The Toxicity of Sodium Pentachlorophenolate for Three Species of Decapod Crustaceans and Their Larvae

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Introduction

Pentachlorophenol (PCP) is used extensively as a biocide and has been detected in toxic concentrations in aquatic environments.

PCP is a potent uncoupler of oxidative phosphorylation (WEINBACH 1954, 1956) and this effect has been considered to be at least partly responsible for its toxic effects (WEINBACH and NOLAN 1956). Apart from the effect on oxidative phosphorylation stimulation or inhibition of a large number of enzyme systems has been described (KRUEGER et al. 1966, ISHAK et al. 1970, ISHAK 1972, BOSTRÖM and JOHANSSON 1972). According to HOLMBERG et al. (1972) the effect of subtlethal concentrations of PCP in the eel Anguilla anguilla L. is comparable to an exercise or stress situation.

According to GOODNIGHT (1942), MANN (1957) and WEBER (1965) the lethal concentrations of PCP for a number of fishes vary between 0.2 and 1 ppm. For Anguilla anguilla it must lie somewhat above 0.1 ppm (HOLMBERG et al. 1972) while DAVIS and HOOS (1975) showed that for a number of salmonids the LC_{50} 96 h at 10°C varies from 0.037 to 0.130 ppm.

WEINBACH and NOLAN (1956) mention that a concentration of 3 ppm kills a number of water snails. According to MANN (1957) and WEBER (1965) Tubifex sp. can survive at 0.5 ppm. According to GOODNIGHT (1942) insect larvae survive at 5 ppm and according to MANN (1957) at 1.1 to 2.2 ppm.

GOODNIGHT (1942) stated that Cambaris virilis Hagen, the amphipod Hyelella knickerbockeri (Bate), Daphnia pulex (de Geer) and Asellus communis Say survived at 5 ppm Na-PCP. MANN (1957) mentions that Cladocerans and Amphipods survive at 0.5 ppm, while WEBER (1965) exposed Daphnia magna to 1.0 ppm and found 30% mortality after 6 h and 100% mortality after 21 h, but no mortality at 0.5 ppm.

In the present paper the toxicity of Na-PCP for two marine decapods i.e. Crangon crangon (Linnaeus) and Palaemon elegans (Rathke) and one brackish water decapod i.e. Palaemonetes varians (Leach) has been

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compared using adults as well as 1st instar larvae.

Methods

The following decapod Crustaceans were used. Crangon crangon (Linnaeus, 1758), obtained from the National Institute for Sea Research (NIOZ) at den Helder. Palaemon elegans (Rathke, 1837), captured in the Oosterschelde near Yerseke. Palaemonetes varians (Leach, 1814), captured in brackish water on the island of Tholen

Before the experiments the adult animals were kept in natural sea water at 15°C for at least two weeks. They were fed with Enchytraeus albidus Henle.

Females carrying eggs were isolated and the hatched larvae were used on either the first or the second day after hatching.

The adults were put in groups of 5 animals, irrespective of sex, in glass aquaria containing 5 l natural sea water unless otherwise stated. Usually 10 animals (2 aquaria) were used as controls while a number of groups of 10 animals were exposed to different concentrations of Sodium pentachlorophenolate. The adults were fed daily with E.albidus.

The larvae were kept in glass jars (inner diameter 76 mm) each containing either 5 or 10 larvae in 100 ml natural sea water. During the experiment the larvae were offered Artemia salina L. nauplii, finely cut E. albidus and phytoplankton mainly consisting of Nannochloris sp..

The temperature was 15°C unless otherwise stated, the illumination 10h with 2 Philips TL 40 W/33 fluorescent tubes 100 cm above the containers.

The aquaria were aerated by passing large air bubbles at a low rate. The jars containing the larvae were not aerated.

The pH of the water remained essentially constant, it varied only with the batch of natural sea water used, between 7.5 and 8.0.

In most experiments the water was not changed. In a few experiments in which fresh solutions were used daily, the mortality rates were not essentially different.

Sodium pentachlorophenolate (Na-PCP) (Merck-Schuchardt zur Synthese) was dissolved in natural sea water. A stock solution of 1000 ppm was made freshly every 2 weeks, and diluted just before each experiment.

Percentage mortality was corrected for natural mortality (FINNEY 1952), which was only observed in the experiments with C.crangon larvae (10% in 96 h).

Dose-mortality data were analysed and compared using the probit analysis (FINNEY 1952). If more than two regression lines were compared, Bonferroni statistics were used (MILLER 1966). If a comparison of regression lines was impossible, (multiple) 2 x 2 tables were used,

using Fisher's exact method and Stouffer's method for combining independent tests of significance (DE JONGE 1964) and, if appropriate, Bonferroni statistics. If time-mortality data were evaluated the Lt_{50} was estimated by fitting a straight line by eye through points plotted on semilogarithmic probability paper. Comparison of survival times was performed using Wilcoxon's rank sum test (WILCOXON 1945), exact probabilities being read from the tables constructed by WABEKE and VAN EEDEN (1955).

An overall level of significance of 0.05 was adopted.

Results

Mortality caused by Na-PCP in natural sea water at 15°C.

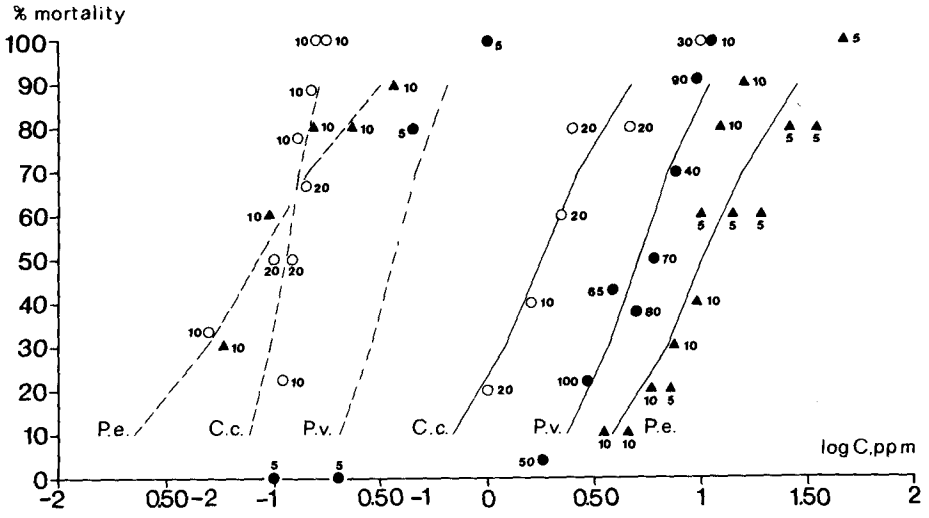

fig.1 Percentage 96 h mortality plotted against log. concentration (in ppm) of Na-PCP. Natural sea water, 15°C.
O Crangon crangon, ▲ Palaemon elegans,
● Palaemonetes varians
—— adults, --- 1st instar larvae

Fig. 1 shows the observed 96 h mortalities at different Na-PCP final concentrations for the adults and the 1st instar larvae of C.crangon, P.elegans and P.varians in 100% natural sea water at 15°C. The lines in fig. 1 are drawn between the LC_{10}, LC_{30}, LC_{50}, LC_{70} and LC_{90} values taken from the calculated log.concentration-probit (straight) regression lines. The LC_{50}'s and the slopes of the calculated probit regression lines are presented in table 1.

TABLE 1

LC_{50} and 95% confidence limits (lower limit: ll; upper limit: ul) and slope of the log.concentration-probit regression lines for adults (a) and 1st instar larvae (l) of C.crangon, P.elegans and P.varians exposed to Na-PCP for 96 h at 15°C in natural sea water.

species		LC_{50}, ppm	ll, ppm	ul, ppm	slope(b)	standard error of b
C.crangon	a	1.79	1.36	2.21	3.05	0.566
	l	0.112	0.097	0.122	7.89	1.741
P.elegans	a	10.39	8.33	13.19	2.95	0.563
	l	0.084	0.027	0.125	2.22	0.766
P.varians	a	5.09	4.72	5.50	3.77	0.327
	l	0.363	0.200	0.680	5.11	2.000

TABLE 2

Ratio's (R) and overall 95% confidence limits of the LC_{50}'s of adults (a) and 1st instar larvae (l) of C. crangon, P.elegans and P.varians exposed to Na-PCP in natural sea water for 96 h at 15°C. For each separate ratio the confidence limits were 100(1-0.05/9)=99.44%. For the calculation of the ratio's the common slope (b=3.44) was used.

Comparison	R	ll	ul
P.elegans(a) vs.C.crangon(a)	5.61	3.88	8.24
P.varians(a) vs.C.crangon(a)	2.77	2.08	3.76
P.elegans(a) vs.P.varians(a)	2.02	1.52	2.69
P.elegans(l) vs.C.crangon(l)	1.04	0.66	1.65
P.varians(l) vs.C.crangon(l)	3.89	1.64	9.49
P.varians(l) vs.P.elegans(l)	3.74	1.51	9.46
C.crangon(a) vs.C.crangon(l)	19.60	13.54	28.65
P.elegans(a) vs.P.elegans(l)	105.55	67.40	169.35
P.varians(a) vs.P.varians(l)	13.98	6.01	32.54

Tests on linearity and parallelism permitted the calculation of ratio's between LC_{50}'s, using the common slope (see table 2). All ratio's were significantly different from 1 except the ratio for larvae of P.elegans and larvae of C.crangon.

Therefore, among the adults C.crangon was the most sensitive (lowest LC_{50}), while P.elegans was in average 5.6 and P.varians 2.8 times less sensitive than C.crangon. Among the 1st instar larvae C.crangon and P.elegans were about equally sensitive while P. varians was in average 3.8 times less sensitive. All 1st instar larvae were more sensitive than the corresponding adults, the average ratio being for C.crangon 20, for P.elegans 106 and for P.varians 14.

The adults of C.crangon surviving exposure to Na-PCP for 96 h nearly all died within 24 h after being transferred to normal sea water. All adults of P. elegans and P.varians (and also the larvae) surviving exposure for 96 h remained alive for at least a further 3 days in normal sea water.

The effect of body weight was investigated using P.varians adults exposed to 100 ppm Na-PCP in natural sea water at 15°C. In a group of 10 animals with an average body weight of 140 mg the Lt_{50} was 58 min, whereas in 10 animals with an average body weight of 620 mg the Lt_{50} was 78 min. According to Wilcoxon's test the difference between the Lt_{50}'s was not significant (P_2=0.6).

The effect of moulting was investigated using larvae of P.elegans. Since all surviving larvae had moulted after 96 h, the number of moulted larvae, either dead or alive, was counted after 72 h. The results are summarized in table 3. Statistical evaluation by combination of the 5 separate 2 x 2 tables showed that the number of moulted dead larvae did not differ significantly from the number of moulted living larvae (P_2=0.2).

TABLE 3

P.elegans 1st instar larvae. Number of moulted larvae observed in groups of larvae that either died or stayed alive after exposure to Na-PCP for 72 h in natural sea water at 15°C.

concentration, ppm	number of larvae				
	total	dead	moulted	alive	moulted
0	10	0	0	10	7
0.06	20	10	6	10	10
0.09	20	10	8	10	8
0.15	20	8	2	12	10
0.23	20	9	5	11	7
0.38	20	15	15	5	4

The effect of temperature and salinity

To study the effect of temperature, 96 h mortality was determined, using adult P.varians at a number of concentrations of Na-PCP in natural sea water at 5°C, 15°C and 25°C. In the same experiment the survival time at 3.8 ppm Na-PCP was determined. The results are presented in table 4.

TABLE 4

P.varians adults.
96 h mortality after exposure to different concentrations of Na-PCP and mortality at different times after exposure to 3.8 ppm Na-PCP, at 5°C, 15°C and 25°C in natural sea water.

concentration, ppm	mortality ratio 5°C	15°C	25°C	
3.0	1/10		8/10	
3.8	2/10	5/10	9/10	
4.8	4/10	7/10	10/10	96 h
6.0	4/10	8/10	9/10	mortality
7.5	5/10	9/10	10/10	
9.5		10/10		
time, h				
17		1/10		
24			4/10	
40	1/10	3/10		mortality
65	1/10	4/10	7/10	at
89		4/10		
96	2/10	5/10	9/10	3.8 ppm
163	3/10			

96 h mortality was significantly lower at 5°C than at 15°C, the ratio of the LC_{50}'s being 1.88 (ll 1.28, ul 5.31). Since the data at 25°C did not show a significant regression, a comparison was made using a combination of respectively 5 and 4 separate 2 x 2 tables. The mortality was significantly lower at 5°C than at 25°C ($P < 10^{-7}$) but at 15°C not significantly different from that at 25°C (P_2=0.2). The Lt_{50} at 3.8 ppm Na-PCP could not be estimated at 5°C; at 15°C it was 104 h and at 25°C 33 h. Wilcoxon's test showed no significant difference between the data obtained at 5°C and at 15°C (P_2=0.2) and between 15°C and 25°C (P_2=0.08). However, the survival time was significantly higher at 5°C than at 25°C ($P_2=2\times10^{-3}$).

The effect of salinity was also studied using adult P.varians. 96 h mortality was determined at 15°C in natural sea water and in sea water diluted to 70% and 30% respectively, with distilled water. The results are presented in table 5.

TABLE 5

P.varians adults. Mortality ratio's after exposure to different concentrations of Na-PCP in 100% (data of fig. 1), 70% and 30% natural sea water at 15°C.

concentration, ppm	96 h mortality			192 h mortality
	100%	70%	30%	30%
1.8	2/50	-	-	-
3.0	22/100	0/10	-	-
3.8	28/65	7/10	0/10	2/10
5.0	30/80	-	1/10	3/10
6.0	35/70	8/10	0/10	2/10
7.5	28/40	-	1/10	5/10
9.5	82/90	-	2/10	6/10
16.0	10/10	10/10	-	-

The mortality in 100% sea water was not significantly different from that in 70% sea water. The mortality in 30% sea water was significantly lower than that in 100% sea water ($P_2=3\times10^{-11}$) and in 70% sea water ($P_2=7\times10^{-6}$). After 192 h instead of 96 h in 30% sea water the mortality was somewhat increased (on the borderline of significance) but it was still significantly lower than after 96 h in 70% sea water.

Discussion

Among the adult decapods studied C.crangon was the most sensitive with an LC_{50} 96 h at 15°C of about 2 ppm. Since C.crangon is difficult to keep in the laboratory, its high sensitivity might be due to additional causes. The late mortality found in the survivors might be a confirmation of this notion. On the other hand all animals had been kept in the laboratory for at least two weeks before the experiments started, no mortality was found in the control aquaria containing C.crangon without added Na-PCP, and the data presented in fig. 1 are composed of three separate closely matching experiments. Therefore, the LC_{50} of C.crangon seems sufficiently well established.

Among the 1st instar larvae C.crangon and P. elegans were equally sensitive (LC_{50} 96 h 15°C about 0.1 ppm), while P.varians was less sensitive (LC_{50} about 0.4 ppm). Since the optimal conditions for rearing larvae of C.crangon and P.elegans are not well established rapid deterioration of the larvae might be a factor determining the high sensitivity in these species. On the other hand P.varians larvae are easily reared on either A.salina nauplii or mashed E.albidus alone, and therefore the conditions for these larvae must be considered as satisfactory. Since the P. varians larvae are about 14 times more sensitive than

P.varians adults, the higher sensitivity of the larvae may be real.

The period of moulting had no significant effect on the sensitivity of P.elegans larvae, which is surprising since the toxic effect of Na-PCP is ascribed to an inhibition of a number of metabolic processes.

The effect of temperature was studied using P. varians adults. If all results are combined the toxicity seems to increase with the temperature, roughly by a factor of 2 for each 10 degrees centigrade.

The effect of salinity was also studied using P.varians. No significant difference was observed when the salinity was lowered from 100% sea water to 70%. However, in 30% sea water the sensitivity was significantly decreased. These results are somewhat surprising since the osmotic concentration of the blood of P. varians is practically constant at a value comparable to 70% sea water (PANIKKAR 1941/43) and one would expect at that salinity the lowest toxicity of a compound inhibiting energy producing processes.

Obviously long term studies are needed in order to obtain a more complete picture of the toxicity of Na-PCP. However, long term experiments are difficult when larvae are used, which are relatively difficult to keep a.o. because they survive only for a few days without adequate food.

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