control	($6x10^{-6}$)	10^{-5}	$2x10^{-5}$	$3x10^{-5}$
3.3	Et50	12.4	17.1	18.9	28.9	no
	Et20-Et80	11.4-13.4	14.5-20.2	15.6-22.9	25.0-33.6	postl.
	Mortality	0-0	11-16	17-20	33-60	100
P.elegans						
		Control	10^{-5}	($3x10^{-5}$)		10^{-4}
33	Et50	25.8	25.3	31.2		no
	Et20-Et80	23.9-27.4	23.8-26.9	28.7-34.0		postl.
	Mortality	0-0	7-7	7-7		100

A total of 90 adults of Neomysis integer was exposed for 14 days at 3.3 o/oo salinity to chromate from 10^{-6} to 3 x 10^{-5} mol/L with an average group size of 13. Control mortality was 15% at 14 days. Table 1 shows that chromate was about equally toxic to N.integer adults and P.varians larvae. Four day LC50 could not be estimated.

A total of 60 adult Praunus flexuosus was exposed for 6 to 23 days to chromate from $2x10^{-5}$ to $6x10^{-4}$ mol/L at 33 and 23 o/oo salinity, with an average group size of 6. Table 1 shows that chromate is about equally toxic at both salinities, while in both media P.flexuosus adults are possibly slightly more sensitive than P.varians adults or larvae.

A total of 165 young Daphnia magna was exposed for 16 to 30 days to chromate from 10^{-6} to 10^{-4} mol/L at 3.3 o/oo salinity. Average group size was 24. Control mortality was 2% at 4-10 days, 4% at 11 to 14 days and 7% at 15 to 30 days. Chromate is about equally toxic to D.magna, P.varians larvae and N.integer adults (Table 1).

Table 3. Chromate, exposure for 8 h, 1 and 2 days. Effect on larval development. Et20, Et50, Et80 = time (days) at which 20%, 50% or 80% of the larvae reached the 1st postlarval stage. Mortality (M), during the period from Et20 to Et80. P_1 = one tailed probability.

Salinity, o/oo	Species	Period of Exposure	Concentration, mol/L	P_1	Et50,d	M, o/o
		-	Controls	-	8.6	0-0
33	P.varians	day 1 and 2	$6x10^{-4}$	<0.0001	12.5	0-0
		day 1 and 2	$3x10^{-4}$	0.0005	11.0	0-0
		day 1 and 2	10^{-4}	0.4878	8.3	0-0
		-	Controls	-	12.6	0-0
		first 8h	$6x10^{-5}$	0.0028	14.4	0-6
3.3	P.varians	day 1	$6x10^{-5}$	0.0016	14.9	87-87
		day 1 and 2	$6x10^{-5}$	0.0002	16.4	73-73
		day 1 and 2	$3x10^{-5}$	<0.0001	14.6	11-13

The results of experiments with chromate may not be extrapolated to other heavy metals. In sea water P.flexuosus adults, which are available the year round, were about equally sensitive as P.elegans or P.varians larvae which are only available during the summer. In "fresh" water none of the organisms tested were more sensitive than D.magna, and since this species is easy to culture and has been used widely, it is to be preferred. There is no indication that mysids are more sensitive than other crustaceans as suggested by Nimmo et al. (1978) and Buikema et al. (1981).

A total of 30 young adults of Palaemonetes varians was exposed for 14 to 20 days to 3,4-DCA from 10^{-5} to 10^{-4} mol/L at 3.3 o/oo salinity. Average group size was 10 (see Table 4). (10^{-5} mol/L corresponds to 1.6 ppm).

In all, 105 Palaemonetes varians larvae were exposed for 12 to 30 days to 3,4-DCA from 10^{-6} to 10^{-4} mol/L at 33 and 3.3 o/oo salinity. Average group size was 15. Table 4 shows that in both media MEC and NOEC for mortality and larval development are identical, while the difference in salinity has no effect. At 3.3 o/oo the larvae are somewhat more sensitive than the adults. Table 5 shows that only a very small range of concentrations can be used to study the effect on larval development.

Table 4. 3,4-dichloroaniline added daily. Effect on mortality (M) and larval development (LD). Compare with caption of Table 1. LC50's for N.integer and D.magna corrected for control mortality.

Sal., o/oo	Species	M or LD	MEC, mol/L	NOEC, mol/L	Estimated LC50, mol/L 4d	10d
33	P.var,l	M	6x10^{-6} (30)	3x10^{-6} (30)	2.0x10^{-5}	1.1x10^{-5}
	P.var,l	LD	6x10^{-6} (21)	3x10^{-6} (12)*	-	-
3.3	P.var,a	M	3x10^{-5} (14)	10^{-5} (20)	4.0x10^{-5}	1.9x10^{-5}
	P.var,l	M	6x10^{-6} (30)	3x10^{-6} (30)	8.1x10^{-6}	5.8x10^{-6}
	P.var,l	LD	6x10^{-6} (25)	3x10^{-6} (15)**	-	-
	N.int,a	M	10^{-5} (6)	3x10^{-6} (14)	9.1x10^{-6}	6.3x10^{-6}
	D.mag,y	M	10^{-7} (30)	3x10^{-8} (30)***	7.6x10^{-7}	2.6x10^{-7}

*P_1=0.034 **P_1=0.331 ***P_1=0.160.

Table 5. 3,4-dichloroaniline added daily at the indicated concentrations. Effect on larval development of Palaemonetes varians. Compare with caption of Table 2. MEC (Table 4) in parentheses.

Sal., o/oo		Control	3,4-DCA, mol/L 10^{-6}	3x10^{-6}	(6x10^{-6})	10^{-5}
33	Et50	11.2	-	10.0	15.0	18.7*
	Et20-Et80	10.3-11.5	-	9.1-11.1	12.7-17.7	16.6-20.6*
	Mortality	0-0	-	0-0	37-53	80-87*
3.3	Et50	11.8	12.5	11.8	25.2*	no
	Et20-Et80	10.9-12.9	11.0-13.8	10.9-12.8	22.6-27.2*	postl.
	Mortality	0-0	0-0	0-0	80-80*	100

*rough estimate

Eighty adult Neomysis integer were exposed for 6 to 14 days to 3,4-DCA from 3 x 10^{-6} to 10^{-4} mol/L at 3.3 o/oo salinity. Average group size was 13. Control mortality was 15% on day 14. N.integer is about equally sensitive as P.varians larvae (Table 4).

A total of 135 young Daphnia magna was exposed for 30 days to 3,4-DCA from 3 x 10^{-8} to 10^{-6} mol/l. Average group size was 27. Mortality in the control groups was 2% at 4-10 days, 4% at 11-14 days and 7% at 15-30 days. Table 4 shows that at 3.3 o/oo D.magna is about 100 times more sensitive than the other species tested.
The results obtained with 3,4-DCA indicate that in "fresh" water D.magna is the organism of choice. It is noteworthy that,

in contrast to the results obtained with chromate, P.varians larvae are equally sensitive at 33 o/oo and 3.3 o/oo.

Acknowledgment. The authors are indebted to Dr. M. Wijnans for his statistical advise.

REFERENCES

Anderson BG (1950) The apparent tresholds of toxicity to Daphnia magna for chlorides of various metals when added to lake Erie water. Trans Am Fisheries Soc 78:96-113

Berglind R and Dave G (1984) Acute toxicity of Chromate, DDT, PCP, TPBS and Zinc to Daphnia magna cultured in hard and soft water. Bull Environ Contam Toxicol 33:63-68

Bookhout CG, Monroe RJ, Forward RB, Costlow JD (1984) Effects of hexavalent chromium on development of crabs, Rhitropanopeus harrisii and Callinectus sapidus. Water, Air and Soil Pollution 21:199-216

Buikema AL, Niederlehner BR, Cairns J (1981) The effects of a simulated refinery effluent and its components on the estuarine crustacean, Mysidopsis bahia. Arch Environ Contam Toxicol 10:231-240

Greenwood JG, Fielder DR (1983) Acute toxicity of zinc and cadmium to zoeae of three species of portund crabs (Crustacea:Brachyura). Comp Biochem Physiol 75C: 141-144

Litchfield JT, Wilcoxon F (1949) A simplified method of evaluating dose-effect experiments. J.Pharmacol Exp Ther 96: 99-113

Müller HG (1980a) Acute toxicity of potassium dichromate to Daphnia magna as a function of water quality. Bull Environ Contam Toxicol 25: 113-117

Müller HG (1980b) Experiences with test systems using Daphnia magna. Ecotoxicol Environ Safety 4: 21-25

Nimmo DR, Rigby RA, Bahner LH, Sheppard JM (1978) The acute and chronic effects of Cadmium on the estuarine mysid, Mysidopsis bahia. Bull Environ Contam Toxicol 19: 80-85

Parkhurst BR, Forte JL, Wright GP (1981) Reproducibility of a life-cycle toxicity test with Daphnia magna. Bull Environ Contam Toxicol 26: 1-8

Peto R, Pike MC, Day NE, Gray RG, Lee PN, Parish S, Peto J, Richards S, Wahrendorf J (1980) Guidelines for simple, sensitive significance tests for carcinogenic effects in long-term animal experiments. Annex IARC Monographs, Supplement 2, Lyon, France

Trabalka JR and Gehrs CW (1977) An observation on the toxicity of hexavalent chromium to Daphnia magna. Toxicol Lett 1:131-134

Van Weerelt M, Pfeiffer WC, Fiszman M (1984) Uptake and release of ^{51}Cr (VI) and ^{51}Cr (III) by Barnacles (Balanus sp). Mar Environ Res 11:201-211

Verriopoulos G and Moraïtou-Apostopoulou M (1981) Impact of chromium to the population dynamics of Tisbe holothuriae. Arch Hydrobiol 93: 59-67

Wayne Nichols H (1973) Growth media-fresh water. In Stein IR Handbook of Phycological methods p 7-25. Cambridge University Press, Cambridge, UK

Received May 5,1987;accepted November 9,1987