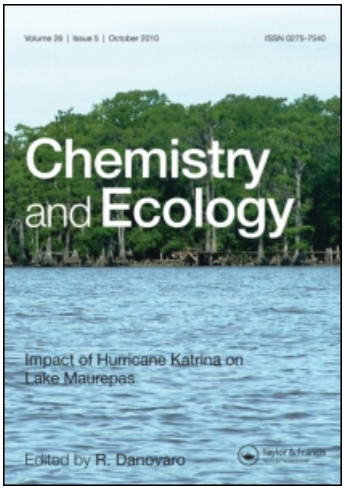

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Elimination of Pentachlorobiphenyls by *Nereis virens* (Polychaeta) in the Laboratory and the Marine Environment

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Fifty sandworms, *Nereis virens*, were maintained in a closed aquarium system with continuous charcoal filtration at 12°C and 27.6‰ S. They were dosed orally for 10 consecutive days with a mixture of three pentachlorobiphenyls: 35 ng 2,4,6,2′,4′-pentachloro[U-^{14}C]biphenyl, 106 ng 2,4,5,2′,5′-pentachlorobiphenyl, and 106 ng 2,3,4,2′,5′-pentachlorobiphenyl per g of *Nereis virens* per day. At the end of the dosing period the accumulated compounds were measured in 5 worms, the remaining 45 specimens were divided into three groups for determining the PCB elimination under different conditions for 14 to 26 weeks: in the laboratory with feeding, in the laboratory without feeding, and in a cage moored in the Weser estuary.

The accumulation percentages for these PCB compounds were 41, 26 and 4% respectively. Times for the initial 50% decrease t_e50 were 4.4, 2.8 and 1.9 weeks respectively, and appeared the same in all three experimental groups. However, in the laboratory experiments the metabolites of the ^{14}C-labelled compound amounted to ~60%, compared to the field experiment with ~30%. This extended the t_e50 for ^{14}C activity (metabolites included) to ~9 weeks in the laboratory experiments.

INTRODUCTION

The sandworm, *Nereis virens*, is a most suitable laboratory species for investigating the fate of xenobiotic compounds in marine

invertebrates. Accumulation, elimination and transformation of PCBs in this species have already been studied in detail, on the assumption that the results are related to natural conditions (Ernst *et al.*, 1977; Goerke and Ernst, 1977; Goerke, 1979, 1984a, b). This assumption is supported by the fact that the animals exhibit the full spectrum of species specific behaviour, a natural growth rate, and no mortality within the life span once the acclimation phase is finished. In order to validate the use of these laboratory experiments, the elimination of three PCB isomers was compared in laboratory and field experiments.

A preceding experiment had shown that the device used in the field allowed the worms to feed on the low amounts of sediment and detritus entrapped in the experimental glass tubes but these were not sufficient for normal growth. Therefore, the parallel laboratory experiments included two feeding regimes: for one group maintenance rations were approximated, the other group was deprived of food.

METHODS AND MATERIALS

General experimental procedure

Fifty *Nereis virens* were dosed orally for 10 consecutive days with a mixture of three pentachlorobiphenyls. After an additional 24 hr digestive period five specimens were analysed to determine the PCB concentrations achieved at the beginning of the elimination period (20 to 300 ng/g). The remaining 45 specimens were divided into 3 groups and allowed to eliminate the test compounds for up to 26 weeks. Group A lived in charcoal filtered recirculated water on a diet (maintenance ration) free of the test compounds, group B lived in an identical aquarium system, but was deprived of any food, and group C was transferred to a cage that was then moored in the Weser estuary (Figure 1). At regular intervals during the elimination period individual worms were withdrawn from all groups and analysed for the three compounds. The experiments were carried out between August 19, 1980 and February 27, 1981. The experiment with group C was finished on December 4, 1980, when the low temperature period had already begun.

Test animals and their maintenance

Nereis virens Sars were collected on July 22 and 23, 1980 from a population on the NE coast of Helgoland near low water mark (Gillandt, 1979). They were acclimated to the experimental conditions thus: living in glass tubes, feeding on muscle and mantle tissues of *Mytilus edulis* five days per week (~1% wet weight of *Nereis* per day), salinity 27.6‰ (sea water of the German Bight diluted with non-chlorinated tap water), 12°C, aeration, natural changes of dim daylight and darkness (1.5 lux). At the start of the experiments, the test animals had wet weights of 4.1 to 7.3 g (∅ 5.8 g) and living tubes of 7.5 to 9.5 mm inner diameter.

Two closed aquarium systems, previously described (Goerke, 1979), were used. In each 45 l of water passed continuously through charcoal (500 g, renewed once during the elimination period), then recirculated (~200 l/hr). Every 10 days ~15% of the water was renewed. Temperature and salinity were maintained at 12 ± 0.5°C and 27.6 ± 0.7‰ in the laboratory experiments. The worms were kept in U-shaped glass tubes fitted with extension tubes and lids and fixed to the aquarium walls by stainless steel wires (Figure 2; Goerke, 1984). For elimination under field conditions, group C was also maintained in glass tubes, which were fixed by clamps to a metal frame (AlMg3) and protected by a stainless steel cage (Figure 1). Individual glass lids whilst not interfering with the animals' irrigation currents prevented the worms from leaving their tubes (Figure 2c). The frame was moored at 4 m chart depth on the bottom of the field station in the Weser estuary at 53°45′22″N, 8°10′36″E. In the course of a long term program (Krause, 1981), temperature and salinity at this position were recorded simultaneously (Figure 4c) by an Aanderaa current meter with conductivity cell and temperature probe, which was installed on a second frame.

Administration of PCBs

During 10 consecutive days *Nereis virens* received 10 oral doses of a mixture of 35 ng 2,4,6,2′,4′-pentachloro[U-^{14}C]biphenyl, 106 ng 2,4,5,2′,5′-pentachlorobiphenyl and 106 ng 2,3,4,2′,5′-pentachlorobiphenyl per g worm in 0.2 µl ethanol. The ^{14}C-labelled PCB,

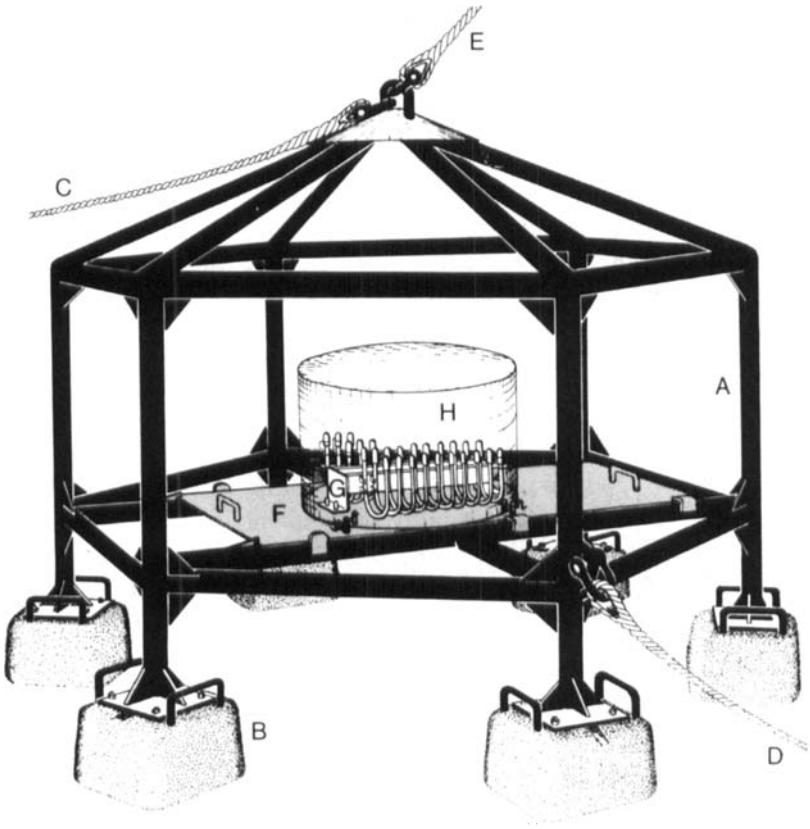


FIGURE 1 Frame with cage for exposing *Nereis virens* during depuration to environmental marine conditions. A = tube construction from alloy of aluminium, B = concrete weight, C = 1st 60 m wire with buoy unit (concrete weight, 15 m nylon rope, plastic buoy), D = 2nd 60 m wire with buoy unit, E = 15 m nylon rope with plastic buoy, F = platform for mounting rack and cage, G = rack with dwelling tubes of *Nereis virens*, H = cage.

specific activity 566 Bq/μg, was provided by the Gesellschaft für Strahlen- und Umweltforschung München and did not contain any significant impurities as revealed by gas chromatographic analysis. The two non-labelled PCBs were purchased from Analabs, North Haven. The latter two occur in technical PCB mixtures and environmental samples in significant amounts (Zell *et al.*, 1978; peaks 52, 57). Depending on the worms' wet weights 0.8 to 1.5 μl of

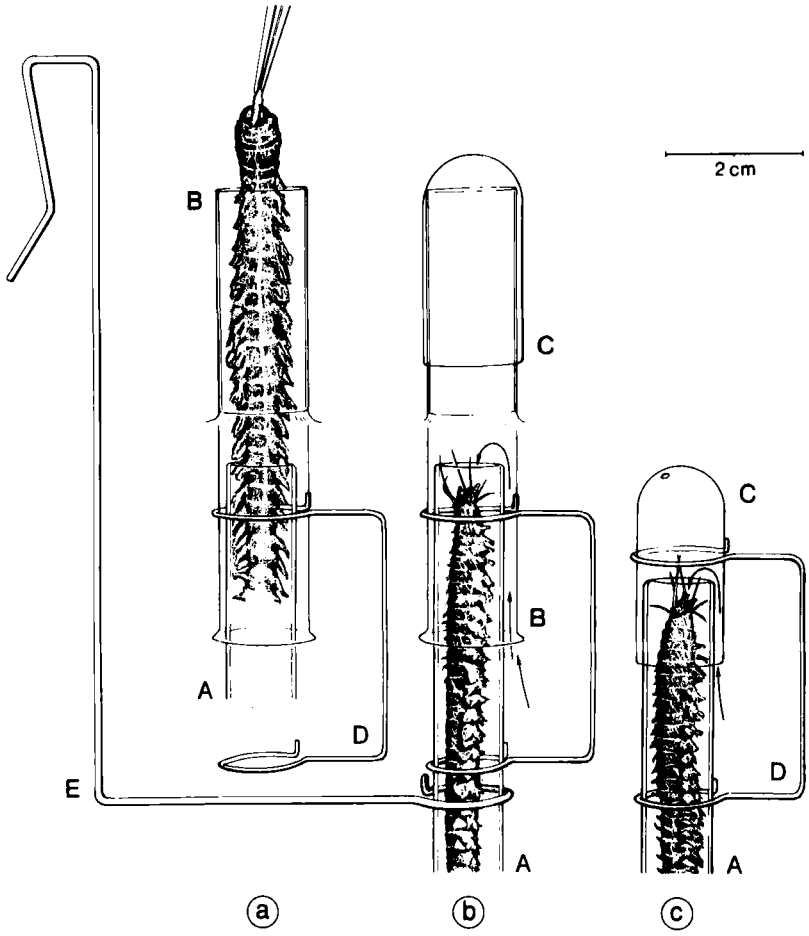


FIGURE 2 Devices for maintenance of *Nereis virens* in dwelling tubes from glass. (a) The worm seizes the dosed food with its jaws outside the water after having crawled up the extension tube. (b) Lid on extension tube prevents *Nereis virens* from leaving its dwelling tube in laboratory experiments. (c) Lid on dwelling tube prevents leaving in submerged cage of field experiment. Hole in the lid allows entrapped air to escape. Arrows indicate flow of water, when the worm irrigates its tube. A = Dwelling tube. B = Extension tube. C = Lid. D = Connector clamp. E = Holding clamp. (Modified after Goerke, 1984b.)

the solution were applied with a 5 μl syringe to the surface of excised foot retractor muscles of the blue mussel, *Mytilus edulis*. After evaporation of the solvent in a gentle stream of air for 1.5 min, the dosed food pieces were fed to the worms, after these had been baited by undosed food to crawl up the extension tubes (Figure 2a). Since part of the swallowing occurred out of water, no losses of PCB occurred during swallowing (Table I). The only losses occurred when dosing the food pieces with the compounds.

Analytical procedure for worms

The animals were weighed live after blotting off surface water, wrapped in aluminium foil, and frozen at −20°C for 1 to 22 days until the time of analysis. Chemical analysis was carried out according to the flow diagram of Figure 3. The PCBs were measured by gas chromatography (GC), in addition the radioactive compounds were measured by liquid scintillation counting (LSC). See also Ernst *et al.* (1974) for details of extraction and clean-up. If not stated otherwise, chemicals of analytical grade from Merck, Darmstadt, were used. Solvents were redistilled.

Four control worms were analysed for background levels of PCB.

Gas chromatography

After clean-up of the extracts on Al_2O_3, the PCBs were analysed by packed column gas chromatography with electron capture detection (Packard 428; column all glass, 1.8 m, 2 mm i.d., OV 101 on Chromosorb WHP 100/120 mesh, 180°–200°C; inlet 210°–250°C; ^{63}Ni-ECD 250°–290°C; carrier gas nitrogen 40 ml/min).

Measurements of radioactivity of worms

Aliquots of the extracts, the non-extracted tissue powder and the extracted tissue powder were measured by LSC (Betaszint BF 5000, Berthold/Frieseke, Karlsruhe), the former with Quickszint 501 (Zinsser, Frankfurt), the latter two with Dimilume (Zinsser) upon

solubilization with Soluene 350 (Packard Instr. Comp., Frankfurt). All values were corrected for quenching by external standards. The values derived from ^{14}C measurements refer to 2,4,6,2′,4′-pentachloro[U-^{14}C]biphenyl in terms of compound equivalents.

Detection of metabolites of ^{14}C-PCB

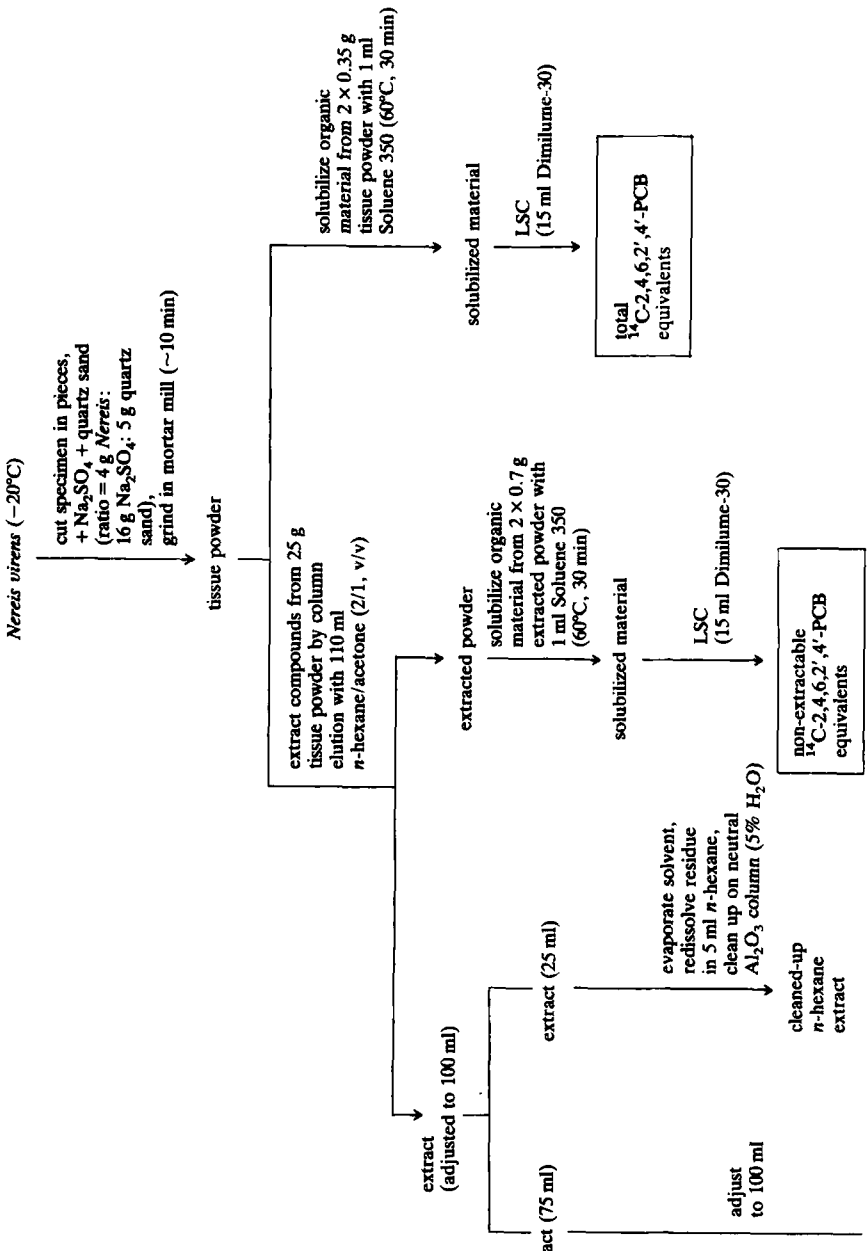
The extracts of about two thirds of the worms were analysed by TLC. The solvents of aliquots were evaporated by a rotary evaporator (EL, Büchi, Flawil, Switzerland) at 20°C nearly to dryness and the residues redissolved in *n*-hexane. TLC was performed on silica gel G (20 × 20 cm glass plates, 250 μm thick, Macherey-Nagel & Co., Düren) that were activated at 105°C for 30 min before use. Plates were developed in *n*-hexane/chloroform (9/1, v/v). After the compounds were detected by a radioscanner (Desaga, Heidelberg) the spots located were scraped off for quantitative LSC measurements.

Determination of lipids (EOM)

The solvents of aliquots of the extracts (~50%) were removed by rotary vacuum evaporation. The extractable organic matter (EOM) was dried at 60°C to constant weight and referred to as "lipid."

Extraction and measurement of ^{14}C-PCB from water

On 5 occasions during the elimination period 800 ml of water of the two aquarium systems in the laboratory (groups A and B) were extracted with 50 ml *n*-hexane and subsequently with 50 ml dichloromethane upon acidification with 40 ml 10 N H_2SO_4. The hexane extract was dried with anhydrous sodium sulphate and concentrated to 5 ml by rotary vacuum evaporation; 3 ml were measured by LSC with 15 ml Quickszint 501 (Zinsser, Frankfurt). The dichloromethane of the second extract was removed by rotary vacuum evaporation nearly to dryness; the residues were redissolved in 5 ml methanol, 3 ml of which were taken for LSC with 10 ml Unisolve (Zinsser, Frankfurt).



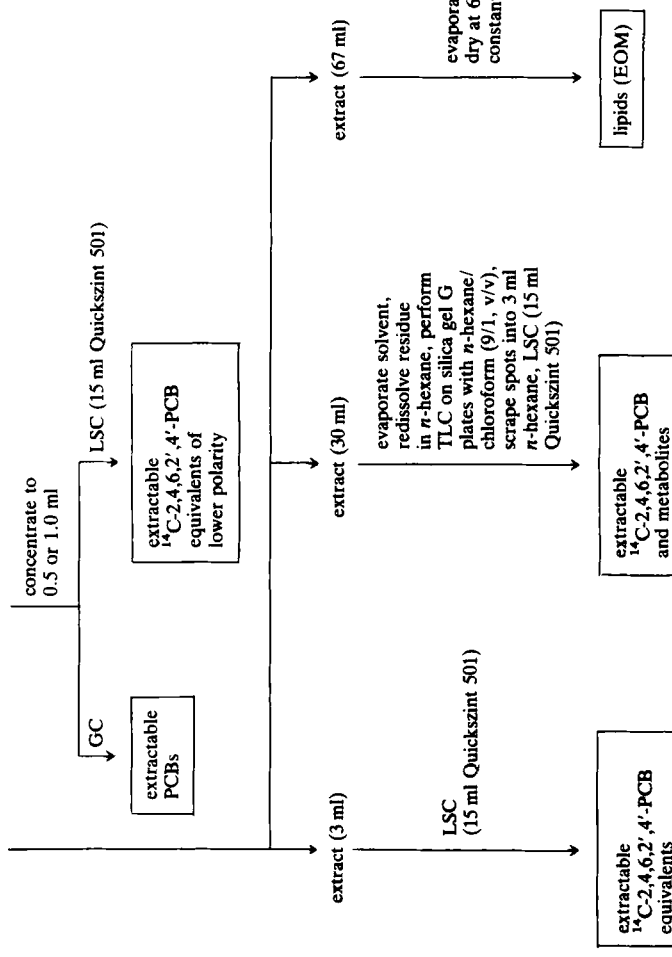


FIGURE 3 Analytical procedure for worms.

RESULTS

Background levels of PCB in worms

Two untreated worms which had been acclimatized together with the experimental animals, though kept untreated separate from them during PCB administration, were analysed for PCB contents at the beginning of the elimination period. The PCB levels were 33 and 53 ng/g wet weight. A further two untreated specimens which had been transferred to the cage for the whole elimination period exhibited PCB levels of 57 and 66 ng/g wet weight. The PCB patterns of these four animals revealed striking similarities with Clophen A60 used as basis of evaluation.

Of the three test compounds, according to GC data only 2,4,5,2′,5′- and 2,3,4,2′,5′-pentachlorobiphenyl might be present in the control animals, each at a level of much less than 5% of the total PCB load. The resulting concentrations of these compounds in control worms represent less than 10% of the low concentrations in the experimental animals. Therefore, the "natural" background levels of PCB can be regarded as not interfering with the test compounds in the experiment.

Absorption

The oral method of PCB administration was equally good for all the applied compounds. The recoveries of the three pentachlorobiphenyls—as determined by gas chromatography—from the food pieces and as well from *Nereis virens* after swallowing the dosed food are statistically not different: analysis of variance in the latter case (Table I, line 3) resulted in $F = 0.5$. There is no difference in average total ^{14}C recovery after one dose between worms analysed upon swallowing and upon defaecation (Table I, lines 3 and 5), which indicates that the worms absorbed the swallowed 2,4,6,2′,4′-pentachloro[U-^{14}C]biphenyl quantitatively. In fact, the faeces originating from the food pieces used for dosing the compounds to the latter 5 worms contained only 0.1% of the swallowed ^{14}C on average. The difference noticed in the respective GC derived recoveries of this compound, 70% and 54%, is interpreted as

TABLE I

Recovery tests of PCBs from food and *Nereis virens* after oral dosage (0.7 to 1.1 µl of PCB solution depending on wet weights of worms). Values in brackets are standard deviations. Italics indicate additional tests carried out with the two non-labelled PCBs and *Nereis virens* of 1.3 to 5.0 g wet weight; in the one dose tests of this series GC analysis was not completely satisfactory due to relatively high background contamination. However, the figures may be reliably compared on a relative basis. Letters indicate methods of measurement: *a* = LSC of ^{14}C in tissue or tissue powder, *b* = LSC of ^{14}C in extract, *c* = GC of compound in extract after clean-up

			Average percentage of applied dose				
			^{14}C-2,4,6,2′,4′-PCB			2,4,5,2′,5′-PCB	2,3,4,2′,5′-PCB
	Line	Number of measurements	*a*	*b*	*c*	*c*	*c*
PCBs on food pieces before offering	1 2	15 3	83(10)		70(12)	73(11)	74(12)
PCBs in *N. virens* upon swallowing 1 dose	3 4	4 5	82(9.4)	80(3.1)	70(7.7)	67(7.2) *62(7.1)*	73(7.7) *86(5.7)*
PCBs in *N. virens* upon defaecation 26 to 32 h after swallowing 1 dose	5 6	5 5	83(6.3)	59(11)	54(15)	46(12) *30(4.7)*	12(2.5) *7(1.4)*
PCBs in *N. virens* 30 h after swallowing the last of 10 doses on 10 consecutive days	7 8	5 5	74(4.7)	48(5.9)	41(6.5)	26(6.6) *30(7.1)*	4(1.5) *3(2.5)*

metabolic elimination. This is also the most probable interpretation for the analogous decreases of the other two pentachlorobiphenyls.

Accumulation

Using Duncan's multiple range test, it was found that recoveries in worms exposed for 10 days to the three compounds differed significantly (Table I, line 7; Table II), in other words: the three pentachlorobiphenyls are accumulated to differing degrees. The particularly low percentage of 2,3,4,2′,5′-PCB is observed already after defaecation following 1 dose. The recoveries after 10 doses are lower than after 1 dose (Table I, lines 7 and 5) due to elimination during the dosing period.

The different results obtained in the recovery tests, when 2,4,6,2′,4′-PCB is analysed by different methods, are probably due to methodical deviations in GC analysis (Table I, line 3), or they must be explained by transformation of the parent compound in the animal (Table I, lines 5 and 7): most polar transformation products remain on the extraction or clean-up columns.

Elimination

For calculating the PCB elimination the constants of the model function $y = a + b \cdot e^{-cx}$ (y: standardized amount or concentration

TABLE II

Results of Duncan's multiple range test on the differences between the PCB mean recoveries $\bar{x}_i$, $\bar{x}_j$ (as determined by GC) in *Nereis virens* upon digestion of the (last) dosed food piece. Test value $q_{i,j} = \frac{x_i - x_j}{s_0}\left(\frac{2n_i n_j}{n_i + n_j}\right)^{1/2}$ in italics indicates non-significant difference, $q_{i,j}$ in boldface indicates significant difference (n_i, n_j are numbers in corresponding groups; s_0 is standard deviation within groups). Indices of q denote compound: *1* = 2,4,6,2′,4′-pentachloro[U-^{14}C]biphenyl, *2* = 2,4,5,2′,5′-pentachlorobiphenyl, *3* = 2,3,4,2′,5′-pentachlorobiphenyl

	Line of Table I	$q_{1,2}$	$q_{1,3}$	$q_{2,3}$
1 dose	5	*1.7*	**8.6**	**6.9**
10 doses	7	**5.8**	**15.2**	**9.3**
p		2	3	2
q (99, p, 12)		4.3	4.5	4.3

of test compound, x: time) are evaluated by computer using the method of least squares and an iterative approximation. After 8 weeks of elimination, concentrations higher than the lowest initial concentration were considered to be abnormal and therefore omitted in the calculation. The model is the most simple one allowing the elimination rate to decrease unproportionally to the PCB concentration with time. Generally the fit is better if $a \neq 0$ is allowed. In the cases $a \neq 0$ no elimination half-life time can be calculated. The time t_e50 for eliminating 50% of the initial load is used instead. A more complicated model is not justified in view of the considerable scatter in the data. Nevertheless, calculations based on first order multicompartment kinetics were carried out on trial, but the functions were not sustained by the distribution of the values.

For all *Nereis virens* the PCB doses were established individually, attaining the same initial concentrations in all worms. Due to the 3 different treatments and individual characteristics, during the elimination period variable weight changes occurred which, in addition, to elimination would influence the PCB concentrations. Therefore, when calculating the elimination kinetics amounts of PCB standardized on the doses of a 5 g worm are used instead of its concentrations. This procedure is supported by the percentage standard deviations giving lower results in 8 out of 13 cases.

The comparison of the elimination kinetics in the three experimental groups A, B, C (Figures 4, 5) may refer to the individual PCBs as measured by GC or to equivalents of the ^{14}C-labelled compound as measured by LSC before and after extraction comprising the parent compound and its transformation products. 2,3,4,2',5'-PCB cannot be included in this comparison because due to its low initial concentration during the elimination period only few measurements could be made; these did not allow reliable evaluation of separate elimination kinetics. Figures 4, 5 and Table III do not reveal any significant differences in the elimination of any of the three test compounds between the three groups, except if—as is possible for the ^{14}C-labelled PCB—all transformation products are included: the two groups in the laboratory show considerably more ^{14}C-labelled metabolites than the group in the field (Figure 6; see also below). In the laboratory, either more or other ^{14}C-labelled metabolites are formed or their excretion occurs more slowly than

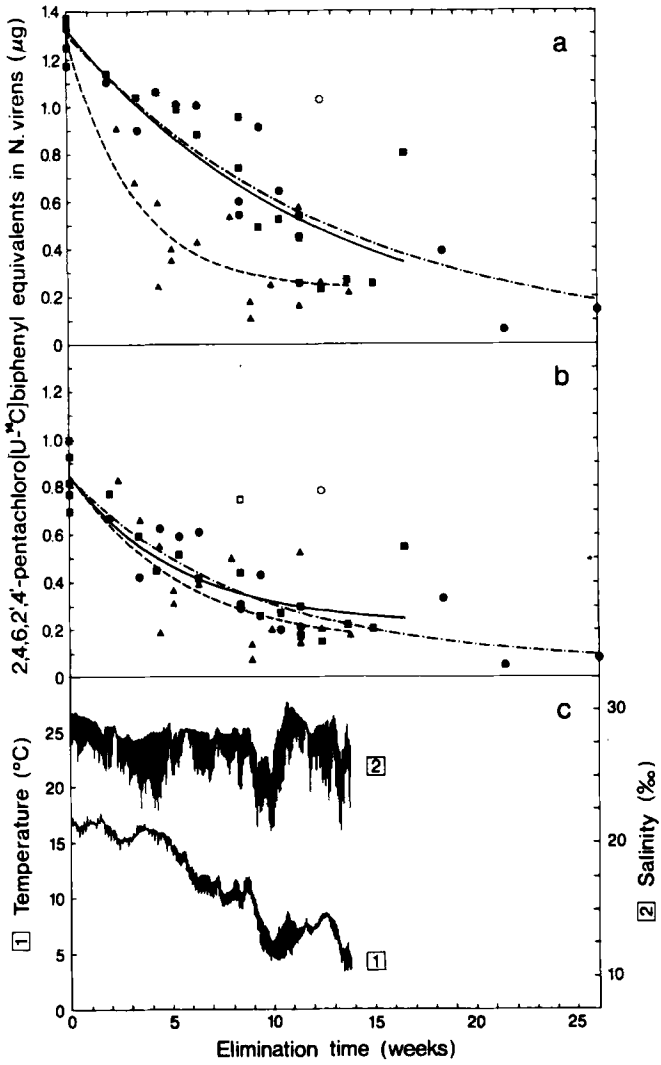


FIGURE 4 Elimination of 2,4,6,2′,4′-pentachloro[U-^{14}C]biphenyl equivalents by *Nereis virens* as measured by LSC in tissue powder (a) and in extract (b) in three experimental groups A, B, C. The lower graphs (c) allow to relate the temperature and salinity fluctuations prevailing with group C *in situ* (courtesy of G. Krause, R. Plugge, M. Walter). PCB amounts are adjusted to the dosis of a 5 g worm. Values indicated by white symbols are considered exceptual and are therefore omitted from the calculation. See Figure 5 for explanation of symbols.

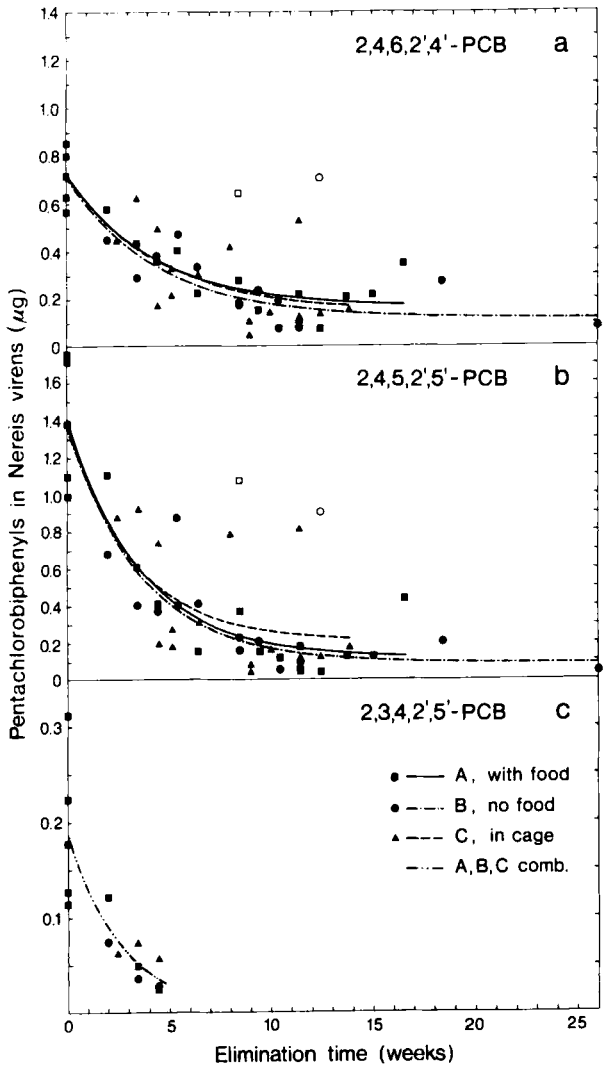


FIGURE 5 Elimination of three pentachlorobiphenyls (a, b, c) by *Nereis virens* as measured by GC in three experimental groups A, B, C. PCB amounts are adjusted to the dosis of a 5 g worm. Values indicated by white symbols are considered exceptual and are therefore omitted from the calculation.

in the field. The elimination times $t_e50 = 8.6$ weeks (Group A, Table III) and $t_e50 = 9.4$ weeks (Group B, Table III) for the total ^{14}C-labelled compounds are longer than those of the parent compound, $t_e50 = 4.5$ weeks and $t_e50 = 4.2$ weeks.

In this context it may be questioned whether the water filtration by charcoal in the laboratory was sufficient. On six occasions the radioactivity of the water was measured in order to test the filtration

TABLE III

Elimination characteristics of PCBs in *Nereis virens*. t_e50 = time for 50% decrease of initial amount. A, B, C = experimental groups (see text). a = LSC of ^{14}C in tissue powder, b = LSC of ^{14}C in extract, c = GC of compound in extract after clean-up

		^{14}C-2,4,6,2',4'-PCB			2,4,5,2',5'-PCB	2,3,4,2',5'-PCB
		a	*b*	*c*	*c*	*c*
Average percentage of recovery of PCB after 10 doses		74	48	41	26	4
Average initial concentration (μg/g)		0.23	0.15	0.13	0.25	0.03
t_e50 (weeks)						
A, with food		8.6	6.1	4.5	2.8	
B, no food		9.4	7.0	4.2	2.8	1.9
C, in cage		3.1	5.0	4.5	2.9	
Constants a, b, c of elimination equation $y = a + b \cdot e^{-cx}$, standard deviation s						
A, with food	*a*	0	0.22	0.16	0.11	
	b	1.33	0.63	0.56	1.30	
	c	0.081	0.18	0.23	0.28	
	s	0.18	0.12	0.09	0.21	
B, no food	*a*	0	0.04	0.11	0.09	0
	b	1.31	0.79	0.59	1.28	0.19
	c	0.074	0.11	0.22	0.27	0.37
	s	0.13	0.12	0.10	0.24	0.05
C, in cage	*a*	0.23	0.12	0.14	0.20	
	b	1.08	0.74	0.57	1.20	
	c	0.30	0.18	0.22	0.30	
	s	0.15	0.16	0.15	0.30	

efficacy. During the elimination period the ratio of the concentration of ^{14}C-PCB equivalents in animals to that in water increased from $\sim 2 \cdot 10^3$ to $\sim 5 \cdot 10^5$. From the identity of the elimination kinetics of each PCB compound in the three experimental groups it is concluded that the low ratio initially is not detrimental in the case of the parent compounds. However, it cannot be excluded that it is detrimental for the elimination of the metabolites, because the low ratio initially could result particularly from any of the latter.

Independent of the three experimemtal groups, *Nereis virens* eliminates the three pentachlorobiphenyl isomers, 2,4,6,2′,4′-, 2,4,5,2′,5′- and 2,3,4,2′,5′-PCB, significantly differently: the average elimination times $t_e 50$ are 4.4, 2.8 and 1.9 weeks respectively. The same order was noted above in relation to the absorption of one dose and the accumulation of 10 doses (Table I).

The individual worms had lipid contents varying from 0.7 to 1.6% with no observable trend during the elimination period. The average percentages of the three groups do not differ significantly (analysis of variance: $F = 0.18$, $f_1 = 2$, $f_2 = 42$). In order to detect any influence of lipid content on PCB elimination, the abscissa was transformed by dividing elimination time by lipid content, and the calculations of the kinetics were repeated. In 9 out of 13 cases the percentage standard deviations increased, indicating in the majority that the variation of the values cannot be attributed to the lipid contents of the worms.

Beginning after the ninth week of elimination, the sexes of the worms were determined by microscopy of the coelomic contents. No correlations between sexes and levels of the test compounds were detected.

Biotransformation

Concentrations of the PCBs applied decrease because the compounds are both excreted and metabolized. These experiments do not aim to separate the two processes quantitatively. They do, however, give some insight concerning the importance of PCB metabolization.

Metabolization of 2,4,6,2′,4′-pentachloro[U-^{14}C]biphenyl is apparent during the 10-day dosing period already, even after one dosage (see above, Table I). Figure 6 allows us to recognize the

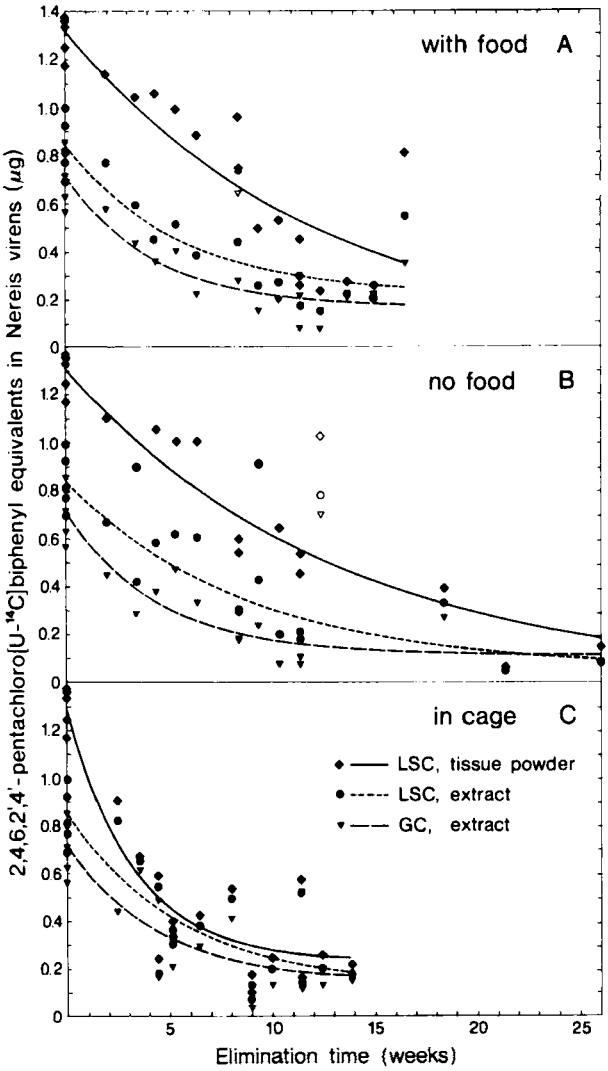


FIGURE 6 Elimination of 2,4,6,2′,4′-pentachloro[U-^{14}C]biphenyl equivalents by *Nereis virens* in three experimental groups A, B, C as measured by three different methods including (◆, ●) or excluding (▼) various metabolites. PCB amounts are adjusted to the dosis of a 5 g worm. Values indicated by white symbols are considered exceptual and are therefore omitted from the calculation.

relation between 2,4,6,2′,4′-pentachloro[U-^{14}C]biphenyl and its transformation products throughout the elimination period. The latter are represented by the differences between the highest and the lowest curves of each group A, B, C; the corresponding high values represent the sum of the parent compound and its metabolites, while the low values represent the parent compound only. The worms contain more radioactive compounds than are extractable, and the amounts of extractable compounds exceed those of the parent compounds. These differences are particularly pronounced in the laboratory experiments.

Here, the amount of all metabolites, if combined and expressed as PCB equivalents, is greater than that of the parent compound. At the end of the 10-day application period, the metabolites represent already 59% of the total radioactivity, i.e. 35% nonextracted, probably polar, compounds, 8% extracted metabolites not moving on thin layer chromatography plates and 16% extracted metabolites with R_f value lower than that of the parent compound. These three fractions very probably also represent three different metabolites. At the end of the elimination periods the metabolites amount to $0.12\,\mu g = 62\%$ (Group B—no food; TLC data insufficient for Group A—with food) and to $0.07\,\mu g = 30\%$ of all ^{14}C-labelled compounds (Group C—in cage); there is much variation but no trend in the individual percentages throughout the elimination period. Minima of 0.57 or $0.62\,\mu g$ respectively of the initially formed amount of $0.69\,\mu g$ metabolites were excreted. This indicates that considerable excretion is preceded by metabolization.

DISCUSSION

Elimination

When comparing the two laboratory experiments, with and without food, no differences in the elimination of any of the PCB isomers were noted. This is remarkable because the faeces have been recognized as a significant route of PCB excretion in *Nereis virens* (Ernst *et al.*, 1977). More than 40% of the excreted 2,4,6,2′,4′-pentachloro[U-^{14}C]biphenyl were recovered from the faeces (Goerke, 1984a). For worms deprived of food, the faecal route of

excretion is not available. This lack is possibly compensated for by enhanced PCB mobilization due to starvation (Roberts *et al.*, 1978). However, McManus *et al.* (1983) observed that copepods *Acartia tonsa* allowed to feed during depuration eliminated Aroclor 1254 more rapidly than unfed copepods.

Because PCB elimination did not differ in the two feeding regimes, the resulting similar elimination rates could also be expected for the feeding conditions of the caged worms. Temperatures during the initial weeks of the field experiment were ~4°C higher compared to the laboratory experiments. This temperature difference should actually have influenced the PCB elimination rate, as a temperature effect was recently demonstrated (Goerke, 1984a). In these experiments the effect might be obscured by influences of transition from the laboratory to the field conditions, and might not show up because the regression analysis was carried out for the full elimination time, i.e. over a great range of temperatures with an average close to 12°C.

In an earlier experiment, in which 2,4,6,2′,4′-pentachloro[U-^{14}C]-biphenyl was orally administered as a single compound in higher dosage (average initial concentration 3.3 μg/g), *Nereis virens* exhibited the same time of initial 50% ^{14}C elimination $t_e50 = 8.7$ weeks (Goerke and Ernst, 1977) as reported here for laboratory experiments with and without food (average initial concentration 0.23 μg/g). This identity is unexpected because (1) very different percentages of polar compounds were recovered from the worms on the two occasions, namely on average 16% (Ernst *et al.*, 1977) and 62% or 30% (these experiments) indicating differences in elimination routes, and (2) in further experiments a concentration dependent elimination rate of this PCB was demonstrated: $t_e50 = 26.5$ weeks at average initial concentration of 0.65 μg/g (Goerke, 1984a). There is no definite explanation for the phenomenon described. Maybe, the worms' lipid contents are of some influence; in the experiment just cited they were higher (∅ 2.5%) than in the present investigations (∅ 0.9%).

The times $t_e50 = 2.8$ weeks and $t_e50 = 1.9$ weeks needed for elimination the initial 50% of 2,4,5,2′,5′-PCB and 2,3,4,2′,5′-PCB are considerably shorter than the half-life times $t_{1/2}$ which are known for these compounds in other aquatic species. In carp,

Cyprinus carpio, the two compounds were eliminated with $t_{1/2}$ = 19.9 weeks and $t_{1/2}$ = 18.9 weeks (Tanabe *et al.*, 1982); in rainbow trout, *Salmo gairdneri*, the respective half-life times were $t_{1/2}$ = 22.1 weeks and $t_{1/2}$ = 2.7 years (Niimi and Oliver, 1983). Lobster, *Homarus americanus*, had $t_{1/2} \sim 9$ weeks for amounts of radioactivity of ^{14}C-2,4,5,2′,5′-PCB (Bend *et al.*, 1973). All cited values were adjusted for growth dilution. Obviously, the elimination constants of identical polychlorinated biphenyls vary greatly in different aquatic animals.

Biotransformation

Quantitative information on biotransformation of the two non-labelled pentachlorobiphenyl isomers can only be obtained indirectly by comparison of the elimination rates. PCBs are known to be transformed in organisms to more polar, less lipid soluble compounds, which may then be excreted (Roberts *et al.*, 1978). If, therefore, better elimination is an indication of better metabolization, the latter should be enhanced in the order of enhanced elimination: 2,4,6,2′,4′-PCB < 2,4,5,2′,5′-PCB < 2,3,4,2′,5′-PCB. This order is in agreement with one of the views on structural prerequisites for appreciable biotransformation, i.e. there are two unsubstituted vicinal carbon atoms in at least one ring of the PCB (Roberts *et al.*, 1978; Zell *et al.*, 1978), metabolization is hereby enhanced if meta and para positions are free of chlorine atoms instead of ortho and meta (Schulte and Acker, 1974; Matthews and Dedrick, 1984).

It is not known why the average percentages of ^{14}C-labelled metabolites in worms were considerably higher in these experiments (62%, 30%) than in the earlier ones (16%; Ernst *et al.*, 1977). The main difference between these and the earlier experiments apart from different average initial 2,4,6,2′,4′-pentachloro[U-^{14}C]-biphenyl concentrations (0.23–3.3 μg/g) is the coadministered PCB isomers in these investigations. Apart from concentration-dependent metabolization, induction of ^{14}C-PCB metabolizing enzymes by 2,3,4,2′,5′-PCB may be suggested, because the latter isomer is the compound with an extremely low accumulation percentage with the highest elimination rate.

CONCLUSIONS

The *Nereis virens* test system was used again successfully to study the elimination of xenobiotics. Considerable deviations of the values from the elimination curves were observed and these could not be explained by lipid content or sex. Apparently individual variations in metabolism and excretion of PCB occur. PCB elimination kinetics determined in the laboratory can be considered valid for natural conditions.

There were significant differences in the elimination of the three applied pentachlorobiphenyls indicating the importance of vicinal unsubstituted carbon atoms and hence of metabolic degradation in the process of elimination. It could not be determined why in the laboratory experiments compared (1) to the field experiment and (2) to the earlier laboratory experiment (Ernst *et al.*, 1977) much higher percentages of PCB metabolites occurred. This demonstrates the present limited insight in PCB metabolization. Cometabolism of mixed PCBs may be an important factor. When experimenters try to make the best use out of studies on xenobiotic elimination by applying several compounds together, they have to consider possible synergistic effects instead of independent behaviour.

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