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Accumulation and elimination of ^{14}C-γ-HCH (lindane) in *Nereis virens* (Polychaeta) with consideration of metabolites

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ABSTRACT: In two experiments, conducted at 6° and 16 °C, 43 *Nereis virens* were exposed to ^{14}C-labelled γ-HCH (lindane) at a concentration of 1 µg/l, which was maintained by intermittent monitoring and redosing. A closed system with individuals living in glass tubes was used. At equilibrium and during the subsequent phase of elimination, both γ-HCH and metabolites were determined in individual worms as well as in the water and faeces throughout the entire experiment. The bioconcentration factors of γ-HCH were 480 and 440 at 6° and 16 °C, respectively; those based on total radioactivity were 500 and 410. These factors were in the upper range of those known for other aquatic animals. The elimination may be described by an exponential function. The initial 50 % decrease of γ-HCH and γ-HCH + metabolites in the worms occurred at both temperatures in 2 and 3 days, respectively. The percentage of γ-HCH metabolites in worms increased considerably during the elimination period. At least four metabolites were detected in worms and water. If pollutants are evaluated by accumulation and elimination kinetics, metabolization has to be taken into account.

INTRODUCTION

During a recent investigation on levels of organochlorine residues in animals of the Weser estuary seven compounds were found, one of which was γ-HCH (Goerke et al., 1979). Various invertebrate species carried this substance at levels of 2 to 3 ng/g. Stadler (1977) determined γ-HCH concentrations of 5 to 6 ng/l in North Sea water. These values result from the wide application of lindane as an insecticide and from its persistence.

Analyses of environmental samples support our understanding of the fate of these xenobiotic substances. However, only controlled laboratory experiments will give detailed information on the basic processes governing bioaccumulation, elimination and metabolization of the foreign compounds. Therefore, the fate of γ-HCH in *Nereis virens* was investigated in the laboratory after this species had proved extremely suitable in experiments on the fate of three polychlorinated biphenyls (Goerke & Ernst, 1977; Goerke, 1979).

MATERIAL AND METHODS

In two experiments, conducted at 6° and 16 °C, 43 *Nereis virens* were exposed to ^{14}C-γ-HCH at a concentration of 1 µg/l. At equilibrium 5 worms per experiment were removed for determining the concentration factors. The remaining worms were transferred to vessels the water of which was continuously filtered over charcoal; in these

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vessels they stayed for various periods for measuring the rates of elimination. Worms, water, and faeces were additionally analysed for ^{14}C-labelled metabolites.

Test animals and their maintenance

Nereis (Neanthes) virens Sars (Polychaeta, Nereidae) naturally adapted to low and high temperatures were used for the two experiments. They were collected in November 1976 for the 6 °C experiment (Feb./March 1977) and in July 1976 for the 16 °C experiment (Sept./Nov. 1976) in the Kieler Bucht (see Goerke, 1979). They were acclimated to the conditions of the experiment: living in glass tubes, feeding on pieces of the polychaete *Lanice conchilega*, salinity 20 ‰ S (sea water of the German Bight, dilution with tap water), constant temperature, aeration, natural change of dim daylight and darkness. During the dark period indirect low illumination (~ 1.5 lx) helped to prevent worms from leaving their tubes (Goerke, 1979).

Exposure and clearance

At 6 °C, 22 worms (∅ 4.5 g, *s* 0.5 g) in 15 l water were used, at 16 °C, 21 worms (∅ 8.9 g, *s* 2.2 g) in 30 l water. The glass aquaria (36 × 23 × 26 cm, 1 at 6 °C, 2 at 16 °C) were maintained in a water bath at constant temperature (Cryostat FK2, Colora, Lorch). ^{14}C-γ-HCH in ethanolic solution was added to the water using a microlitre syringe to attain a concentration of 1 µg/l. A circulation pump (No. 381, Eheim, Deizisau) was installed to ensure uniform concentration in all parts of the test vessels. A nylon gauze filter prevented escaped worms from creeping into the circulation tubes. At frequent intervals the radioactivity in the water was measured and maintained within the levels indicated in Figures 2 and 3 by redosing with ^{14}C-γ-HCH.

At equilibrium *Nereis virens* were exposed for at least another week before either concentration factor determination or transfer to another aquarium fitted with a charcoal power filter (No. 386 SE, Eheim, Deizisau). As the 16 °C experiment was used to determine the appropriate periods the animals should spend in the elimination vessel, the worms had to be exposed for different periods at 16 °C, contrary to one period at 6 °C. The concentrations of ^{14}C-γ-HCH equivalents in the clearance water dropped from 3 to 0.2 ng/l within 4 days.

Test compound

^{14}C-γ-HCH (= 1,2,3,4,5,6-γ-hexachlorocyclohexane, γ-BHC, lindane), uniformly labelled, specific activity of 164 µCi/mg, from the Radiochemical Centre Amersham (Amersham Buchler, Braunschweig) was used in ethanolic solution (0.1 µg/µl). Before use, the compound was purified on an aluminium oxide column using n-hexane (batch No. 1, 16 °C experiment) or by thin layer chromatography (TLC) on silica gel G (Macherey-Nagel & Co., Düren; 250 µm on 20 × 20 cm glass plates) using n-hexane/benzene (3 : 1, v/v) as solvent; the separated ^{14}C-γ-HCH was detected by a radioscanner (Desaga, Heidelberg) and dissolved in n-hexane/acetone (2 : 1, v/v) (batch No. 2, 6 °C experiment). All solvents were of analytical reagent grade (Merck, Darmstadt). The values derived from ^{14}C measurements in the following refer to ^{14}C-γ-HCH in terms of equivalents.

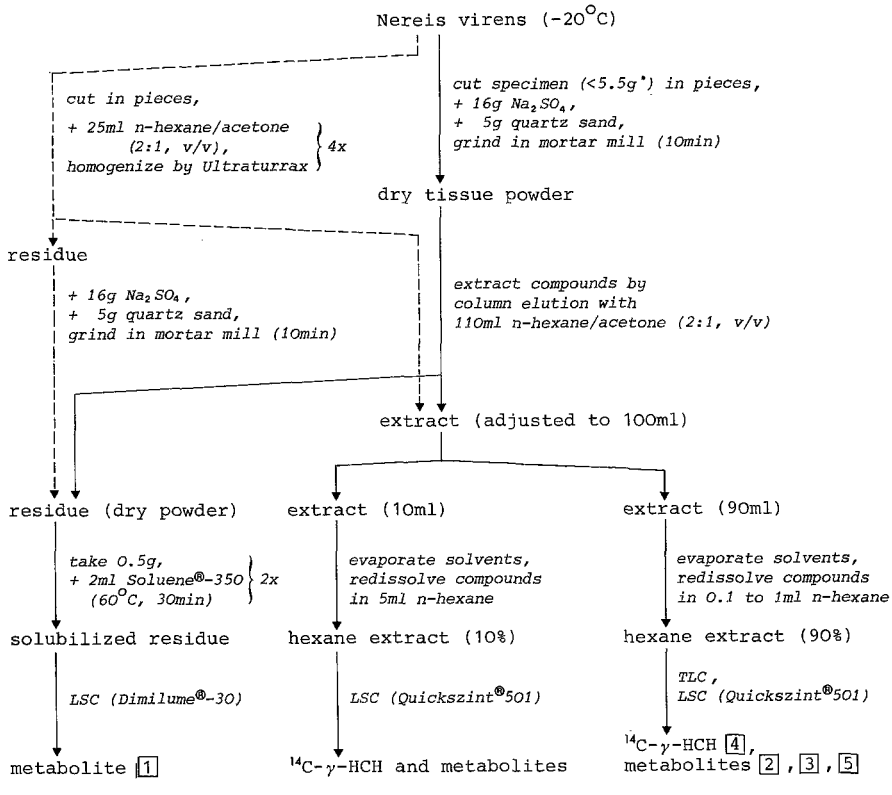

Fig. 1. Analytical procedure for worms. Dashed lines indicate an alternative, which was used in the 16 °C experiment (* preferably subsamples ≤ 4g)

Measurements of radioactivity

W a t e r. Samples of 1 and 5 ml water were added to 10 ml Insta-Gel® (Packard Instr. Comp., Frankfurt) in a scintillation vial and measured by liquid scintillation counting (LSC) (Betaszint BF 5000, Berthold/Frieseke, Karlsruhe). For measuring the radioactivity in the clearance vessels, a 45-ml sample was extracted with 5 ml n-hexane, 3 ml of which were taken for LSC with 15 ml Quickszint® 501 (PPO/POPOP scintillator, Zinsser, Frankfurt).

A n i m a l s. Worms were weighed live after blotting off surface water, wrapped in aluminium foil, and stored for 1 to 21 days at − 20 °C until analysed. Length of storage period did not affect the amount of radioactivity or percentage of metabolites.

Residues were extracted with n-hexane/acetone (2 : 1, v/v) by either of two methods (Fig. 1): (a) using an Ultraturrax (Janke & Kunkel, Staufen) with 4 × 25 ml solvent (16 °C experiment), or (b) grinding in a mortar mill with anhydrous sodium sulphate and quartz sand, and extracting the resulting powder in a cold column (6 °C experiment) (Ernst et al., 1974; Goerke & Ernst, 1977). The solvent was removed from an aliquot (10 ml) of the 100 ml extract and the residue redissolved in 5 ml n-hexane, of which 3 ml were used for LSC with 15 ml Quickszint® 501 (Zinsser, Frankfurt).

Aliquots of 0.5 g of the residue, which contained part of the polar metabolites, were dissolved in 2 ml Soluene®-350 (Packard Instr. Comp., Frankfurt) at 60 °C within 30 min. For LSC 15 ml Dimilume®-30 (Packard Instr. Comp.) were added to 1 ml of the Soluene®-350 extract.

Detection of metabolites

Animals. After quantitative measurement of the radioactivity, the remaining extract (90 %) was used for TLC examination (Fig. 1). The solvents were evaporated and the residues redissolved in 0.1 to 1 ml n-hexane according to radioactivity present in the samples. The compounds were separated by thin layer chromatography and detected as described above. Quantitative estimations were made by measurement of peak areas after radioscanning (16 °C experiment) or by liquid scintillation counting of the scraped spots (6 °C experiment).

Water. During the exposure of the animals, water samples of usually 100 ml were acidified with 4 ml 15N H_2SO_4, and extracted with n-hexane and ethyl ether. When changing the water of the aquaria, larger volumes of 1 to 16 l were extracted with n-hexane and ethyl ether after acidification. Extracts were dried over anhydrous sodium sulphate, concentrated in vacuo and subjected to TLC, as described.

Faeces. Faeces were collected by a pipette 5 times per day and stored at − 20 °C. Faeces of 7 days were combined, allowed to thaw and centrifuged. The faecal residue (~ 4 g) was analysed by column extraction, LSC and TLC, as described for worms. The supernatant was acidified to 1N H_2SO_4, extracted by n-hexane and ethyl ether; the extracts were then measured by LSC and TLC, the residue by LSC (Insta-Gel®).

RESULTS AND DISCUSSION

Accumulation

It took about 14 (6 °C) and 10 days (16 °C) until *Nereis virens* carried a load of ^{14}C-γ-HCH appearing to be in equilibrium with the concentration in the water of 1 μg/l (Figs 2, 3). The worms were exposed for another week before the concentration factors were determined. Even during this additional period of exposure, ^{14}C-γ-HCH had to be added for maintenance of the standard concentration; the reductions are thought to be due to metabolization and subsequent evaporation. Figure 3 shows a decrease in concentration from day 23 on when no worms were left and no ^{14}C-γ-HCH was added to the experimental vessel.

Concentration factors were calculated for ^{14}C-γ-HCH and tentatively for the sum of ^{14}C-γ-HCH equivalents (Table 1). A significant difference was not revealed by the *t*-test, but could have occurred if considerable amounts of metabolites had been produced with bioconcentration potentials other than that of the parent compound.

The average concentration factors of ^{14}C-γ-HCH were 480 and 440 at 6° and 16 °C, respectively (Table 1); the difference did not prove to be statistically significant (*t*-test). When metabolites were included, the average concentration factor at 6 °C, 500, was about 25 % higher than that at 16 °C, 410. This difference cannot be due to different averages of animal weights because: (1) in the 16 °C experiment there is no clear relation between concentration factor and weight (Table 1) and (2) animals in the 16 °C experi-

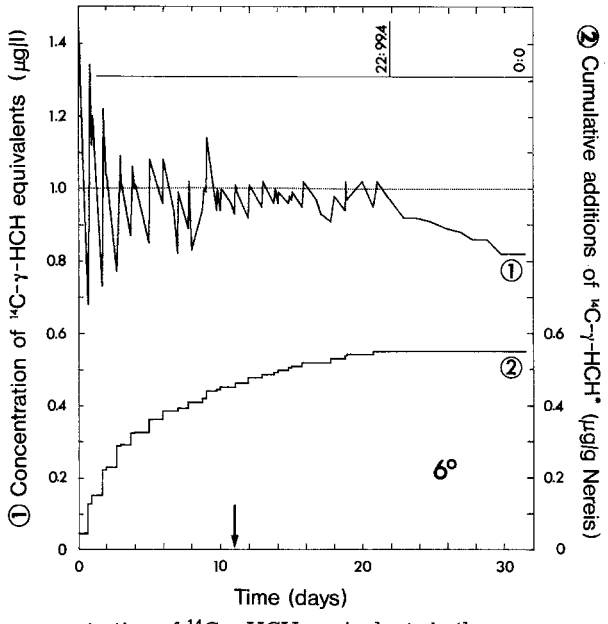

Fig. 2. Variation in concentration of ^{14}C-γ-HCH equivalents in the exposure water ① and cumulative additions of ^{14}C-γ-HCH ② (* above the initial level of 1 µg/l) at 6 °C. Arrow marks the time when the water was changed and ^{14}C-γ-HCH was redosed. Numbers separated by colons indicate number and total wet weight (g) of worms

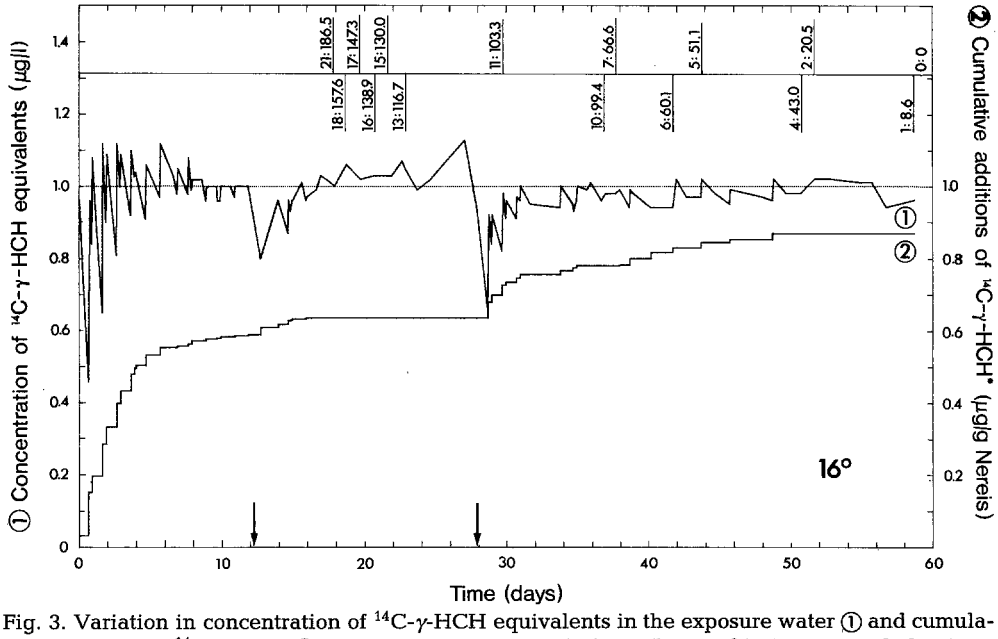

Fig. 3. Variation in concentration of ^{14}C-γ-HCH equivalents in the exposure water ① and cumulative additions of ^{14}C-γ-HCH ② (* above the initial level of 1 µg/l) at 16 °C. Arrows mark the times when the water was changed and ^{14}C-γ-HCH was redosed. Numbers separated by colons indicate number and total wet weight (g) of worms. Concentration decreases between days 17 and 28 are due to dilution

ment were on average heavier and thus most likely had somewhat higher lipid contents, which would favour higher concentration factors than in the 6 °C experiment. The significant difference between 500 and 410 in comparison with the insignificant difference between 480 and 440 suggests that temperature influences the storage of metabolites of ^{14}C-γ-HCH; however, at neither temperature were there significant differences between concentration factors of ^{14}C-γ-HCH equivalents and ^{14}C-γ-HCH (Table 1). Therefore, at present no definite conclusion on the influence of temperature on concentration factors of γ-HCH metabolites can be drawn. Temperature does not influence the concentration factor of the parent compound γ-HCH in *Nereis virens*. Similarly, no influence was found in *Mytilus edulis* and *Lanice conchilega* (Ernst, 1979).

Table 1. Accumulation of ^{14}C-γ-HCH and equivalents in *Nereis virens*. n. d. = not determined, s = standard deviation, t = t-test. * additional small-scale experiment, ** significantly different averages at $P = 0.02$, *** not significantly different averages at $P = 0.1$

	^{14}C-γ-HCH and equivalents		^{14}C-γ-HCH		Wet weight of *Nereis virens* (g)	
	6 °C	16 °C	6 °C	16 °C	6 °C	16°C
Level in *Nereis virens* (ng/g)	479	369	311	272	4.2	11.6
	458	458	309	332	4.1	9.1
	468	332	325	n.d.	4.0	8.5
	476	442	369	n.d.	4.0	4.9
	553	319	421	n.d.	3.8	3.9
Concentration in water (μg/l)	0.97	0.92–1.03	0.72	0.57		
Level in *Nereis virens* (ng/g)*		505		339		7.4
		474		392		6.8
		437		292		6.4
		393		266		4.9
		318		166		2.9
Concentration in water (μg/l)*		0.97–1.03		0.64		
Concentration factor	494	369	432	477		
	472	458	429	582		
	482	332	451	n.d.		
	491	429	512	n.d.		
	570	347	584	n.d.		
Concentration factor*		521		n.d.		
		489		n.d.		
		424		456		
		405		n.d.		
		309		259		
∅	502	408	482	444	4.0	6.6
s	39	69	66	135	0.1	2.6
t		2.77**		0.56***		
				−0.66***		
		0.59***				

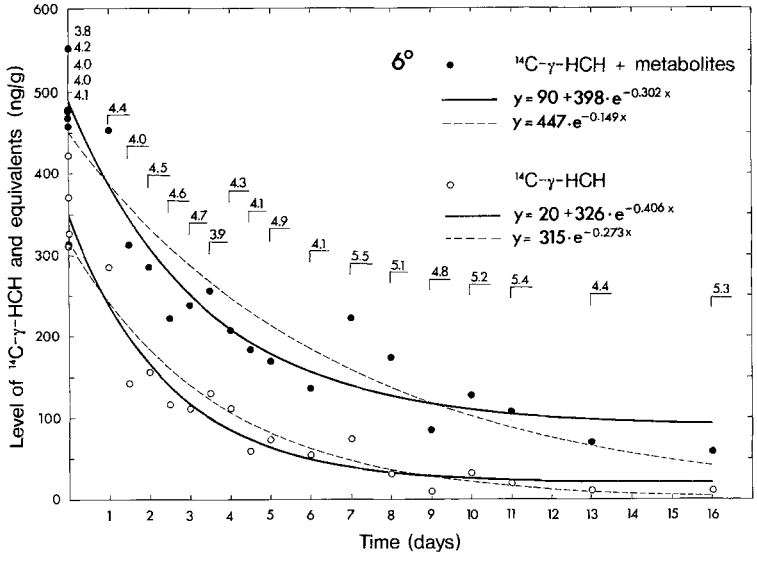

Fig. 4. Elimination of ^{14}C-γ-HCH and its metabolites in *Nereis virens* at 6 °C. Values above points are wet weights of worms (g). Levels are on wet tissue basis

The concentration factors (CF) of γ-HCH 440 to 480 determined in *Nereis virens* compare well with those in other aquatic invertebrate species. In zooplankton of an experimental field study, Hamelink & Waybrant (1976) found CF = 170 to 450; Ernst (1975, 1977, 1979) determined CF = 100 to 180 in *Mytilus edulis* and CF = 1200 in *Lanice conchilega*, Hansen (1980) CF = 40 in *Daphnia magna*. Analyses in *Cerastoderma edule, Mya arenaria, Arenicola marina, Crangon crangon* and *Solea solea* of the Weser estuary indicate CF = 300 to 600 (Stadler, 1977; Goerke et al., 1979). Concentration factors in various species of freshwater fish appear to vary from 420 to 1500 (Hamelink & Waybrant, 1976). Schimmel et al. (1977) measured CF = 320 for technical grade BHC (mixture of isomers) in *Crassostrea virginica*; Canton et al. (1975) determined CF ~ 60 for α-HCH in *Daphnia magna* and CF ~ 140 in *Lebistes reticulatus*, Canton et al. (1978) CF ~ 60–90 for α-HCH in *Artemia salina* and CF ~ 500 in saltwater-adapted *Lebistes reticulatus*.

Elimination

The elimination of ^{14}C-γ-HCH and all ^{14}C-labelled compounds is assumed to occur according to the function $y = a + b \cdot e^{-cx}$ (y compound level on wet tissue basis, x time); this assumption appears justified from the appreciable approximation of the measurements by the curves. The calculation of constants was carried out by the method of the least squares and iterative approximation; the latter is superior to a linear approximation after logarithmic transformation.

The equations are stated in Figures 4 and 5; a is relatively small in three out of the four equations of the general form stated above. In these cases, but not in the case "6°, γ-HCH + metabolites", $y = b \cdot e^{-cx}$ (a = O) may also be accepted. For the purpose of

Table 2. Elimination of ^{14}C-γ-HCH and equivalents by *Nereis virens*

	Time (days)			
	^{14}C-γ-HCH and equivalents		^{14}C-γ-HCH	
	6 °C	16 °C	6 °C	16 °C
$y = a + b \cdot e^{-cx}$				
t_e50	3.2	3.2	1.9	2.1
t_e75	8.4	6.9	3.9	4.1
t_e87.5			6.5	6.0
$y = b \cdot e^{-cx}$				
Elimination half-life	(4.7)	3.0	(2.5)	1.8

equal treatment, only the equations with $a \neq 0$ are considered in the following. It should be noted that calculations were carried out only for short periods of elimination. Therefore, it is not justified to extrapolate the curves $x \rightarrow \infty$ and to conclude for a residue that *Nereis virens* cannot eliminate at all.

At both temperatures, the total of ^{14}C-γ-HCH and metabolites was eliminated more slowly than ^{14}C-γ-HCH decreased by elimination and metabolization (Figs 4, 5, 6). $a \neq 0$ indicates that no half-life time can be calculated; therefore, in Table 2 elimination times t_e50 etc., corresponding to successive 50 % lowering of the levels, are compared, and a half-life time is stated only for 16 °C. The initial 50 % decrease of ^{14}C-γ-HCH and of ^{14}C-γ-HCH + metabolites in the worms occurred in 2 and 3 days, respectively, at both temperatures. The levels of ^{14}C-γ-HCH metabolites in the worms decrease extremely

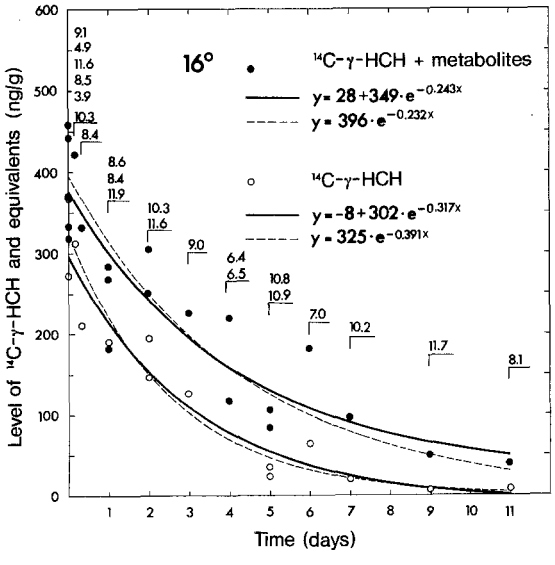

Fig. 5. Elimination of ^{14}C-γ-HCH and its metabolites in *Nereis virens* at 16 °C. Values above points are wet weights of worms (g). Levels are on wet tissue basis

slowly compared with those of ^{14}C-γ-HCH; this is seen from the comparison of the difference between the two solid curves with the lower curve in Figures 4 or 5.

The period of $t_e50 = 2$ days that *Nereis virens* exhibited for γ-HCH is longer than the values $t_e50 < 12$ h and $= 22$ h known in *Carassius auratus, Lepomis macrochirus* and *Mytilus edulis* (Gakstatter & Weiss, 1967; Ernst, 1977). Various initial concentrations of α-HCH in *Artemia salina* and *Lebistes reticulatus* were halved within $t_e50 = 48 - 72$ h and $t_e50 = 10$ h, respectively (Canton et al., 1978).

Contrary to various polychlorinated biphenyls (Ernst et al., 1977), which are less soluble in water, the percentage of eliminated ^{14}C-γ-HCH equivalents recovered from the faeces was extremely small (about 0.3 %). This indicates that there may be routes of more importance than the faecal one for γ-HCH elimination in *Nereis virens*.

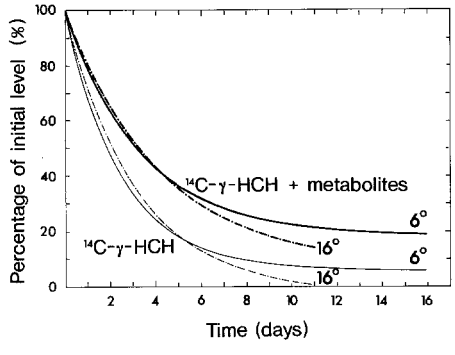

Fig. 6. Elimination of ^{14}C-γ-HCH and its metabolites in *Nereis virens* at 6° and 16 °C

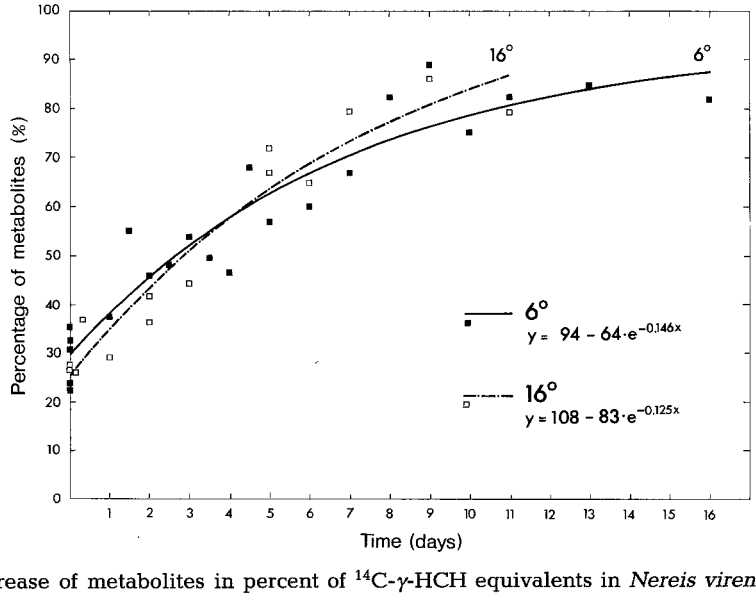

Fig. 7. Increase of metabolites in percent of ^{14}C-γ-HCH equivalents in *Nereis virens* during the elimination period at 6° and 16 °C

Transformation

Animals. As the levels of ^{14}C-γ-HCH metabolites in *Nereis virens* decrease extremely slowly compared with those of ^{14}C-γ-HCH, there is a steady increase in the percentage of the metabolites in the worms during the elimination period (Fig. 7). The percentages are about the same at both temperatures.

The chromatographic separation of ^{14}C-labelled substances of animal extracts from the 6 °C experiment exhibited three metabolites (2, 3, 5 in Fig. 8c) in addition to a further metabolite (1) which was not extractable by n-hexane/acetone; thus, at 6 °C *Nereis virens* contained at least four metabolites of ^{14}C-γ-HCH, the levels of which are shown in Figure 9 in comparison with those of all ^{14}C-γ-HCH equivalents (^{14}C-γ-HCH + metabolites) throughout the elimination period. Metabolite 1 represents 22 % of the radioactivity at the beginning; substances 5, 2 and 3 represent 3 %, 2 % and 2 %. The relatively

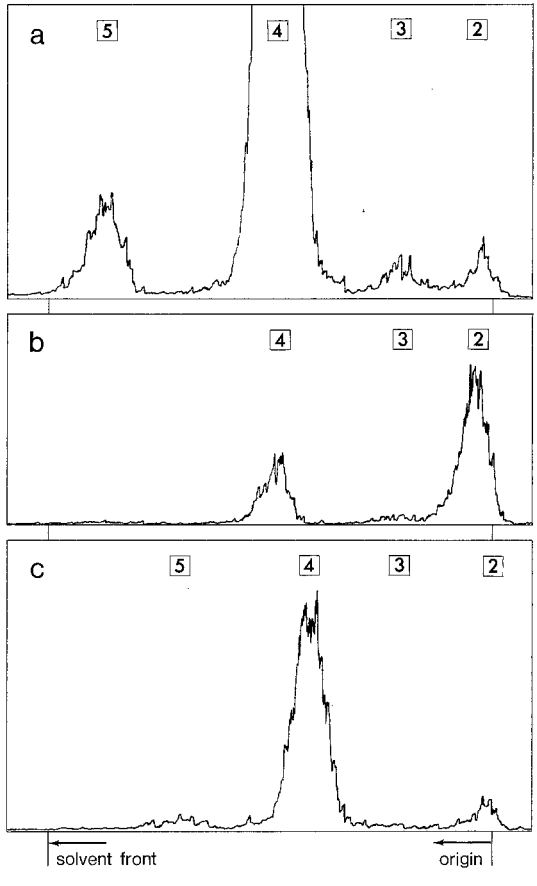

Fig. 8. Thin layer chromatograms presenting ^{14}C-labelled compounds of: (a) water extracted by n-hexane (end of 11-day exposure period, 6 °C, (b) water extracted by ethyl ether after acidification (specification as in a), (c) *Nereis virens* extracted by n-hexane/acetone (1.5 days after beginning of elimination period, 6 °C). Numbers in squares refer to different compounds, 4 represents ^{14}C-γ-HCH. Differences in R_f values between chromatograms c and a, b are attributed to matrix effects

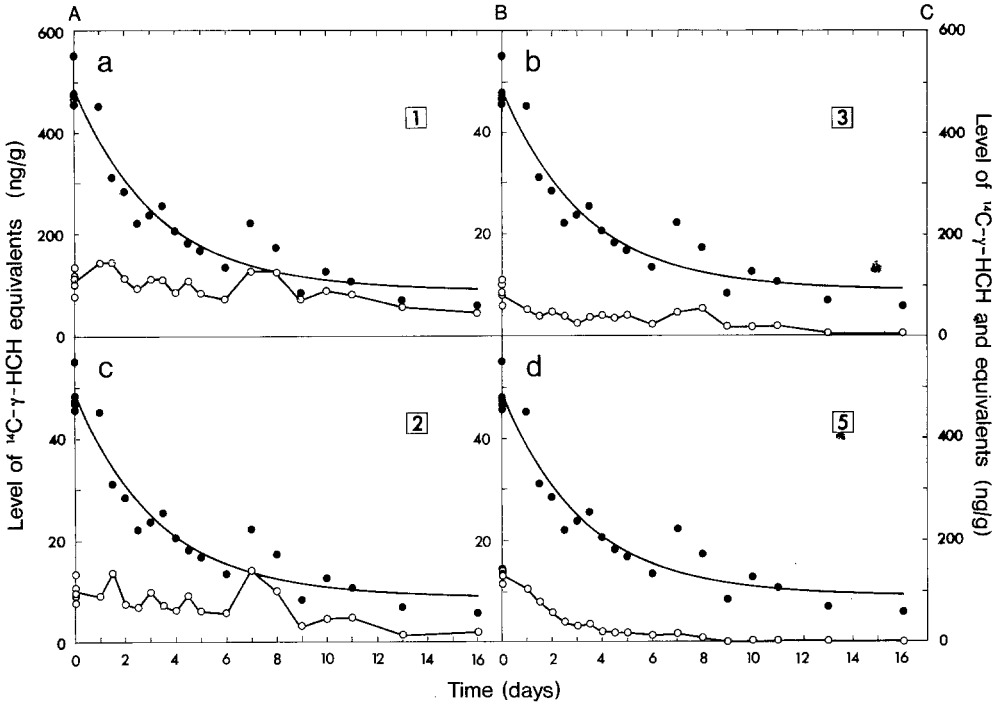

Fig. 9. Decrease in levels of metabolic compounds (O, scales A and B) in comparison to decrease in concentration of ^{14}C-γ-HCH equivalents (●, scale C) in *Nereis virens* at 6 °C during the elimination period. Numbers in squares refer to different metabolites

slow decrease, stated for all metabolites combined, is true for individual metabolites except for substance 5. At 16 °C, only metabolites 1 and 3 were detected in *Nereis virens*; metabolite 3 was present in a considerably higher percentage compared with the values at 6° (Fig. 10). It is concluded that *Nereis virens* has different metabolic capabilities concerning γ-HCH at different temperatures (and/or different animal weights). The rate of conversion of *p,p'*-DDT to *p,p'*-DDE in the fish *Salvelinus fontinalis* also varied with temperature (Zinck & Addison, 1975).

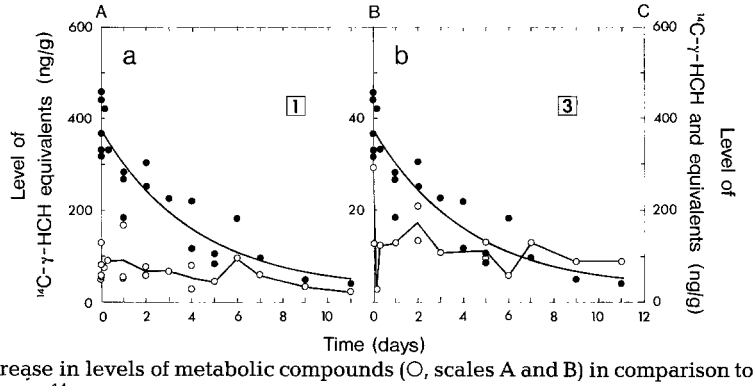

Fig. 10. Decrease in levels of metabolic compounds (O, scales A and B) in comparison to decrease in concentration of ^{14}C-γ-HCH equivalents (●, scale C) in *Nereis virens* at 16 °C during the elimination period. Numbers in squares refer to different metabolites

Water (6 °C experiment). During an exposure period of 11 days extracts of the water with n-hexane and ethyl ether were made daily. After changing the exposure water, extracts were made only at the end of another 11-day exposure period.

Chromatographic separation of the water extracts and subsequent radioscanning showed that at least the three metabolites found in worms were also present in the water (2, 3, 5 in Fig. 8a, b). Additionally the presence of a polar metabolite, 6 – not extractable with ethyl ether, could be assumed from comparing the measurements of radioactivity in extracts and directly in the water. Substance 6 might be identical with substance 1.

Unchanged ^{14}C-γ-HCH (substance 4) and substance 5 were preferably removed from the water with n-hexane (Fig. 8a), whereas ethyl ether extraction yielded the major part of the more polar metabolites (Fig. 8b). A considerable amount, more than 16 % of the total radioactivity in the water, was not extractable with ethyl ether from the acidified water. The patterns of metabolites at the end of the first and second 11-day exposure periods largely resembled one another. There was a continuous build-up of the different metabolites in the exposure water derived from daily measurements during the first 11-day period finally resulting in the values given in Table 3.

Water (16 °C experiment). The 16 °C experiment could not be fully evaluated from TLC data because the incompletely purified ^{14}C-γ-HCH batch No. 1 was used. This led to some difficulties in identifying metabolite 5. Therefore, a small-scale experiment with 5 animals in 4 l water was run additionally under the same conditions as before but using ^{14}C-γ-HCH batch No. 2. Qualitatively the same substances as indicated in Table 3 were detected with the exception that substance 5 could not be traced by radioscanning of the TLC plates. After scraping the corresponding spot from the plate and measuring the radioactivity by scintillation counting, the percentage of substance 5 could be assumed to be about 0.2 %. The percentage of the nonextractable metabolite (substance 6) was considerably higher: 37 % of the total radioactivity.

This high percentage of metabolites is thought to have caused the remarkable uptake of ^{14}C-γ-HCH by *Nereis virens* at 16 °C when the water was changed during the exposure period (Fig. 3): equilibrium of ^{14}C-labelled compounds between animals and water concerns both ^{14}C-γ-HCH and the metabolites. If in this phase, in which considerable quantities of metabolites are present in the water and in the worms, the water is changed and ^{14}C-γ-HCH is redosed, the latter is taken up from the water by the worms readily; and a decrease in concentration of the radioactivity in the water is observed if the worms take up the parent compound faster than they eliminate the metabolites.

Faeces. In the extracts of faeces only unchanged ^{14}C-γ-HCH could be detected, and the radioactivity remaining in the residual matter was assigned to polar metabolites. At 6 °C, total amounts from 3 weeks of collection of faeces during the exposure period

Table 3. Percentage of ^{14}C-labelled substances (^{14}C-γ-HCH equivalents) detected in the exposure water at 6 °C

Exposure period	Percentage of substance				
	2	3	4	5	6
First 11 days	3.2	3.9	82.4	0.4	10.1
Second 11 days	5.3	1.3	74.3	2.7	16.4

Table 4. Percentages of ^{14}C-γ-HCH and ^{14}C-labelled polar metabolites in faeces of *Nereis virens*

Temperature (°C)	Experimental period	^{14}C-γ-HCH equivalents (ng)	^{14}C-γ-HCH (%)	Metabolites (%)
6	Exposure period:			
	first week	220.7	92.3	7.7
	second week	430.0	84.2	15.8
	third week	168.2	77.5	22.5
	Elimination period	66.5	25.0	75.0
16	Exposure period:			
	first week	308.4	82.4	17.6
	second week	366.9	63.1	36.9
	third week	377.1	51.5	48.5

were: 676 ng ^{14}C-γ-HCH and 123 ng polar metabolites. At 16 °C, corresponding data were: 680 ng ^{14}C-γ-HCH and 373 ng polar metabolites. At both temperatures, the percentage of metabolites in faeces steadily increased during the experimental period (Table 4), as was described for the water.

The results obtained so far allow only some suggestions on metabolic pathways of ^{14}C-γ-HCH in the experimental system. Although γ-HCH is known to undergo a number of metabolic transformations in soil microbes, plants, and mammals (Engst et al., 1977, 1979), only little knowledge exists about its transformation in aquatic organisms. The formation of 1,3,4,5,6,-pentachlorocyclohexene-(1) is described by Sweeney (1969) for unicellular freshwater algae. However, in the common mussel, *Mytilus edulis*, no substantial transformation of γ-HCH has been observed (Ernst, 1975). *Nereis virens* is more likely to transform γ-HCH compounds than *Mytilus edulis* because it is capable of transforming lower chlorinated biphenyls considerably (Ernst et al., 1977).

From TLC data it may be assumed that pentachlorocyclohexene was formed, but additional experiments are necessary to confirm this structure. Further compounds, probably chlorinated benzenes, may be involved as well as chlorophenols, which may be in a conjugated form and be responsible for the inextractable ^{14}C-labelled substances. Hydrolysis of the exposure water after exhaustive extraction with ethyl ether liberated additional polar compounds, which could then be extracted with n-hexane and ethyl ether; this suggests that acid labile compounds have been formed.

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