Polychlorinated Biphenyls (PCB) in Sea Water and Their Effect on Reproduction of *Gammarus oceanicus*

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Concentrations of PCB in sea water lethal to *Gammarus oceanicus* Sergestråle have been determined in continuous exposure experiments (1). This paper describes the effect of short-term exposure to PCB in sea water on the median lethal time and reproductive performance of *G. oceanicus*.

The solubility of Aroclor 1254 in sea water is inversely proportional to the degree of chlorination and in two batches was 0.3 to 1.5 mg/ℓ (2). Field samples from Escambia Bay, Florida, contained 0.0001 to 0.275 mg/ℓ of PCB with detection limits of 0.1 ppb (3). In the Firth of Clyde, Scotland, estuarine water contained no PCB with detection limits of 1 ppb (4). Using a more sensitive technique, PCB in the ppt range have been detected in lake and tap water (5).

Methods and Materials

Stock and test solutions were made up as described previously (1) with Aroclor 1254:Corexit 7664 (1:19 w/w ratio) in sea water (S = 29 ± 1‰). A temperature of 5.0 ± 0.5°C and a 12:12 hr light:dark photoperiod were provided throughout the experiments.

In experiment I, 2 groups of 5 animals were exposed for 1, 10, 32, 56, and 100 hr to Aroclor 1254 (1, 10, or 100 mg/ℓ) and transferred to 2-ℓ beakers containing clean sea water. Ten control animals for each exposure time were treated in clean sea water. The sea water in all beakers was changed weekly. Observations to determine the survival time were begun on exposure and continued for 30 days. The median lethal time (LT50) for *G. oceanicus* was estimated graphically from plots of the percentage of animals dead against time on probit and log scales, respectively (6).

In experiment II, two pairs of animals were exposed in 2-ℓ beakers for 1 or 10 hr to Aroclor 1254 (1, 10, or 100 mg/ℓ). Ten pairs were used at each concentration and exposure time. Each pair consisted of a male and

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female in precopula. Twenty pairs were treated in clean sea water and 10 pairs in 1.9 g/ℓ Corexit 7664 in sea water for 10 hr as controls (2 pairs/2-ℓ beaker). Observations were made to determine the survival time, mating success, brood size, and wet body weight of each female.

In experiment III, 14 females were removed from experiment II after producing a brood. They were pinned out in a wax dish containing filtered sea water (S = 29 ± 1‰) to which 100 mg/ℓ of chloromycetin had been added. Eggs were removed from the marsupium with a camel-hair brush and blunt needle under a binocular microscope. Eggs from each brood were transferred in a Pasteur pipette to a Petri dish containing filter paper wetted with the antibiotic sea water. Water evaporating from the dishes was replaced with distilled water. Observations with a binocular microscope were made through the Petri dish lid to determine the development and hatching success of the eggs.

Results

Experiment I

The results in Table 1 show that the LT50 was below the lower 95% confidence limits of the controls at 10 mg/ℓ (56 and 100 hr) and 100 mg/ℓ (32, 56, and 100 hr). Control mortality was due mainly to cannibalism by males which is known to be a function of density (7). Cannibalism was virtually absent in PCB-dosed animals, perhaps due to reduced palatability of, or interest by, them.

Survival time of PCB-dosed *G. oceanicus* depended on the initial concentration (C_0) and exposure time (t) and by plotting log LT50 (y) against log C_0t(x) a linear relationship was obtained. The fitted regression line was:

$$y = 1.62-0.25\ x\ (t = 8.52,\ d.f. = 13,\ p > 0.001)$$

This result suggested that Aroclor 1254 is a cumulative poison with a time lag before the poisoning mechanism causes death. It also demonstrated the difficulty of defining a sublethal dose. At 30 days 50% mortality had just occurred and it was estimated that C_0t = 1 mg/ℓ hr had an LT50 = 35.3 days (LT50 = 41.6 days calculated from the regression equation).

In previously obtained data below the 'critical concentration' for *G. oceanicus* (1) at 0.1 mg/ℓ, C_0t = 31.2 mg/ℓ hr with an LT50 = 13.0 days, and at

0.01 mg/ℓ C_0t = 5.0 mg/ℓ hr with an LT50 = 21.0 days, both of which fit the described regression equation. Values calculated from the regression equation are 17.6 and 27.8 days respectively.

TABLE 1

LT50 (days) and 95% confidence limits for groups of 10 *G. oceanicus* exposed to Aroclor 1254 in sea water

Exposure hr	LT50 days 1 mg/ℓ	10 mg/ℓ	100 mg/ℓ
1	35.3(10.7-116.0)	13.7(8.4-22.5)	13.6(10.0-18.5)
10	24.0(7.1-81.6)	12.0(6.1-23.8)	9.3(6.8-12.7)
32	17.0(9.0-32.0)	9.8(7.2-13.4)	5.9(3.2-10.8)
56	15.3(9.9-23.7)	6.0(3.7-9.8)	6.0(4.9-7.4)
100	10.7(6.9-16.7)	5.2(3.7-7.4)	4.4(4.0-4.8)

Control LT50 = 13.1(6.3-27.5, N = 50)

Experiment II

Populations of *G. oceanicus* normally breed throughout the year with a 3-month resting stage in the summer (8). A female in mid-intermoult is caught and held by a male with the specially adapted second pair of gnathopodia. The pair remain in this precopula position until the female moults, after which time copulation, release of the female, and egg laying rapidly ensue. Eggs are carried by the female ventrally and held in place by flat outgrowths from the coxae which bear long interlocking setae and form a brood pouch.

The percentage of females which successfully completed mating in experiment II are shown in Table 2.

In unsuccessful matings males abandoned females without copulating, either before or immediately after the moult. Such males frequently died a few days later. There was evidence (Table 3) that male mortality was higher than female mortality at concentrations of 10 mg/ℓ of Aroclor 1254 and above. A possible physiological explanation for this is that some PCB is concentrated by developing oogonia and thus unavailable for direct poisoning of the females. In treated females which mated and produced eggs, and in the few unmated, treated females which laid infertile eggs spontaneously, some PCB may have been eliminated from their bodies via the eggs.

TABLE 2

The mating success of *G. oceanicus* exposed to Aroclor 1254 in sea water

C_0t (mg/ℓ hr)	% mating success	N
Control (sea water)	100	20
Control (sea water + Corexit 7664)	100	10
1	100	10
10	85	20
100	45	20
1000	40	10

No significant differences in respect of brood numbers between dosed and control groups were found. For both sets of data, regardless of dose, the brood number (y) was dependent upon wet body weight (x) in mg. The relationship was:

$$y = 7.48 + 0.91\ x \quad (t = 4.43,\ d.f. = 20,\ p < 0.001)$$

TABLE 3

Number of male and female *G. oceanicus* alive after 30 days in experiment II

Concentration of Aroclor 1254	Exposure time 1 hr		10 hr	
	Male	Female	Male	Female
1	-	4/6	2/8	5/8
10	-	4/6	0/10	6/9
100	0/10	2/8	0/7	2/9

Experiment III

After approximately 1 month in the culture conditions embryonic development had proceeded normally (Table 4) in the controls. Development was also normal in embryos from PCB-dosed parents except for the two broods from parents exposed to 10 and 100 mg/ℓ for 10 hr.

Soon after these observations were made the cultures became infected with an unidentified fungal infection and all embryos failed to develop further.

TABLE 4

Mortality of embryos derived from PCB-dosed parents

Maternal parent			Embryos	
Concentration mg/ℓ	Exposure time (hr)	Wet body weight (mg)	Total number	Number dead
1	1	59	69	0
1	1	62	41	0
10	1	62	58	0
10	1	78	101	0
10	1	49	54	0
1	10	47	64	0
1	10	55	79	0
10	10	69	33	25
100	10	54	38	38
	Controls	100	129	2
		97	131	0
		78	87	0
		93	12	0
		60	62	0

Conclusions

It can be calculated from the results of experiment I that exposure of *G. oceanicus* to 0.2 mg/ℓ of Aroclor 1254 solubilized in Corexit 7664 in sea water for 10 hr will produce an LT50 = 35.1 days. The data suggest that no significant biological detoxification process occurs in the animals during the experiments. However, nothing is known about the biological half-life or possible bio-transformation of PCB in animal tissues.

The effects on mating success described seem best interpreted as responses of the male to impending death caused by PCB poisoning. Such effects should therefore depend on the same dose: survival time relationship, with C_0t = 10 mg/ℓ hr as the minimum effective dose. Assuming that the maximum time in precopula is 20 days, the minimum concentration possible to produce this effect is 0.021 mg/ℓ of Aroclor 1254 solubilized in Corexit 7664 in sea water.

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