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Elimination of 2,4,5,2',4',5'-Hexachlorobiphenyl by the Purple Sea Urchin, *Strongylocentrotus purpuratus*, Following Single Exposure

Ronald S. Tjeerdema* and Robert S. Jacobs

Marine Science Institute and Department of Biological Sciences, University of California, Santa Barbara, California 93106

Polychlorinated biphenyls (PCB's), a group of industrially synthesized chlorinated hydrocarbons, were once widely used due to their outstanding physical and chemical properties. During the 1970's, PCB's were widespread pollutants of California coastal waters (Schmidt et al. 1971; Young et al. 1976), and worldwide, residues were common in all forms of marine and estuarine life (Stainken and Rollwagen 1979; O'Shea et al. 1980; Shaw and Connell 1980). Although use has decreased, PCB incineration has occurred aboard vessels in the Gulf of Mexico since the early 1980's (Zurer 1985), and residues are still widely found (Richardson and Waid 1983; Tanabe et al. 1983). In the future, PCB incineration off the California coast may become reality, impacting the local marine community (California Air Resources Board 1981).

Understanding the fate of a single PCB isomer in a resident species may aid in assessing the risk to the marine community. Therefore, the elimination of 2,4,5,2',4',5'-hexachlorobiphenyl (HCBP) by the purple sea urchin, *Strongylocentrotus purpuratus*, following a single exposure, was investigated. The purple sea urchin was chosen because of its economic importance (Kato 1972) and ability to proliferate in certain polluted conditions (Leighton et al. 1966). Single exposure may best mimic the effects of intermittent oceanic incineration or disposal, and 2,4,5,2',4',5'-HCBP was chosen due to its presence in common PCB mixtures and high chlorine content, thus strong lipophilicity (Hutzinger et al. 1974).

MATERIALS AND METHODS

Purple sea urchins (*S. purpuratus*; mean weight, 3.7 ± 0.7 g) were collected from a lagoon on the UCSB campus. They were housed in a running-seawater Fiberglas® tank, fed giant kelp, *Macrocystis* sp., and acclimated for two months at 18°C prior to study. Both 2,4,5,2',4',5'- and 2,4,6,2',4',6'-HCBP were purchased from Analabs-Foxboro Analytical, North Haven, CT.

Within a second tank were placed 15 2-gal glass jars filled with 6 L of seawater and equipped with glass aerators. Into each of nine jars were

*Present address and reprint requests: Department of Environmental Toxicology, University of California, Davis, CA 95616.

placed 16 urchins, previously fasted for 24 h, and enough 2,4,5,2',4',5'-HCBP in acetone to produce an initial concentration of 10.0 ± 0.2 μg L^{-1}. The level was similar to those previously measured near sewage outfalls (Schmidt et al. 1971; Young et al. 1976). Another three jars received only 2,4,5,2',4',5'-HCBP and served to estimate loss from glass adsorption and volatilization; it was anticipated and utilized to create the single nature of the exposure. Only urchins were placed into the last three jars, which served as controls. Sampling commenced immediately following chemical administration; after 6 h the jars were refilled with clean seawater. After 0, 0.5, 1, 1.5, 2, 2.5, 3 h, and thereafter every hour until 12 h, an urchin was removed from each of the 12 jars, and a 2-mL water sample was taken from every jar. Urchins were immediately dissected, placed in aluminum foil, and frozen; water samples were pipetted into 1-dr vials, covered with teflon-lined caps, and also frozen. All instruments and glassware were disposed of or decontaminated by the method of Bevenue et al. (1971).

For analysis, tissue was thawed, weighed, and homogenized with acetonitrile in a Polytron® tissue homogenizer (Brinkmann Instruments, Westbury, NY). Residue was removed by filtration; a 1.25-mL aliquot of the filtrate was placed into a 1-dr vial and extracted three-fold with 300 μL of hexane. Extracts were aspirated, combined, volume-reduced to 100 μL under a stream of N_2 gas, and transferred to individual Florisil® (E. Merck, Darmstadt, Germany) columns for cleanup. The columns were pasteur pipettes packed at the tips with glass wool, filled with activated Florisil®, and topped with anh. sodium sulfate; Florisil® was activated by heating at 130°C for 5 h, followed by immersion in hexane. Following extract elution, the columns were flushed with 6% diethylether-in-hexane; the eluent and wash from each column were combined, evaporated to dryness, and adjusted to 25 μL with hexane.

Seawater was extracted sequentially with 300 μL of 15% diethylether-in-hexane, 6% diethylether-in-hexane, and hexane; extracts were aspirated, combined, volume-reduced to 100 μL, and passed through Florisil® columns. Florisil® cleanup was identical to that used for tissue; eluents and washes were again combined, evaporated, and adjusted to 25 μL with hexane.

Samples were analyzed with a Hewlett-Packard Model 5840A gas chromatograph, equipped with a 50.0 x 0.2 mm (i.d.) capillary column coated with methyl silicone, and an electron capture detector. Helium and argon-methane (95:5; 31 mL min^{-1}) served as the carrier and detector make-up gasses, respectively. Column temperature was programmed to rise from 80.0°C to 250°C at 10°C min^{-1} for the first 10 min, and 3°C min^{-1} thereafter. The injector was in the splitless mode (180°C), and the inlet pressure was 25 psi. Retention times were 38.2 min for 2,4,5,2',4',5'-HCBP and 32.5 min for 2,4,6,2',4',6'-HCBP, which served as a method internal standard. Amounts as low as 0.15 ng g^{-1} in tissue and 0.01 μg L^{-1} in seawater could be detected accurately and reproducibly, and recoveries above 90% were routine.

A two-compartment body model was used to describe the kinetics of 2,4,5,2',4',5'-HCBP (O'Flaherty 1981; Fig. 1). While their mathematical

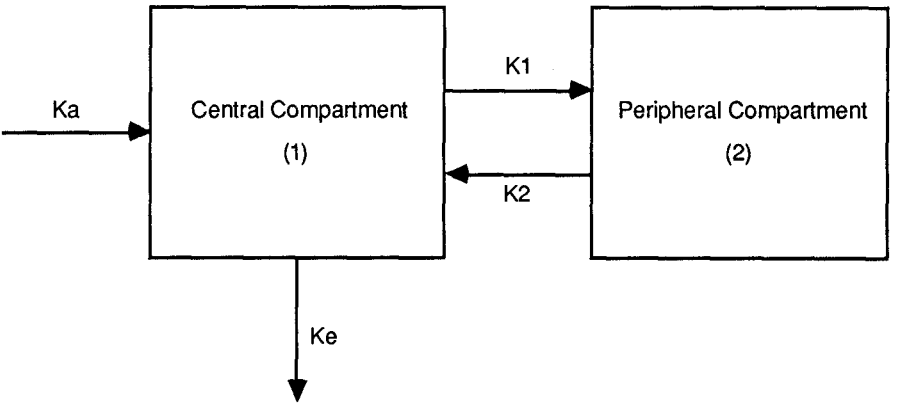

Figure 1. The linear two-compartment open model with first-order absorption and elimination.

derivations may be found elsewhere (O'Flaherty 1981), the following equations were utilized:

$$C_t = A_o e^{-\alpha t} + B_o e^{-\beta t} \quad (1)$$

$$A_o;B_o = \text{antilog } b \quad (2)$$

$$\alpha;\beta = -2.303\ a \quad (3)$$

$$t_{50a};t_{50b} = \frac{0.693}{\alpha;\beta} \quad (4)$$

$$k_1 = \alpha + \beta - k_2 - k_e \quad (5)$$

$$k_2 = \frac{A_o \beta + B_o \alpha}{A_o + B_o} \quad (6)$$

$$k_e = \frac{\alpha \beta}{k_2} \quad (7)$$

$$F_a = \frac{\beta}{k_e} \quad (8)$$

C_t - tissue concentration at time t.
A_o - tissue t = 0 concentration intercept for the disposition curve.
B_o - tissue t = 0 concentration intercept for the dieaway curve.
α - first-order rate constant for disposition from the central to the peripheral compartment.
β - first-order rate constant for whole-body elimination.
a - slope.
b - y-intercept.
t_{50a} - half-life in the central compartment.
t_{50b} - half-life in the peripheral compartment (biological half-life).
$k_1;k_2$ - first-order disposition microconstants.

k_e - first-order disposition whole-body elimination microconstant.
F_a - total body burden fraction in the central compartment during the terminal dieaway phase.

The two decaying exponentials in the expression for C_t allowed the curve to be resolved into two basic straight line segments by a process referred to as feathering, curve-stripping, or the method of residuals (O'Flaherty 1981).

RESULTS AND DISCUSSION

Table 1 presents the seawater and urchin tissue 2,4,5,2',4',5'-HCBP concentrations. During the first 6 h, the seawater concentration diminished by more than 60%, creating the single exposure; it was most rapid during the first hour. The decrease, primarily due to glass adsorption and volatilization, has been previously described (Hutzinger 1974). Highly chlorinated PCB isomers, being very hydrophobic, will adhere to almost any surface. Following the 6-h flush, a much lower PCB concentration was re-established; it probably leached back into the seawater from the jars' glass surfaces. The levels, divided into initial (A, 1-6 h) and final (B, 6-12 h) segments, were compared to the controls by

Table 1. Seawater and urchin 2,4,5,2',4',5'-HCBP concentrations measured during the 12-h study. Seawater levels at 6 h represent both before (A) and after (B) the fresh seawater flush.

Time (h)	2,4,5,2',4',5'-HCBP concentration	
	Seawater ($\mu g\ L^{-1}$)[a]	Tissue ($ng\ g^{-1}$)[a,b]
0.0	10.01 (0.20)	0.00 (0.00)
0.5	5.63 (0.45)	58.82 (4.27)
1.0	4.89 (0.51)	35.49 (2.65)
1.5	4.61 (0.26)	28.76 (3.13)
2.0	4.47 (0.31)	23.56 (3.48)
2.5	4.27 (0.27)	21.30 (3.21)
3.0	4.03 (0.38)	20.77 (2.96)
4.0	3.63 (0.41)	19.80 (2.22)
5.0	3.43 (0.38)	18.81 (2.62)
6.0 (A)	3.23 (0.43)	18.28 (3.03)
6.0 (B)	0.00 (0.00)	---- ----
7.0	0.46 (0.07)	17.12 (3.04)
8.0	0.45 (0.09)	15.09 (2.72)
9.0	0.42 (0.12)	13.99 (1.59)
10.0	0.45 (0.12)	12.88 (3.42)
11.0	0.46 (0.16)	12.20 (1.65)
12.0	0.38 (0.11)	11.94 (3.66)

[a] Mean (SD), N = 9.
[b] Wet weight.

analysis of variance (ANOVA), with one between-subjects and one-within subjects factor (Sokal and Rohlf 1981). Differences in parallelism (A: F=0.41, P=0.9269, DF=9,90; B: F=0.18, P=0.9820, DF=6,60) and profile (A: F=0.15, P=0.7088, DF=1,10; B: F=0.46, P=0.5113, DF=1,10) were not significant; changes over time (A: F=670.72, P<0.0001, DF=9,90; B: F=43.59, P=0.0001, DF=6,60) were significant. Therefore, when compared to the controls, both segments were parallel, with overlapping profiles that changed with time.

Figure 2 presents the tissue 2,4,5,2',4',5'-HCBP concentrations; a two-compartment model of elimination best fit the curve. Tissue level peaked at 0.5 h, then rapidly diminished for 2 h; it gradually declined thereafter. Immediately following the 6-h flush, the slope increased. Tissue concentration did not rapidly decrease after the flush. Therefore, the initial decline may not simply be an artifact of the decreasing water level, and a two-compartment model may be justified. Absorption appeared to be first-order and rapid; however, lack of data prevented its description. Also, the controls showed no traces of the PCB isomer.

The kinetic parameters are presented in Table 2. During the initial, or distribution, phase (first 2.5 h), 2,4,5,2',4',5'-HCBP excretion and transfer from the central to the peripheral compartment were very rapid, as reflected by a large α and small t_{50a}. A_o was almost four times larger than B_{o_1}, indicating a large difference in the initial concentrations of the two compartments. During the initial dieaway phase (2.5 to 6 h), both a small β_1 and large t_{50b_1} reflected a slow whole-body loss. Also, k_1 was larger than both k_2 and k_e, indicating distribution from the central to the peripheral compartment was larger than either the reverse or whole-body elimination. In addition, F_a was relatively small. Following the 6-h flush, slope increased. Correspondingly, β_2 was larger than β_1, t_{50b_2} was smaller than t_{50b_1}, and B_{o_2} was larger than B_{o_1}. In essence, whole-body elimination roughly doubled following the infusion of clean water. Also, k_1 decreased, while both k_2 and k_e increased, indicating increased redistribution from the peripheral to the central compartment and increased whole-body elimination; F_a decreased as expected.

PCB kinetics have been described in a number of aquatic organisms, including the American oyster, *Crassostrea virginica* (Lowe et al. 1972), the rainbow trout, *Salmo gairdneri* (Branson et al. 1975), and the bay mussel, *Mytilus edulis* (Calambokidis et al. 1979). American oysters exposed to 1.0 and 5.0 $\mu g\ L^{-1}$ of Aroclor® 1254 eliminated it to below detectable levels in 25 and 32 weeks, respectively; elimination was dependent upon the exposure length and concentration. Rainbow trout exposed to 1.6 and 9.0 $\mu g\ L^{-1}$ of 2,4,2',4'-tetrachlorobiphenyl eliminated it slowly, with rate constants (k_e's) of 9.66×10^{-4} and $1.53 \times 10^{-3}\ h^{-1}$, respectively; elimination was first-order, dependent upon exposure concentration, and fit a one-compartment model. Elimination from bay mussels exposed to a PCB mixture was first-order and dependent on the chlorine content of the individual isomers; retention increased for higher chlorinated biphenyls. Three of the components, Aroclor® 1242, 1254, and 1260, produced t_{50}'s of 8, 23, and 39 d, respectively.

Elimination of 2,4,5,2',4',5'-HCBP from purple sea urchins cannot easily

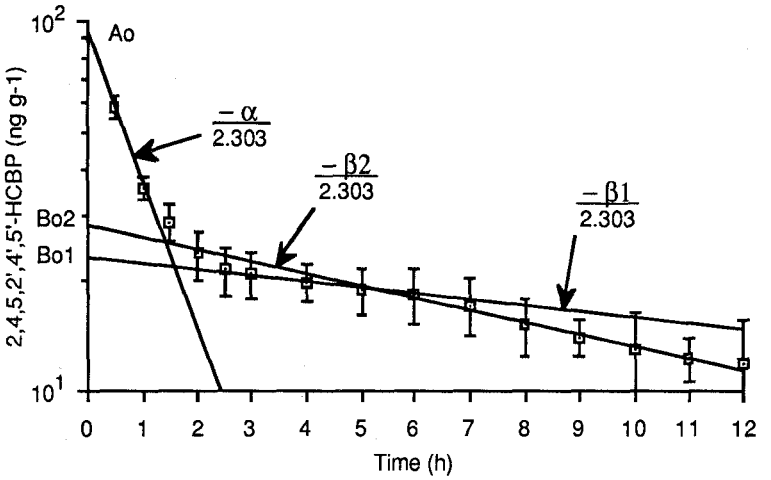

Figure 2. The two-compartment body model of elimination derived from urchin 2,4,5,2',4',5'-HCBP concentrations; intervals are in standard deviations. The lines are as follows: $-\alpha \cdot 2.303^{-1}$, distribution phase (0 to 2.5 h); $-\beta_1 \cdot 2.303^{-1}$, initial dieaway phase (2.5 to 6 h); and $-\beta_2 \cdot 2.303^{-1}$, terminal dieaway phase (6 to 12 h).

be compared with other animals because description occurred with PCB still present, and absorption occurred briefly during a concentration decline. Elimination is usually described in clean water, during a clearance, or depuration, phase, with absorption occurring within a period of stable concentration, during an exposure, or uptake, phase.

Table 2. The kinetic parameters calculated for 2,4,5,2',4',5'-HCBP in purple sea urchins (see text for definitions).

Parameter	Phase[a]	
	First	Second
A_o ($ng\ g^{-1}$)	93.11	---
α (h^{-1})	1.90	---
t_{50a} (h)	0.37	---
B_o ($ng\ g^{-1}$)	23.71	28.31
β (h^{-1})	0.04	0.08
t_{50b} (h)	15.75	8.66
k_1 (h^{-1})	1.34	1.18
k_2 (h^{-1})	0.42	0.50
k_e (h^{-1})	0.18	0.30
F_a	0.22	0.16

[a] Each 6 h in duration.

In conclusion, 2,4,5,2',4',5'-HCBP elimination by purple sea urchins fits a two-compartment model. Rapid elimination may depend upon low PCB chlorination, short exposure time, and decreasing exposure concentration; the result is reduced lipid sequestration. Therefore, water levels must remain elevated for urchins to retain possibly harmful levels. When exposure is periodic, urchins may quickly eliminate residues, reducing the threat to themselves and consumers, including humans and California sea otters, *Enhydra Lutris* (Ebert 1968).

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